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OP 01 Incretins: new clinical evidence

1

Time to insulin in the Trial Evaluating Cardiovascular Outomes with Sitagliptin (TECOS)

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Background and aims: TECOS was a randomized, placebo-controlled trial assessing the impact of sitagliptin on cardiovascular outcomes when added to usual care in patients with type 2 diabetes (T2DM). Among those not using insulin at baseline (n=11,263), we report the risk for progression to insulin during follow-up.

Materials and methods: TECOS enrolled 14,671 participants with HbA1c 6.5-8.0% on monotherapy with metformin (MET), pioglitazone, sulfonylurea (SU) or insulin, or dual combination with two oral agents or insulin with MET. They were randomized double-blind to sitagliptin or placebo, with subsequent diabetes management by the participants' usual care physician. Time to initiation of insulin was estimated using a Cox proportional hazards model.

Results: 5739 and 5472 participants were on mono- (MET 4435 [77%], SU 1246 [22%]) and dual- (SU + MET 5152 [94%]) oral agent therapy, respectively. Monotherapy patients had similar mean age (66 vs 65 years) but shorter median T2DM duration (6 vs 11 years), compared to dual therapy patients. MET monotherapy users were slightly younger (65 vs 68 years), had shorter T2DM duration (6 vs 8 years), similar HbA1c (7.1% vs 7.2%) and higher eGFR (77.4 vs 70.7) compared to SU monotherapy users. Overall, 4.7% of MET monotherapy users, 11.0% of SU monotherapy users and 17.2% of MET + SU users initiated insulin over a median duration of 3.1 years. Randomization to sitagliptin delayed the time to progression to insulin when added to MET monotherapy (1.3 vs 2.0 events per 100 pyrs; HR 0.67 [95% CI 0.51-0.89]) or SU + MET dual therapy (5.1 vs 7.8 events per 100 pyrs; 0.64 [0.56-0.73]), but not to SU monotherapy (4.0 vs 4.2 events per 100 pyrs; 0.96 [0.68-1.34]).

Conclusion: Among a cohort of international clinical trial participants with T2DM well controlled with MET or SU mono- or dual oral agent therapy, the rate of initiation of insulin was higher among SU users (as mono- or dual therapy). Randomization to sitagliptin treatment delayed progression to insulin among MET and MET + SU users.

Clinical Trial Registration Number: NCT00790205

Supported by: Merck & Co., Inc., Kenilworth, NJ, USA

Disclosure: **S.S. Engel:** Employment/Consultancy; Merck & Co., Inc.

2

Comparable glycaemic control with once weekly dulaglutide versus insulin glargine, both combined with lispro, in type 2 diabetes and chronic kidney disease (AWARD-7)

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Background and aims: This phase 3 study compared once weekly dulaglutide (DU) to titrated daily insulin glargine, both combined with insulin lispro, in people with type 2 diabetes (T2D) and moderate to severe chronic kidney disease (CKD) stages 3-4.

Materials and methods: Participants were randomised (1:1:1) to DU 1.5 mg or DU 0.75 mg or titrated insulin glargine. The objective was to demonstrate DU noninferiority for HbA_{1c} change from baseline.

Results: Baseline characteristics (N=576) included: [mean±SD] age 64.6 ±8.6 years, HbA_{1c} 8.6±1.0%, eGFR 38.3±12.8 mL/min/1.73m^2, BMI 32.5±5.2 kg/m^2, daily insulin dose 58.2±31.8 units. DU was non-inferior to insulin glargine for HbA_{1c} change from baseline at both 26 weeks (primary timepoint), and 52 weeks (table). Body weight decreased with DU, whereas it increased with insulin glargine at both timepoints. The hypoglycaemia event rate (glucose ≤3.9 mmol/L) was lower with DU 1.5 mg and 0.75 mg versus insulin glargine at 26 weeks (5.5, 7.8 and 17.1 events/participant/year; p<0.001 for both) and 52 weeks (5.8, 7.6 and 14.4 events/participant/year; p<0.001, p=0.004, respectively). Patients experiencing severe hypoglycaemia were fewer in the DU groups compared to the insulin glargine group (0 in DU 1.5 mg; 5 [2.6%] in DU 0.75 mg; and 13 [6.7%] in insulin glargine). Nausea, vomiting and diarrhoea were more common with DU 1.5 mg (19.8%, 13.5%, 17.2%) and DU 0.75 mg (14.2%, 8.4%, 15.8%) versus insulin glargine (4.6%, 4.6%, 7.2%).

Conclusion: In conclusion, DU produced comparable glycaemic control, greater weight loss, and lower hypoglycaemia rate versus insulin glargine in people with T2D and CKD stage 3-4, with the anticipated gastrointestinal side effects.

	Timepoint	DU 1.5 mg	DU 0.75 mg	Insulin Glargine
mITT population (N=549)		**(N=183)**	**(N=180)**	**(N=186)**
[a]HbA_{1c} change	Week 26, %	-1.2 (0.1)[†,††]	-1.1 (0.1)[†]	-1.1 (0.1)
	Week 26, mmol/mol	-13.1 (1.1)[†,††]	-12.0 (1.1)[†]	-12.0 (1.1)
	Week 52, %	-1.1 (0.1)[†,††]	-1.1 (0.1)[†]	-1.0 (0.1)
	Week 52, mmol/mol	-12.0 (1.1)[†,††]	-12.0 (1.1)[†]	-10.9 (1.1)
Proportion (%) with HbA_{1c} <7% (<53 mmol/mol) / HbA_{1c} <8% (<64 mmol/mol)	Week 26	37.5 / 78.3	31.7 / 72.6	34.6 / 75.3
	Week 52	32.9 / 69.1	33.5 / 69.5	29.1 / 70.3
Safety population (N=576)		**(N=192)**	**(N=190)**	**(N=194)**
[b]Weight change, kg	Week 26	-2.8 (0.4)[##]	-2.0 (0.4)[##]	1.1 (0.3)
	Week 52	-2.7 (0.5)[##]	-1.7 (0.4)[##]	1.6 (0.4)

Data are reported as LSM (SE) unless otherwise indicated.
[†, ††] Multiplicity adjusted 1-sided p<0.001 for noninferiority versus insulin glargine with a 0.4% margin or 0.3% margin, respectively, [##]2-sided p<0.001 versus insulin glargine.
[a] HbA_{1c} changes from baseline were significant for all treatment groups (2-sided p<0.001 change from baseline)
[b] Body weight significantly decreased in DU 1.5 mg and DU 0.75 mg (2-sided p<0.001 change from baseline); and significantly increased in the insulin glargine group (2-sided p<0.05 and p<0.001 change from baseline at Week 26 and Week 52, respectively).
Abbreviations: BMI=body mass index; eGFR=estimated glomerular filtration rate (CKD-EPI creatinine equation); mITT=modified intent-to-treat; LSM=least squares mean; SE=standard error
Definitions: Safety population=randomised patients who received at least one study drug dose; mITT=patients in the safety population who have at least one post-randomisation HbA_{1c} measurement.

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Supported by: Eli Lilly and Company

Disclosure: **K.R. Tuttle:** Employment/Consultancy; Eli Lilly and Company.

3

Individual HbA_{1c} and weight responses to exenatide QW or placebo added to titrated insulin glargine in type 2 diabetes uncontrolled after insulin optimisation in the DURATION-7 study

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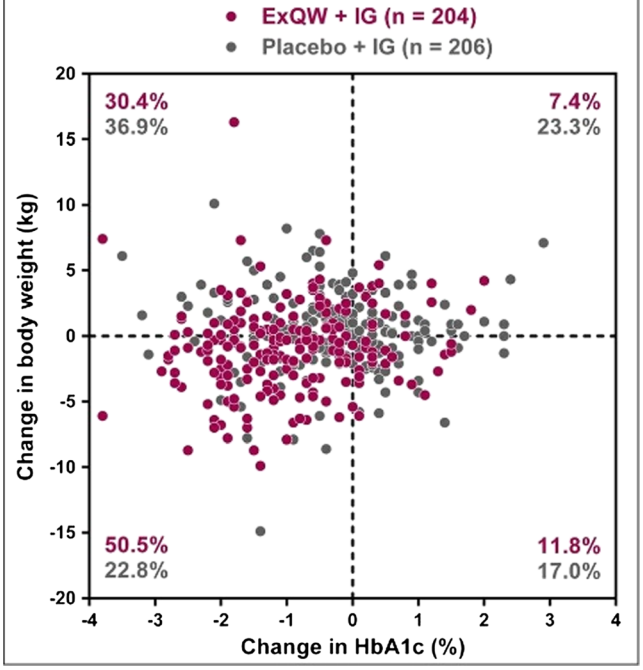
Background and aims: Results of the DURATION-7 trial showed that, compared to placebo, adding exenatide once weekly (ExQW) to titrated insulin glargine (IG) ± metformin resulted in significant HbA1c-lowering and weight loss in patients with type 2 diabetes (T2D) not achieving glycaemic targets on intensified IG. Since mean values do not provide information on distribution of HbA1c and weight responses, in this analysis we examined the associated HbA1c and body weight individual responses to ExQW or placebo added to titrated IG.

Materials and methods: DURATION-7 included 511 T2D patients with screening HbA1c 7.5-12.0% who underwent an 8-week IG dose optimization period during which any sulphonylurea use was discontinued. Subsequently, 464 patients with continued hyperglycaemia (HbA1c 7.0-10.5%) were randomized to ExQW (n=233) or placebo (n=231) added to IG ± metformin while IG titration continued over 28 weeks.

Results: Of 464 randomized patients, 91% completed 28 weeks. Mean baseline values in patients who received treatment and had at least one post-baseline HbA1c measurement (n=461) were: age 57.7 years; diabetes duration 11.3 years; HbA1c 8.5%; body weight 94.0 kg; BMI 33.7 kg/m^2; IG dose 51 U/day. At 28 weeks, a greater proportion of patients achieved any HbA1c reduction with ExQW + IG vs placebo + IG (165/204, 81% vs 123/206, 60%, respectively), while half the proportion of patients treated with ExQW + IG experienced an increase in HbA1c versus those treated with placebo + IG (39/204, 19% vs 83/206, 40%, respectively; Figure). The proportion of patients experiencing any body weight loss was greater with ExQW + IG vs placebo + IG (127/204, 62% vs 82/206, 40%, respectively), while almost half the proportion of patients treated with EQW + IG experienced body weight gain versus those treated with placebo + IG (77/204, 38% vs 124/206, 60%, respectively; Figure). The proportion of patients achieving both HbA1c and body weight reduction with EQW + IG was more than double that observed with placebo + IG (103/204, 51% vs 47/206, 23%, respectively; Figure).

Conclusion: While patients with inadequately controlled T2D despite optimized IG ± metformin, demonstrated a wide range of HbA1c and body weight responses with ExQW or placebo addition to ongoing titrated IG, patients receiving ExQW were twice as likely to experience an improvement in HbA1c and body weight reductions than those remaining on titrated IG alone.

Figure. Individual HbA1c and Body Weight Responses at 28 Weeks. One ExQW-treated patient with changes in body weight of −34 kg and HbA1c of −4.4% not shown.



Clinical Trial Registration Number: NCT02229383
Supported by: AstraZeneca
Disclosure: **E. Hardy:** Employment/Consultancy; AstraZeneca. Stock/Shareholding; AstraZeneca.

4

Semaglutide provides sustained reductions in body weight over 2 years in subjects with type 2 diabetes (SUSTAIN 6)

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Background and aims: Semaglutide, a glucagon-like peptide-1 analogue in development for the treatment of type 2 diabetes (T2D), has demonstrated superior body weight loss vs placebo and active comparators across the SUSTAIN phase 3a clinical trial programme. Excess weight is often a concomitant complication in patients with T2D and is associated with increased cardiovascular risk.

Materials and methods: SUSTAIN 6 was a 2-year cardiovascular outcomes trial that was conducted in 3,297 subjects with T2D at high risk of cardiovascular events. The primary outcome was a composite of the first occurrence of CV death, non-fatal myocardial infarction or non-fatal stroke. Secondary endpoints included glycaemic and weight-related endpoints. Key inclusion criteria were subjects ≥50 years old with established cardiovascular disease (previous cardio-, cerebro-, or peripheral-vascular disease), chronic heart failure (New York Heart Association class II or III), or chronic kidney disease (stage 3 or higher) or ≥60 years old with at least one cardiovascular risk factor. Subjects were randomised to once-weekly, s.c. semaglutide 0.5 or 1.0 mg, or volume-matched placebo, added to standard of care, without any lifestyle intervention, for 104 weeks.

Results: At baseline, overall mean body weight, age, duration of diabetes and HbA_{1c} were 92.1 kg, 65 years, 13.9 years and 8.7%, respectively. Semaglutide significantly reduced the risk of the primary composite outcome vs placebo (hazard ratio, 0.74; 95% CI, 0.58-0.95; p<0.001 for non-inferiority with a margin of 1.8). Treatment with semaglutide, vs placebo, led to significantly reduced body weight, BMI and waist circumference at 2 years vs placebo (p<0.001; Table). BW loss plateaued at Week 44 and was sustained throughout the remainder of the trial until Week 104. The proportion of subjects achieving ≥5% and ≥10% reduction in body weight was more than two-fold greater with semaglutide vs placebo. For semaglutide 0.5 and 1.0 mg, 77% and 81% of subjects, respectively, had no weight gain at 2 years, compared with 52% and 53% of subjects receiving placebo 0.5 and 1.0 mg, respectively. A dose-response effect with semaglutide was observed for body weight reduction.

Conclusion: Semaglutide treatment, added to standard of care, led to clinically meaningful and sustained reductions in body weight, BMI and waist circumference at 2 years in subjects with T2D at high cardiovascular risk.

Table. Body weight-related endpoints: Change from baseline at Week 104

	Overall mean at baseline	Semaglutide 0.5 mg	Semaglutide 1.0 mg	Placebo 0.5 mg	Placebo 1.0 mg
Number of randomised subjects		826	822	824	825
Body weight, kg					
	92.1	−3.6	−4.9	−0.7	−0.5
ETD vs placebo [95% CI]	-	−2.87* [−3.47; −2.28]	−4.35* [−4.94; −3.75]		
BMI, kg/m²†					
	32.8	−1.3	−1.8	−0.2	−0.2
ETD vs placebo [95% CI]	-	−1.06* [−1.28; −0.85]	−1.59* [−1.80; −1.37]		
Waist circumference, cm					
	110.2	−2.7	−4.2	−0.6	−0.9
ETD vs placebo [95% CI]	-	−2.17* [−2.82; −1.53]	−3.25* [−3.89; −2.60]		
Weight loss category, n (%)					
≥5%		297 (36)*	383 (47)*	144 (18)	154 (19)
≥10%		109 (13)*	168 (20)*	47 (6)	54 (7)

*p<0.0001. †*Post-hoc* defined endpoint. Data are in trial, including all scheduled assessments from randomisation to last subject-site contact or death, estimated mean changes from baseline, and treatment differences with CIs from mixed models for repeated measures. For weight loss categories, data are observed proportions; p-values are from logistic regressions where missing data were imputed as predictions from a mixed model for repeated measures. BMI, body mass index; CI, confidence interval; ETD, estimated treatment difference.

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5

The effects of GIP/GLP-1 receptor co-activation on appetite and food intake in overweight/obese subjects

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Background and aims: Glucagon-like peptide-1 (GLP-1) is a potent suppressor of appetite and food intake in humans whereas the other incretin hormone, glucose-dependent insulinotropic polypeptide (GIP), does not seem to have independent effects on eating behaviour. Interestingly, rodent data have shown that concomitant activation of the GIP and the GLP-1 receptors may potentiate the satiety-promoting effects of GLP-1. The aim of this study was to delineate the effects of GIP/GLP-1 receptor co-activation on food intake, appetite, and resting energy expenditure (REE) in overweight/obese humans.

Materials and methods: We examined 18 overweight/obese men (age: 38 (25-70) years [median (range)]; BMI: 33 (26-36) kg/m^2) during five experimental days separated by at least 72 hours: 50-g OGTT followed by isoglycaemic i.v. glucose infusion (IIGI)+saline (placebo), IIGI+GIP (4 pmol/kg/min), IIGI+GLP-1 (1 pmol/kg/min) and IIGI+GIP+GLP-1 (4 and 1 pmol/kg/min, respectively) performed in a double-blinded and randomised order. The primary endpoint was food intake measured by an *ad libitum* meal after 240 minutes of infusion. Secondary endpoints included appetite ratings on visual analogue scales (VAS) and REE measured by indirect calorimetry at baseline and after 210 minutes.

Results: Food intake was significantly less on the IIGI+GLP-1 day, compared to placebo (2,736±1,637 kJ vs. 4,391±2,306 kJ [mean±SD], p=0.035) while no significant differences were seen on the IIGI+GIP (4,048±2080 kJ) and the IIGI+GIP+GLP-1 day (3,827±1,815 kJ), compared to placebo. On the IIGI+GLP-1 day participants reported less hunger, compared to placebo (-6.9 [-2.4 to -11.4] (mean difference [95% CI]), p<0.001), a higher degree of satiety (4.6 [8.6 to 0.5], p<0.05) and lower prospective food consumption (-5.9 [-2.4 to -9.5], p<0.0001). The IIGI+GIP day and the IIGI+GIP+GLP-1 day, did not differ from placebo on any of the above ratings. Ratings of comfort and nausea, and measures of REE were similar between all study days.

Conclusion: While GLP-1 receptor activation lowers food intake and appetite in overweight/obese male subjects, simultaneous activation of the GIP receptor did not potentiate these GLP-1-mediated effects.

Clinical Trial Registration Number: NCT02598791

Supported by: The Innovation Fund Denmark, Zealand Pharma A/S, The Vissing Foundation

Disclosure: **N.C. Bergmann:** Employment/Consultancy; Zealand Pharma A/S.

6

DURATION-8 randomised controlled trial 1-year results: efficacy and safety of once-weekly exenatide plus once-daily dapagliflozin versus exenatide or dapagliflozin alone

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Background and aims: In patients with T2D uncontrolled by metformin (baseline HbA1c 8-12%), dual therapy for 28 weeks with once-weekly exenatide (ExQW) plus once-daily dapagliflozin (DAPA) reduced glycaemia, body weight and systolic blood pressure (SBP) significantly more than with ExQW + placebo or DAPA + placebo alone with no unexpected safety signals (DURATION-8 trial). Here, we examined these outcomes after a further 24 weeks of double-blind therapy.

Materials and methods: DURATION-8 was a phase 3, multicentre, double-blind, randomised, 28-week study with a 24-week double-blind extension. Adults with T2D (n=695; 1 not treated) were randomised to ExQW (2-mg subcutaneous injection) plus DAPA (10-mg oral tablet) (n=231), ExQW with oral placebo (n=230), or DAPA with injected placebo (n=233).

Results: Of 695 patients randomized, 525 (75.5%) completed 52 weeks with 3.7% discontinuing due to adverse events (AEs). At Week 52, greater reductions in HbA1c, FPG, 2-h PPG, body weight and SBP were observed with ExQW + DAPA versus ExQW + placebo or DAPA+ placebo. Compared with Week 28, reductions in HbA1c in all treatment groups and treatment differences were maintained at Week 52. AEs occurring in ≥5% of patients were diarrhoea, headache, injection site nodule, nausea, upper respiratory tract infection, and urinary tract infection. Serious AEs occurred in 4.8%, 5.2%, and 5.2%, respectively. Minor hypoglycaemia occurred in 1.3%, 0%, and 0.4%. No major hypoglycaemia was recorded. Reductions in estimated glomerular filtration rate at Week 1 with ExQW + DAPA and DAPA + placebo returned to baseline by Week 52 (Table).

Conclusion: ExQW + DAPA was well tolerated with no unexpected AEs, and the glycaemic, weight, and SBP effects observed at Week 28 were maintained over 52 weeks.

Table. Changes in efficacy-related endpoints from baseline to Week 52

	ExQW+DAPA N=228 n=180	ExQW+PBO N=227 n=163	DAPA+PBO N=230 n=182
HbA1c, %			
BL mean (SD)	9.34 (1.07)	9.30 (1.06)	9.30 (1.03)
28 week LSM change (SE)	−1.96 (0.09)	−1.59 (0.09)	−1.38 (0.09)
Diff vs ExQW+DAPA (SE)		−0.37 (0.13)**	−0.58 (0.13)***
52 week LSM change (SE)	−1.75 (0.10)	−1.38 (0.10)	−1.23 (0.10)
Diff vs ExQW+DAPA (SE)		−0.37 (0.14)**	−0.52 (0.13)***
FPG, mg/dL			
BL mean (SD)	198.8 (46.9)	195.0 (43.5)	195.6 (42.0)
28 week LSM change (SE)	−65.0 (2.9)	−44.8.0 (3.0)	−48.4 (2.9)
Diff vs ExQW+DAPA (SE)		−20.2 (4.0)***	−16.6 (4.0)***
52 week LSM change (SE)	−63.0 (2.9)	−45.7 (3.1)	−39.7 (3.0)
Diff vs ExQW+DAPA (SE)		−17.3 (4.1)***	−23.3 (4.0)***
2h-PPG, mg/dL			
BL mean (SD)	270.7 (66.4)	269.3 (66.6)	262.5 (60.8)
28 week LSM change (SE)	−87.0 (4.1)	−59.6 (4.3)	−61.4 (4.2)
Diff vs ExQW+DAPA (SE)		−27.4 (5.2)***	−25.7 (5.2)***
52 week LSM change (SE)	−82.4 (4.8)	−64.0 (5.1)	−59.6 (5.0)
Diff vs ExQW+DAPA (SE)		−18.4 (6.3)**	−22.8 (6.2)***
Body weight, kg			
BL mean (SD)	91.8 (22.2)	89.8 (20.2)	91.1 (19.7)
28 week LSM change (SE)	−3.6 (0.3)	−1.6 (0.3)	−2.2 (0.3)
Diff vs ExQW+DAPA (SE)		−2.0 (0.4)***	−1.3 (0.4)***
52 week LSM change (SE)	−3.3 (0.4)	−1.5 (0.4)	−2.3 (0.4)
Diff vs ExQW+DAPA (SE)		−1.8 (0.5)***	−1.0- (0.5)
Systolic BP, mmHg			
BL mean (SD)	130.1 (12.7)	129.1 (13.1)	130.0 (12.9)
28 week LSM change (SE)	−4.3 (0.8)	−1.2 (0.8)	−1.8 (0.8)
Diff vs ExQW+DAPA (SE)		−3.1 (1.1)**	−2.5 (1.1)*
52 week LSM change (SE)	−4.5 (0.8)	−0.7 (0.9)	−2.8 (0.8)
Diff vs ExQW+DAPA (SE)		−3.8 (1.1)***	−1.7 (1.1)
eGFR (CKD-EPI), ml/min/1.73m^2			
BL mean (SD)	98.0 (17.2)	97.7 (17.5)	97.1 (17.4)
1 week mean change (SD)	−3.8 (9.2)	−1.0 (10.6)	−3.9 (8.7)
28 week mean change (SD)	−1.0 (9.5)	−0.1 (8.5)	−1.3 (8.7)
52 week mean change (SD)	−2.0 (9.0)	−1.1 (8.7)	−0.8 (10.4)

*p<0.05, **p<0.01, ***p<0.001 (p-values at Week 52 are nominal).
BL, baseline, BP, blood pressure; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; LSM, least-squares mean; N, number comprising the intention-to-treat analysis set; n, number completing 52 weeks of treatment; 2h- PPG, 2-hour post-prandial glucose; SD, standard deviation; SE, standard error.

Clinical Trial Registration Number: NCT02229396

Supported by: AstraZeneca

Disclosure: **C. Guja:** Honorarium; Quintiles as Investigator in the DURATION 8 trial.

OP 02 Fat in the liver: how it gets in, how it gets out

7

Non-alcoholic fatty liver disease and risk of mortality and cardiovascular disease among people with type 2 diabetes: a national retrospective cohort study

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) occurs frequently in type 2 diabetes (T2DM), but the association between NAFLD and mortality or cardiovascular disease (CVD) in people with T2DM is unclear. Our aim was to investigate whether clinically significant NAFLD as identified from history of hospital admission with NAFLD is associated with mortality or CVD among people with T2DM.

Materials and methods: We used linked routine data from Scottish population-based diabetes, hospital admissions and death registers for people with type 2 diabetes diagnosed between 2004 and 2013 of 40-89 years of age who had a history of at least one hospital admission and complete data available. NAFLD, cause-specific mortality and CVD were identified using International Classification of Diseases codes. Cox proportional hazards models were adjusted for age, sex, an area-based measure of socio-economic status, smoking status, high blood pressure/ anti-hypertensive use, high cholesterol/statin treatment, HbA1c and prevalent CVD at diagnosis of diabetes.

Results: We included 133,312 people with complete data available (88% of potentially eligible people) of whom 1998 had a hospital admission mentioning NAFLD. At diagnosis of diabetes people with NAFLD compared to those without NAFLD were younger (60.0 vs 62.7 years), had higher BMI (32.9 vs 32.1 kg/m2) and HbA1c (64 vs 62 mmol/l) and were more likely to be women (49% vs 45%) and current smokers (25% vs 22%), p<0.01 for all comparisons. Adjusted hazard ratios (95% CIs) and numbers of events by NAFLD status over mean follow-up of 4.7 years are shown in the table.

Conclusion: NAFLD is an independent risk factor for increased mortality and incident/recurrent CVD events in people with type 2 diabetes. Lifestyle interventions should be emphasised for people with type 2 diabetes to prevent NAFLD and effective, safe treatments are urgently needed for NAFLD.

Outcome	HR (95% CI) for NAFLD vs no NAFLD	Number of events	
		NAFLD	No NAFLD
All-cause mortality	2.11 (1.92, 2.32)	434	16,211
CVD mortality	1.39 (1.10, 1.74)	75	4,432
Hepato-cellular cancer (HCC) mortality	41.9 (27.1, 64.8)	33	59
Cancer mortality (excluding HCC)	1.15 (0.92, 1.42)	85	5,570
Other causes of death	3.16 (2.77, 3.59)	241	6,150
Incident or recurrent CVD event	1.62 (1.47, 1.77)	460	21,892

HRs and number of events by NAFLD status for all-cause and cause-specific mortality and CVD events among study cohort of people with type 2 diabetes.

Supported by: Data linkage was supported by the Scottish Government
Disclosure: **S.H. Wild:** Grants; Support for data linkage was provided by the Scottish Government.

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Association between steatohepatitis and left ventricular diastolic function in type 2 diabetes

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Background and aims: Association between hepatic steatosis or fibrosis and cardiac dysfunction in relation to insulin resistance has not been fully investigated. We evaluated whether hepatic steatosis or fibrosis is associated with left ventricular (LV) diastolic dysfunction in patients with type 2 diabetes (T2DM).

Materials and methods: 454 patients with T2DM, aged 55 years or older (men 22.0%, mean age 64.9 years old) were enrolled. All subjects had undergone liver ultrasonography, pulsed-wave doppler echocardiography, short insulin tolerance test (SITT), and bioimpedance analysis. Simple hepatic steatosis and steatohepatitis were defined in the presence or absence of fibrosis according to NAFLD fibrosis scores by liver ultrasonography. Diastolic dysfunction was measured by using peak early (E) to late (A) ventricular filling ratio (E/A) and E-wave deceleration time (DT).

Results: Of 454 patients, 284 (62.6%) had hepatic steatosis, and 273 (60.1%) had diastolic dysfunction. The prevalence of diastolic dysfunction showed a positive correlation with the presence of hepatic steatosis or fibrosis (52.9%, 62.0%, and 65.3%; normal, simple steatosis, and steatohepatitis, respectively; P for trend<0.05). Multivariate logistic regression analysis revealed a significant association between diastolic dysfunction and hepatic steatosis (odds ratio [OR]=1.98, 95% confidence interval [CI]=1.02-3.90, P<0.05), after adjusting for glycometabolic parameters, abdominal fat percentages, and SITT. Furthermore, subjects with steatohepatitis presented a significantly higher odds for diastolic dysfunction (OR=2.06, 95% CI=1.04-4.11, P<0.05) compared to subjects with normal or simple steatosis. This statistical significance was attenuated when including SITT in the model though.

Conclusion: In conclusion, hepatic steatosis and steatohepatitis is an independent predictive marker for LV diastolic dysfunction in older adults with T2DM, and insulin resistance may mediate this correlation between steatohepatitis and diastolic dysfunction.

Disclosure: **K. Huh:** None.

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Fatty liver disease determines the progression of coronary artery calcification in a metabolically healthy obese population

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Background and aims: Metabolically healthy obese (MHO) phenotype describes an obese state with a favorable metabolic profile. However, the prognosis of this subpopulation remains controversial. We aimed to examine whether MHO phenotype is associated with progression of atherosclerotic activity, reflected as the changes in coronary artery calcification (CAC) over time. If so, we sought to determine the role of fatty liver disease (FLD), the hallmark of hepatic steatosis, in this progression.

Materials and methods: We enrolled 1,240 asymptomatic subjects who underwent repeated CAC score measurement during routine health examinations. CAC score progression was defined as either incident CAC in a population free of CAC at baseline, or an increase by ≥2.5 units between the baseline and final square root of CAC scores in participants with detectable CAC at baseline. Subjects were stratified by body mass index (cut-off, 25.0 kg/m^2) and metabolic health state using Adult Treatment Panel-III criteria. FLD was assessed via ultrasonography.

Results: Over 2.9 years of follow-up, 25.2% of total subjects exhibited CAC score progression. The MHO phenotype was not significantly

associated with CAC score progression (multivariate adjusted-odds ratio [OR], 1.45; 95% confidence interval [CI], 0.93-2.25), as compared to the metabolically healthy non-obese (MHNO) phenotype. However, subgroup analysis indicated that the MHO/FLD phenotype was significantly associated with CAC score progression (multivariate adjusted-OR, 2.37; 95% CI, 1.34-4.16), as compared to the MHNO/no FLD phenotype, whereas the MHO/no FLD phenotype was not (multivariate adjusted OR, 1.25; 95% CI, 0.71-2.24).

Conclusion: Obese individuals with FLD have an increased risk of atherosclerosis progression, despite their healthy metabolic profile. Preventive interventions targeting cardiometabolic risk factors should be considered in such individuals, regardless of the weight status.

Disclosure: **W. Lee:** None.

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Unravelling the pathogenetic mechanisms of fructose consumption as multiple hit in the pathogenesis and progression of non-alcoholic fatty liver disease

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Background and aims: High dietary fructose consumption is associated with fatty liver disease (NAFLD) and might contribute to the development of insulin resistance, characterized by ectopic lipid deposition and impaired postprandial glycogen synthesis.

Materials and methods: 5 NAFLD patients (age 49.6±2.3 years; BMI 29.1±2.2 kg/m^2) and 10 healthy controls (CON) (age: 27.5±2,1 years; BMI 21.5±0.5 kg/m^2) underwent stepped hyperinsulinemic euglycemic clamp tests and high caloric mixed meal tests (MMT) in combination with ^{1}H and ^{13}C MRS for measurement of glycogen and lipid content in the liver (HCL[%]) and skeletal muscle (IMCL[%]). Glucose infusion rates at low (15mU/m^2) and high dose (40mU/m^2) of insulin infusion was used to calculate the rate of metabolized glucose [M(mg/kg*min)]. These tests were repeated in CON after 8 weeks of dietary fructose challenge (150g/day).

Results: As expected NAFLD were characterized by decreased hepatic (M_{NAFLD} 3,96±0,44 vs. M_{CON} 6,23±0.8; p=0.07) and skeletal muscle insulin sensitivity (IS)(M_{NAFLD} 9,9±2,5 vs. M_{CON} 15,9±1.8; p=0.049) in comparison to CON. Notably, 8 weeks of high caloric fructose consumption did not affect hepatic (M_{CON} 6,23±0.8 vs. M_{CON_POST} 7,4±1,1; p=0,39) or skeletal muscle IS (M_{CON} 15,9±1.8 vs. M_{CON_POST}16,4±1.8; p=0,88) in CON. Ectopic lipids were higher in NAFLD (HCL_{NAFLD} 19.2±8.84 vs HCL_{CON} 1.19±0.21; p=0.004), ($IMCL_{NAFLD}$ 2.18±0.26 vs $IMCL_{CON}$ 1.19±0.21; p=0.007) at baseline. In the postprandial state ectopic lipids increased in NAFLD, but decreased or remained unchanged in CON: (ΔHCL_{NAFLD} 20.6±13.05 vs. ΔHCL_{CON} -19.54±9.2; p=0.039); ($\Delta IMCL_{NAFLD}$ 17.6±16.6 vs. $\Delta IMCL_{CON}$ 0.61±7.72; p=0.3). Also postprandial glycogen storage was impaired in NAFLD ($\Delta GlycogenLiver_{NAFLD}$ 17.76±7.8 vs. $\Delta GlycogenLiver_{CON}$ 72.06±12.03; p=0.02); ($\Delta GlycogenMuscle_{NAFLD}$ 6.62±10.6 vs. $\Delta GlycogenMuscle_{CON}$ 32.29±9.01; p=0.15) but was not affected by fructose challenge in CON: ($\Delta GlycogenLiver_{CON}$ 72.06±12.03 vs. $\Delta GlycogenLiver_{CON\_POST}$ 51,89±9,23 p=0,11); ($\Delta GlycogenMuscle_{CON}$ 32.29±9.01 vs. $\Delta GlycogenMuscle_{CON\_POST}$ 16,57±5,46 p=0,17).

Conclusion: As expected patients with NAFLD were characterized by severely impaired postprandial lipid and glycogen metabolism. Notably, high dose fructose consumption did not affect insulin sensitivity or postprandial lipid and glucose metabolism higlighting the capacity to compensate for high dietary fructose intake in metabolcally healthy subjects.

Clinical Trial Registration Number: LS12-008

Supported by: WWTF

Disclosure: **S. Smajis:** None.

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Effects of 3-week low carbohydrate diet on endothelial function, visceral fat and liver fat in type 2 diabetes patients compared to a low fat diet

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Background and aims: Increasing fat and carbohydrate consumption are suggested to trigger a complex metabolic pathologic network, which links type 2 diabetes (T2DM) with cardiovascular diseases (CVD) and non-alcoholic fatty liver disease (NAFLD) together. Our objective is to compare the effects of a short term low carbohydrate with a low fat dietary intervention, both hypocaloric, on the endothelial function, visceral fat and intrahepatic lipids of T2DM patients.

Materials and methods: Fifty five T2DM patients were randomized to a short term hypocaloric dietary intervention characterized either by a very low carbohydrate (VLC, n=27) (4-10% E) intake or by a low fat (LF, n=28) intake (<30% E) (age: 62 ±7.8 yr, BMI: 32.5 ±4.8 kg/m^2, HbA1c: 6.6 ±1.0 %). Endothelial function was assessed by a partially automated computer assisted flow mediated-dilation (FMD) method (Brachial Analyzer for Research, Version 5.10.6, Medical Imaging Applications LLC, Iowa City, Iowa, USA). Adipose tissue depots and intrahepatic lipids (IHL) were determinate via magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS), respectively. All data was collected before and after 3 weeks dietary intervention.

Results: Both groups experienced significant reductions of body weight (VLC: 93.7 ± 20.4 kg vs 89.6 ± 19.3 kg, p<0.001; LF: 98.5 ± 19.6 kg vs 89.7 ± 23.6 kg, p<0.05), blood lipids (VLC: Total CHO: p<0.05, LDL: p<0.05, TAG p<0.001; LF: Total CHO: p<0.001, LDL: p<0.001, TAG: p<0.001) and HbA1c (VLC: p<0.001; LF: p<0.001). IHL showed a significant reduction also in both groups (VLC: 12.3 ± 8.6 % vs 7.9 ± 7.3 %, p<0.001; LF: 14.4 ± 11.1 % vs 9.4 ± 9.6 %, p<0.001). We did not observe significant alterations on endothelial function nor visceral fat in either group after 3 weeks on respective macronutrient restriction. However, visceral adipose tissue at umbilical level was reduced only in the LF group (p<0.001). Besides the greater improvement of body weight in the LF group compared with the VLC group (p<0.05), no significant difference was found between dietary groups.

Conclusion: Short-term, hypocaloric VLC/LF diets both stimulated significant metabolic improvements inducing reductions of IHL in T2DM patients suggesting that caloric restriction primarily determines the outcomes. Unexpectedly, an effect on endothelial function could not be appreciated after 3 weeks diet, which may be explained by the absence of CVD in the patients.

Disclosure: **R.L. Barbosa Yañez:** None.

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Effectiveness of dapagliflozin in nonalcoholic fatty liver disease in type 2 diabetes patients compared to sitagliptin and pioglitazone

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Background and aims: Nonalcoholic fatty liver disease (NAFLD) shows a wide disease spectrum ranging from simple steatosis to cirrhosis while it is also associated with the presence and morphology of subclinical coronary atherosclerosis. Dapagliflozin, a selective oral sodium glucose cotransporter 2 (SGLT2) inhibitor lowers blood glucose, significantly reduces body weight and blood pressure and it may also improve high density lipoprotein (HDL) cholesterol and triacylglycerol. Sitagliptin is a widely used DPP-4 inhibitor while pioglitazone already has several clinical evidence in the treatment of NAFLD. The aim of the study was to examine the effectiveness of dapagliflozin in NAFLD patients with type 2 Diabetes Mellitus (T2DM) compared with sitagliptin and pioglitazone.

Materials and methods: 247 T2DM patients with NAFLD were included in the study. 89 patients were under treatment with dapagliflozin, 67 under

treatment with pioglitazone and 91 under treatment with sitagliptin. All patients also received metformin. Mean follow up period was 52 weeks ± 2 weeks. The evaluation of liver fibrosis depended on calculation of aspartase aminotransferase (AST) to platelet counts rAPRI) index. APRI index was calculated as AST level (IU/L) divided by upper limit of AST (37 IU/L) nad platelet counts and finally multiplied bay 102. APRI over 1.5 was considered as bridging fibrosis and over 2.0 as liver cirrhosis. All patients went through an ultrasonography before being included in the study and after the end of the study.

Results: The study's patients were aged 59.6 ± 9.2 years without differences between groups under study and mean duration of T2DM was 6.1±3.4 years without differences between treatment (p=0.312) and with no differences in Body Mass Index (BMI) (p=0.362) and HbA1c (p=0.218) with mean value 7.74±0.89%. HbA1c values improved in all three groups with most patients maintain the therapeutic goal in the dapagliflozin (p=0.036) group after 52 weeks of treatment. APRI index's improvement was significant in the dapagliflozin group (1.12 (0.59-1.22) vs 0.97 (0.32-0.1.01), p=0.016), significant in the pioglitazone group (1.14 (0.49-1.28) vs 0.80 (0.41-1.06), p=0.011) while there was no improvement in the sitagliptin group (1.07 (0.42-1.29) vs 1.05 (0.43-1.27) p=0.455). APRI index's improvement was accompanied by a significant change of fatty liver in ultrasonography. The decrease of body weight in the dapagliflozin group was statistically significant (p<0.001) with most its patients (59 patients) achieving a >3Kg decrease.

Conclusion: Administration of dapagliflozin led not only to good control of T2DM but also improvement of liver inflammation, alteration of liver fibrosis, and reduction of body weight, which are particularly important factors in patients with T2DM. Aggravation of liver fibrosis score might lead to future liver cirrhosis, and body weight gain, observed in the pioglitazone group, could exacerbate liver inflammation and other metabolic disorders. As far as we know, this study is the first to be carried out on the long-term effect of dapagliflozin on liver fibrosis which indicates its positive impact in T2DM with NAFLD. Particularly, body weight reduction was a favorable outcome of applying dapagliflozin in NAFLD patients with T2DM.

Disclosure: **A. Koutsovasilis:** None.

OP 03 Genetics and epigenetics of type 2 diabetes

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Discovery and fine-mapping of type 2 diabetes susceptibility loci across diverse population

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Background and aims: To discover and improve fine-mapping resolution of type 2 diabetes (T2D) loci, we conducted the most comprehensive and diverse trans-ethnic meta-analysis of genome-wide association studies (GWAS).

Materials and methods: 99,265 T2D cases and 545,212 controls were included in this interim meta-analysis. Each GWAS was imputed up to optimal reference panels from the 1000 Genomes Project or Haplotype Reference Consortium. We conducted meta-regression analysis to account for heterogeneity in allelic effects between diverse populations. We performed approximate conditional analyses to identify distinct signals in the loci attaining genome-wide significance ($p<5x10^{-8}$). For each signal, we defined credible sets of variants that accounted for 99% of the posterior probability of driving the association. Across signals, we evaluated enrichment in the posterior probability across genomic annotations

Results: We identified 110 loci at genome-wide significance, including 37 mapping outside regions previously implicated in T2D, with the strongest novel associations at/near *INHBB* (rs58884021, $p=2.8x10^{-12}$), *PLEKHA1* (rs2421016, $p=3.2x10^{-12}$), and *EIF5A2* (rs6804915, $p=3.8x10^{-12}$). We identified 156 distinct association signals across the 110 loci, including 11 at *KCNQ1*, 5 at *INS-IGF2*, and 4 each at *CDKN2A-B* and *CCND2*. Whilst allelic effects on T2D risk of index variants were predominantly consistent across populations, for the first time we observed strong evidence of heterogeneity correlated with ancestry at *LEP* (rs7778167, $p_{HET}=8.2x10^{-16}$, East Asian specific), *UBE2E2* (rs35352848, $p_{HET}=4.2x10^{-11}$, effect strongest in East Asians), and *KCNQ1* (rs11819853, $p_{HET}=2x10^{-10}$, varying direction/magnitude of effect between ethnic groups). We substantially improved fine-mapping resolution compared with previous efforts, highlighting 17 signals with a single variant accounting for >99% of the posterior probability of driving the association, including at *JAZF1* (rs10226758), *CDC123-CAMK1D* (rs11257655), *TCF7L2* (rs7903146), and 2 signals at *KCNQ1* (rs2237884 and rs2237895). The posterior probability of association was significantly enriched in coding exons ($p=1.4x10^{-5}$), for the first time including an index variant at the *APOE-TOMM40* locus, *APOE* p.Cys130Arg (rs429358). After accounting for coding variation, the posterior probability was also significantly jointly enriched for transcription factor binding sites for PDX1 ($p=2.6x10^{-6}$) and FOXA2 ($p=1.8x10^{-5}$).

Conclusion: Our study represents the most comprehensive view of the genetic contribution to T2D in terms of sample size and ethnic diversity, and will be enhanced by GWAS in an additional 400,000 individuals in the coming months.

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Disclosure: **H. Kitajima:** Employment/Consultancy; Manpei Suzuki Diabetes Foundation.

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Novel locus discovery through trans-ethnic association analyses of glycaemic traits using densely imputed genetic data

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Background and aims: To date, genome-wide association studies (GWAS) have identified >120 loci associated with glycaemic traits, most arising from European descent populations. We present the first large-scale trans-ethnic discovery for four glycaemic traits: fasting glucose (FG), fasting insulin (FI), glycated haemoglobin (HbA1c) and 2-hour glucose (2hG). We aimed to identify additional glycaemic trait-associated loci, investigate the portability of signals across ancestries, and leverage linkage disequilibrium differences to conduct fine-mapping.

Materials and methods: Meta-analyses included up to 281,416 individuals without diabetes, from five ancestries (71% Europeans, 13% East Asian, 7% Hispanic, 6% African-American and 3% South Asian) with genetic data imputed to the 1000 Genomes Project (phase 1 v3, March 2012, or later). Genetic association analyses were performed at the cohort level using an additive model, and combined by fixed-effect ancestry-specific meta-analyses, followed by trans-ethnic meta-analyses in MANTRA. A $\log_{10}$ Bayes factor ($\log_{10}$BF) threshold of 6 was used to identify trans-ethnic genome-wide significant signals. HbA1c signals were classified as erythrocytic or glycaemic through hierarchical clustering after lookup in red blood cell traits and glycaemic traits. Lastly, GARFIELD and DEPICT (FDR<5%, unless otherwise stated) were used to perform functional, tissue and pathway enrichment analyses.

Results: We identified 102 trans-ethnic signals associated with FG, of which 48 did not overlap known FG-associated regions. The most significant novel FG signals - not previously known to be involved in diabetes - include those at or close to NFX1 and ZBTB38 genes ($\log_{10}$BF=12.42 and 11.79, respectively). We also identified 62 FI-associated trans-ethnic signals (43 novel), 130 HbA1c-associated trans-ethnic signals (57 novel) and 21 2hG-associated trans-ethnic signals (10 novel). The most significant novel signals for FI, HbA1c and 2hG - not previously known to be involved in diabetes - fall in or near BCL2, LRRC16A and CLEC14A genes ($\log_{10}$BF = 11.66, 18.60 and 9.68, respectively). BCL2 was previously associated with waist-to-hip ratio and body mass index, LRRC16A was shown to be involved in platelet count and volume, and CLEC14A was associated with monoamine metabolite levels in human cerebrospinal fluid. HbA1c signals were mostly classified as influencing HbA1c via erythrocytic pathways (~70%) which was consistent with functional, tissue and pathway enrichment analyses, which identified hemic and immune system signatures, as well as anaemia related pathways. Functional and tissue enrichment analyses also identified a liver signature in all glycaemic traits; pancreas in HbA1c and FG; adipose and fat tissues, as well as, adrenal glands and cortex specific in FI (FDR<20%). Pathway analyses highlighted insulin signalling in HbA1c; insulin and MTOR signalling, maturity onset diabetes of the young, type 2 diabetes and circadian rhythm in FG; and cancer in FI. This large trans-ethnic study, afforded new discoveries including ancestry-specific signals, lower minor allele frequency variants, and common variants of more modest effects.

Conclusion: This large international effort has identified over a hundred novel loci underlying glycaemic traits that pose new hypotheses about the biology and genetic architecture of glucose and insulin related traits.

Disclosure: **I. Barroso:** None.

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Poor glycaemic control is associated with altered blood gene expression levels of cell cycle and immune related genes. The Hoorn Diabetes Care System cohort

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Background and aims: With the global pandemic of obesity, type 2 diabetes is becoming increasingly prevalent. Yet, people with type 2 diabetes are heterogeneous in their disease trajectory. Glycaemic control is tracked by measuring blood HbA1c levels on a regular basis. To predict glycaemic control over a longer period of time, the utility of several molecular measures in blood is increasingly explored, but one of them - gene expression - has been sparsely investigated. To this end, we here investigate the relation between whole blood gene expression and HbA1c at baseline and up to 2-year follow-up in individuals participating in the Hoorn Diabetes Care System cohort.

Materials and methods: Genome-wide gene expression levels in whole blood (15,564 genes, RNA-seq) were measured in 391 individuals with type 2 diabetes. Gene expression levels were compared to baseline (N=391), 1-year (N=372) and 2-year HbA1c levels (N=362), adjusted for age, sex, blood cell composition, BMI, medication use and technical covariates. To investigate the pathways to which identified genes were related with HbA1c at baseline we explored the interrelatedness of genes in a co-expression network. Genes identified at baseline were investigated in external microarray data of target tissues muscle (N=115) and pancreas (N=113).

Results: Gene expression levels were particularly associated with baseline HbA1c (220 genes, 1.4%, fold change (fc): -3.34 - 4.80) and to a lesser extent to one year follow-up HbA1c (25 genes, 0.16%, fc: -2.90 - 3.10) and two-year follow-up HbA1c (9 genes, 0.06%, fc: -3.88 - 2.69). Genes identified at baseline and follow-up overlapped, with 5 genes identified at all time points and 18 additional genes between baseline and 1-year follow-up. After adjustment for baseline HbA1c, the number of genes markedly decreased with 1 gene at 1-year follow-up (NDN, fc:-2.11) and 2 genes at 2-year follow-up (MTND1P23, fc:-3.64, FAM132B, fc:-1.90). Genes associated with baseline HbA1c clustered to three groups of co-expressed genes. Two larger clusters comprised 55 and 42 genes respectively and the third comprised 2 genes. The largest cluster showed strong overrepresentation in cell cycle (checkpoint) pathways ($P_{FDR} < 1{\cdot}10^{-10}$). The second cluster showed overrepresentation in the complement system activation and B-cell signalling pathway ($P_{FDR} < 1{\cdot}10^{-10}$). The third cluster consisted KLF10 and KLF11, that both link to cell cycle regulation. Finally, the association between HbA1c and gene expression was also confirmed for 15 genes (|r|>0.2) in external datasets of target tissues muscle (N=115) and pancreatic islets (N=113).

Conclusion: While gene expression levels are interesting biomarkers for poor glycaemic control, our study suggests that gene expression levels in whole blood are particularly a reflection of the current glycaemic state, rather than predictive of the future glycaemic state. Nonetheless, identified genes provide insight into the relation of glycaemic control with gene expression changes in blood, where some genes also associate with HbA1c levels in target tissues.

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Disclosure: **R.C. Slieker:** None.

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Identification of alleles associated with higher body fat percentage but lower risk of type 2 diabetes

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Background and aims: Recent studies have identified metabolically "healthy obesity" alleles - those associated with higher body fat % but lower risk of type 2 diabetes, hypertension, and heart disease. By definition, the opposite alleles of the same variants are associated with lower BMI but higher risk of disease - "polygenic lipodystrophy" alleles. We aimed to identify novel "healthy obesity" alleles, and test their protective effects on type 2 diabetes.

Materials and methods: First, we identified alleles associated with higher body fat % (p-value < $5x10^{-8}$) using 220,000 individuals from the initial UK Biobank dataset and published studies. Next, we used a multivariate model that jointly tested the effects of these alleles on biomarkers of "healthy obesity": higher HDL-C (N=99,900), lower Triglycerides (N=96,600), lower fasting insulin (N=51,800), higher adiponectin (N=29,400), higher SHBG (N=21,800) and a marker of lower liver fat, lower ALT (N=55,500). We defined "healthy obesity" alleles as those (i) which had a multivariate p-value of < $5x10^{-4}$ (multiple test corrected) and (ii) which were associated with the multivariate model stronger than body fat % alone. The latter criterion was set to uncouple "healthy obesity" from "unhealthy obesity" alleles. To validate the variants as protective of disease, we calculated the association of "healthy obesity" genetic score with type 2 diabetes using independent published type 2 diabetes GWAS data from the DIAGRAM consortium.

Results: We identified 85 independent genetic variants associated with 1.66%-6.44% standard deviation (SD) per allele differences in body fat % (p-value < $5x10^{-8}$). Fourteen of these genetic variants fitted the definition of "healthy

obesity" including 10 known alleles previously published. All of the previously known variants were more strongly associated with the "healthy obesity" phenotype when we used adiponectin, SHBG and ALT in the multivariate statistical model. The multivariate model was able to uncouple "healthy obesity" (e.g. the *PPARG* allele) from "unhealthy obesity" alleles (e.g. *FTO* allele) among alleles associated with higher body fat %. When analysing all 14 genetic variants together as a genetic score, carrying 5 additional "healthy obesity" alleles was associated with a 0.340 [0.338, 0.341] Kg/m^2 higher BMI (p-value = 2x10^{-62}, using data from GIANT consortium) but lower risk of type 2 diabetes (odds ration: 0.882 [0.876, 0.889]; p-value = 2x10^{-11}, using data from DIAGRAM consortium). These effects were primarily driven by the ten known "healthy obesity" variants.

Conclusion: "Healthy obesity" alleles can be defined using adiposity related phenotypes. Adiponectin, SHBG and ALT add extra accuracy to the identification of "healthy obesity" alleles when used in the model. Our study supports the use of a multivariate analysis approach in identifying variants associated with higher body fat % but lower risk of type 2 diabetes.

Disclosure: **H. Yaghootkar:** None.

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DNA Methylome signature in subcutaneous adipose tissue precursor cells identifies individuals with a family history of type 2 diabetes

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Background and aims: First-degree relatives (FDRs) of individuals with type 2 diabetes (T2D) have dysfunctional subcutaneous adipose tissue (sAT). Indeed, they feature significantly larger sAT cells due to impaired adipogenesis and hypertrophy of pre-existing mature sAT cells, which increases their susceptibility to the detrimental effects of weight gain. Despite clear familiarity of these abnormalities, no genotype convincingly accounting for their increased diabetes risk has so far been identified. Since epigenetics might also have a role, we have explored the DNA Methylome and full transcriptome analyses in sAT pre-adipocytes from FDRs and matched subjects lacking a known family history of T2D.

Materials and methods: Stromal Vascular Fraction cells (SVFs, enriched in pre-adipocytes) were isolated from sAT biopsies of 9 FDR and 12 control individuals. DNA Methylome and transcriptome profiles of SVFs were analyzed using MeDIP- and RNA-seq, respectively. Bisulphite sequencing and qPCR were then performed in SVFs of these individuals.

Results: The clinical characteristics of individuals in the study group are shown in Table 1. MeDIP-seq analysis revealed a uniform distribution of differentially methylated regions (DMRs) throughout the entire SVF genome, with a greater number of hypomethylated regions in FDR subjects (fdr<5%). This different methylation pattern was accompanied by a decreased expression of DNMT1 (p<0.05) and DNMT3A (p<0.05) genes in FDRs. Most DMRs were located within gene bodies. DMRs mapped to 3234 unique differentially methylated genes (DMGs, fdr<5%). Of note, pathway analysis revealed that most DMGs were significantly enriched in key cell growth and differentiation pathways, including FGF, PKA and Wnt/β-catenin signaling pathways. The RNA-Seq analysis identified a total of 84 (fdr<5%) differentially expressed genes. Merging DNA Methylome with RNA-seq data identified 12 genes showing concomitant changes in mRNA expression and DNA methylation. These included genes that are biologically relevant to the adipocyte function and development of T2D. Bisulphite sequencing and qPCR assays were used to validate the MeDIP-seq findings at individual gene level. Moreover, 11 obesity and 12 T2D candidate genes, identified by GWAS, had altered methylation in SVF from FDR individuals.

Conclusion: In FDRs, the DNA Methylome signature of sAT pre-adipocytes includes abnormalities in key genes implicated in adipocyte growth and function and in T2D risk. Persistent epigenetic changes may affect the evolution towards T2D and identify potential therapeutic targets.

Measure	FDR n=9	Ctrl n=12
Age, years	42.3±8.7	39.4±7.8
BMI, Kg/m^2	25.4±1.5	24.5±2.2
Cell size,μm	100.2±5.2**	89.6±6.0
GIR/bw,mg/min	7.9 ±1.7**	11.3±2.5
f-insulin,μU/mL	60.1±22.7**	34.0±13.3
fb-glucose,mmol/L	4.8±0.4*	4.4±0.4

Table 1. Clinical features of the FDR and control subjects. Values are mean ±SD, *p<0.05 **p<0.005.

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Disclosure: **L. Parrillo:** None.

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Lower DNA methylation of metformin transporter genes in human liver is associated with metformin therapy in type 2 diabetes subjects

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Background and aims: Given that metformin is the most common pharmacological therapy for type 2 diabetes (T2D), understanding the metabolism of this drug is of great importance. Hepatic metformin transporters are responsible for the pharmacologic action of metformin. Since epigenetics of metformin transporter genes remains uncertain, we wanted to analyze DNA methylation in metformin transporter genes in human liver, and assessed whether epigenetic alterations associate with diabetes medication.

Materials and methods: DNA methylation in metformin transporter genes (OCT1 encoded by *SLC22A1*, OCT3 encoded by *SLC22A3*, MATE1 encoded by *SLC47A1)* was analyzed in human liver of 95 subjects from the Kuopio Obesity Surgery Study using the Infinium HumanMethylation450 BeadChip array. mRNA expression was measured with the HumanHT-12 Expression BeadChip in a subsample of 42 individuals. Thirty T2D patients who were taking metformin or insulin plus metformin were considered for the analyses. A control group of 3 diabetic patients who did not receive any diabetes medication was also included. All analyses were adjusted for age, sex and the presence of nonalcoholic steatohepatitis.

Results: T2D subjects who received just metformin showed lower average and promoter DNA methylation of *SLC22A1*, *SLC22A3* and *SLC47A1* genes compared to patients who received insulin plus metformin or no diabetes medication. There were also 13 individual CpG sites annotated to the hepatic metformin transporter genes with differential DNA methylation according to diabetes medication (FDR<0.05). Notably, multiple regression models showed that DNA methylation was also associated with gene expression of all metformin transporter genes in human liver. In addition, higher methylation levels in these genes were associated with higher fasting glucose levels and higher BMI.

Conclusion: Our study demonstrates that DNA methylation of metformin transporter genes in human liver is different according to diabetes medication and associates with hepatic gene expression, glycaemia and obesity. Importantly, metformin decreases DNA methylation of *SLC22A1*, *SLC22A3* and *SLC47A1* in human liver.

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OP 04 Novel regulators of insulin sensitivity

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The association between insulin resistance and iron overload through the transferrin receptor 1 in human skeletal muscle cells and muscles from patients with type 2 diabetes

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Background and aims: Iron plays an important role in many physiological processes, including redox balance, inflammation, and mitochondrial respiratory metabolism. Altered iron homeostasis is associated with insulin resistance (IR) and diabetes. Iron perturbations are well studied in several tissues, such as adipose tissue and the liver in diabetic animal models. However, the relationship between iron metabolism and IR in skeletal muscle has not been well studied. In this study, we investigated the relationship and molecular mechanism between iron overload and IR in human skeletal muscle cells and muscle biopsies from patients with type 2 diabetes (T2DM).

Materials and methods: To clarify the iron homeostatic brake in IR muscle cells and muscle biopsies from patients with T2DM, we measured iron metabolism-related protein levels such as transferrin receptor 1 (TfR1), heavy chain ferritin (FTH), light chain ferritin (FTL) and divalent metal transporter 1 (DMT1).

Results: Immunoblotting analysis showed that treating human skeletal muscle cells with palmitate increased the levels of TfR1 and FTH, but not iron regulatory protein 1. In addition, the levels of TfR1, FTH, FTL and DMT1 increased in muscle biopsies from patients with T2DM compared to those in normal subjects. In addition, the intracellular labile iron pool increased in palmitate-induced IR skeletal muscle cells. Knockdown of TfR1 using siRNA in human skeletal muscle cells protected against palmitate-induced IR and preserved the intracellular iron pool. Treatment with an iron donor ($FeSo_4$ or $FeCl_3$) stimulated phospho-JNK and phospho-p38 and significantly induced mitochondrial dysfunction in human skeletal muscle cells. In particular, the chemical iron chelator deferoxamine recovered mitochondrial dysfunction and palmitate-induced IR in human skeletal muscle cells.

Conclusion: The current study showed that iron overload in skeletal muscle cells induced mitochondrial dysfunction and IR through TfR1, whereas reducing the intracellular iron overload protected against IR. Therefore, attempts to block iron overload might be a strategy for preventing IR and diabetes.

Disclosure: **K. Lee:** None.

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ApoJ is a novel hepatokine regulating muscle glucose metabolism and insulin sensitivity

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Background and aims: A crosstalk between the liver and skeletal muscles has long been recognized as vital for adequate glucose homeostasis. Critical factors for this interorgan crosstalk are hepatokines, liver-derived proteins that play a pivotal role in regulating glucose metabolism and insulin sensitivity in skeletal muscle. Thus, discovery of a new hepatokine would be of particular interest. The aims of the current study are 1) to determine the physiological role of hepatic ApolipoproteinJ (ApoJ, also called clusterin) in the regulation of glucose homeostasis and insulin sensitivity, and 2) to determine whether the ApoJ → LRP2 signaling pathway is a key component of insulin action in skeletal muscle.

Materials and methods: Mice lacking ApoJ in liver or in muscle and LRP2 in muscle were studied for glucose metabolism and insulin signaling. Cultured muscle C2C12 cells were also studied for the molecular interaction of insulin receptor (IR) with LRP2 and insulin-induced endocytosis. Glucose tolerance (1.0 g/kg) and insulin tolerance test (0.75unit/kg) were performed. Glucose-stimulated insulin secretion (1.0 g/kg) was measured. Glucose uptake into peripheral tissues was determined by ip injection with 2U/kg insulin with 0.33 μCi [^{14}C]2-deoxyglucose. Insulin-stimulated signaling events (10 U/kg) in insulin-sensitive tissues were assessed. In situ proximity-ligation assay were performed. Endocytosis was measured in the presence of an inhibitor of clathrin-mediated endocytosis.

Results: Circulating ApoJ is primarily produced by the liver and transports to metabolically active organs, including muscle. Elevated ApoJ levels in serum but decreased ApoJ levels in muscle are found in mice lacking LRP2 in muscle, indicating that ApoJ is used in muscle via LRP2. Of physiologic significance, selective deletion of hepatic ApoJ or muscle LRP2 leads to systemic insulin resistance and glucose intolerance by suppressing insulin-induced signal transduction and glucose uptake in skeletal muscle. Mechanistically, we found that insulin drives the physical interaction between the IR and LRP2 on the cell surface of muscle and the complex of LRP2 with the IR undergoes co-endocytosis, which is an essential step of insulin signaling in muscle. However, when hepatic ApoJ or muscle LRP2 is absent, insulin-stimulated IR-LRP2 interaction is disrupted.

Conclusion: Our findings identify ApoJ as a novel hepatokine that is required for insulin-mediated glucose metabolism through a LRP2-dependent mechanism, coupled with the IR system. Thus, the ApoJ → LRP2 axis is a novel metabolic signaling pathway that is central for the maintenance of normal glucose homeostasis and insulin sensitivity.

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Carnitine supplementation improves metabolic flexibility and skeletal muscle acetylcarnitine formation

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Background and aims: Insulin resistance and type 2 diabetes are characterized by decreased metabolic flexibility and concomitant disturbances in glucose homeostasis. Recent evidence indicates that low carnitine availability may play a substantial role. When substrate availability is high, accumulation of mitochondrial acetyl-CoA is known to blunt glycolysis. However, acetyl-CoA can also be conjugated to acetylcarnitine, which can leave the mitochondria. Hence, impaired formation of acetylcarnitine formation may underlie compromised metabolic flexibility and therefore impair glucose tolerance (IGT). Here, we investigated whether carnitine supplementation improves acetylcarnitine formation and thereby rescues metabolic flexibility and insulin sensitivity in IGT subjects.

Materials and methods: Eleven IGT subjects followed a 36 day placebo- and L-carnitine treatment (2g/day) in a randomized, placebo-controlled, double blind crossover design. Plasma free carnitine concentrations were determined to check compliance to the intervention. A hyperinsulinemic-euglycemic clamp (40mU/m2/min), combined with indirect calorimetry (ventilated hood) was performed to determine insulin sensitivity and metabolic flexibility. Skeletal muscle acetylcarnitine concentrations were measured in vivo using long echo time proton magnetic resonance spectroscopy (1H-MRS, TE=500ms). To stimulate near maximal acetylcarnitine formation, 1H-MRS was performed before and immediately after a 30-minute cycling exercise

at 70% of the subjects predetermined maximal output (W_{max}). Twelve normal glucose tolerant (NGT) subjects were included without any intervention as control group.

Results: Plasma free carnitine concentrations increased upon carnitine supplementation (from 40.8±1.6 to 50.5±1.7μmol/L, p=<0.01) and did not change after placebo treatment (from 39.4±1.4 to 39.8±1.3 μmol/L, p=0.785) indicating compliance to the carnitine supplementation. Metabolic flexibility (Δ respiratory exchange ratio, ΔRER) was lower in IGT compared to NGT (ΔRER 0.07±0.01 vs. 0.10±0.01 respectively, p=0.022), but was completely restored upon carnitine supplementation (0.10±0.01, p=0.001). Similarly, the insulin stimulated increase in glucose oxidation was higher in IGT after carnitine supplementation compared to placebo (ΔGlucose oxidation 7.08±0.74 vs. 4.84±0.48 μmol/kg/min, p=0.001). No difference in whole-body insulin sensitivity (Δ rate of disappearance, ΔRd) was found upon carnitine supplementation (ΔRd 13.32±3.08 vs. 11.74±1.99 μmol/kg/min after placebo, p=0.513). Finally, carnitine supplementation increased pre-exercise (1.08±0.20 vs. 1.62±0.27 mmol/kgww, p<0.05) as well as post-exercise (3.60±0.49 vs. 4.23±0.53 mmol/kgww, p<0.05) skeletal muscle acetylcarnitine concentrations.

Conclusion: Carnitine supplementation completely restored skeletal muscle acetylcarnitine concentrations and rescued metabolic flexibility in IGT. Why insulin sensitivity was unaffected requires further investigation.

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A novel skin-adipose tissue axis regulates pancreatic beta cell function

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Background and aims: The inflammatory skin disease psoriasis is an independent risk-factor for development of insulin resistance and type 2 diabetes (T2D), and presence of psoriasis is a strong predictor of T2D progression and severity. Moreover, some skin-specific transgenic mouse models display altered whole-body glucose homeostasis. This suggests an important role for skin in regulating whole-body glucose metabolism. We investigated the role of the skin secretome on subcutaneous adipose tissue (sAT) and beta-cell function using human and rodent models.

Materials and methods: Human explant skin, from abdominoplasty surgery, was incubated with the TLR7 agonist immiquimod (IMQ; 50 μg/ml; 24 - 48h) to induce a psoriatic phenotype. Conditioned media (skin-CM) was collected and retained or used to incubate explant human sAT, with sAT-CM also collected. Subsequently, MIN6 beta-cells were incubated with skin-CM and sAT-CM. For treatments, CM was diluted 1:1 with fresh media. All experiments were conducted serum-free. To induce skin inflammation in mice, IMQ (3.125 mg/day) was applied to a shaved dorsal region. After 5 days' treatment, serum, sAT and islets were collected for further analysis. We measured MIN6 glucose-stimulated insulin secretion (RIA), proliferation (BrDU assay) and cell viability (MTT assay) and sAT gene expression changes (qPCR). In the mouse model, sAT gene expression (qPCR), plasma insulin (ELISA) and glucose levels were measured.

Results: IMQ induced an inflammatory phenotype in human and mouse skin, evidenced by elevated pro-inflammatory cytokine gene expression (IL1β, IL6, IL8, IL17). Indicative of a regulatory role for human skin-derived factors, IMQ-skin-CM incubation decreased MIN6 GSIS (IMQ-treated-CM 20mmol/l: 6.1 ± 0.8 ng/ml, vs. CON-skin-CM 20mM: 25.5 ± 3.7 ng/ml, p<0.001, n=1 of 4 observations) as well as MIN6 BrDU incorporation (IMQ-treated CM: 0.2 ± 0.01, vs. CON-skin-CM: 0.8 ± 0.03, p<0.0001, n=1 of 6 observations) and cell viability (IMQ-treated CM: 0.08 ± 0.01,vs. CON-skin-CM: 0.8 ± 0.03, p<0.0001, n=2 of 4 observations), both normalised to fresh media control, which equals 1. Moreover, IMQ-skin-CM increased pro-inflammatory cytokine expression (IL1β, IL6, IL8, TNFα) and reduced markers of insulin sensitivity (GLUT4, adiponectin) in human sAT compared to CON-skin-CM. Incubation with sAT-CM (generated by pre-treatment of sAT with skin-CM) significantly enhanced GSIS (20mmol/l: 26.8 ± 3.1 ng/ml) vs. CON-sAT-CM treated with only fresh media (20mM: 16.9 ± 1.2 ng/ml, p<0.001, n=1 of 4 observations), indicating that skin-derived factors may modulate adipocytokine secretion. These effects were reversed following incubation with IMQ-skin-CM. Similarly, IMQ-treated mice displayed elevated pro-inflammatory cytokine expression (IL1β, IL6, IL17, TNFα) and reduced levels of (GLUT4) in sAT. Moreover, IMQ-mice displayed elevated serum insulin levels. These results are suggestive of insulin resistance in IMQ-mice.

Conclusion: These results support the hypothesis that the skin plays an important role in whole-body glucose homeostasis and that impaired skin health or function may play a direct role in development of insulin resistance and T2D. Skin inflammation impairs insulin secretion, beta-cell proliferation and viability as well as increasing sAT inflammation and potentially modulating adipocytokine secretion. This may constitute a novel pathophysiological pathway mediating T2D development.

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Physical inactivity lowers mitochondrial oxidative capacity and predisposes to muscle fat accumulation and lipid-induced insulin resistance

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Background and aims: Physical inactivity and associated decline in mitochondrial function, increased myocellular fat deposition and reduced insulin sensitivity are common denominators in chronic metabolic disorders like obesity and T2DM. Here we examined if one-legged physical inactivity for one week would result in a decline in mitochondrial oxidative capacity, increase in muscle fat content and reduced insulin sensitivity.

Materials and methods: Ten male subjects (age 22.4 ± 4.2 years, BMI 21.3 ± 2.0 kg/m^2) underwent a 12-day unilateral lower limb suspension to investigate the effect of one-legged physical inactivity on skeletal muscle mitochondrial oxidative capacity (^{31}P-NMRS), lipid and glucose metabolism (ex vivo ^{14}C lipid and glucose oxidation), intramyocellular lipid content (IMCL by ^{1}H-NMRS and histology) and insulin sensitivity (markers of insulin signalling upon an acute intravenous insulin bolus). The contralateral leg served as an active internal control.

Results: In vivo mitochondrial oxidative capacity was lower upon inactivity, as revealed by a significantly longer PCr-recovery half-time compared to the active leg (PCr-t1/2: 21.4 ± 2.3 sec vs. 16.7 ± 1.8 sec, p=0.02). In skeletal muscle biopsies, palmitate oxidation to $14CO_2$ was significantly lower in the suspended leg compared to the active leg (0.14 ± 0.03 nmol/2h/mg vs. 0.18 ± 0.03 nmol/2h/mg, p=0.013) accompanied by a higher 14C-palmitate incorporation into TAG in the suspended leg (ex vivo assays, 0.019 ± 0.005 nmol/2h/

mg vs. 0.010 ± 0.002 nmol/2h/mg), p=0.075). Further, IMCL in both m. tibialis anterior (0.312 ± 0.045% vs 0.239 ± 0.041, p=0.003) and in m. vastus lateralis (2.09 ± 0.47 % lipid fraction vs 0.91 ± 0.27% lipid fraction, p=0.008) was higher in the suspended leg compared with the active leg. Finally, lipid-induced insulin resistance was more pronounced in the suspended leg (p<0.05).

Conclusion: These results indicate that a short episode of inactivity already blunts in vivo mitochondrial oxidative capacity and ex vivo fat oxidative capacity, redirecting fat into IMCL storage, and finally augmenting lipid-induced insulin resistance. Thus, we demonstrate that a physical inactivity-mediated decline in mitochondrial oxidative capacity directly impacts insulin sensitivity under conditions of high lipid availability.

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Disclosure: **L. Bilet:** None.

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Gut ghrelin regulates hepatic glucose production and insulin signalling via a gut-brain-liver pathway in rats

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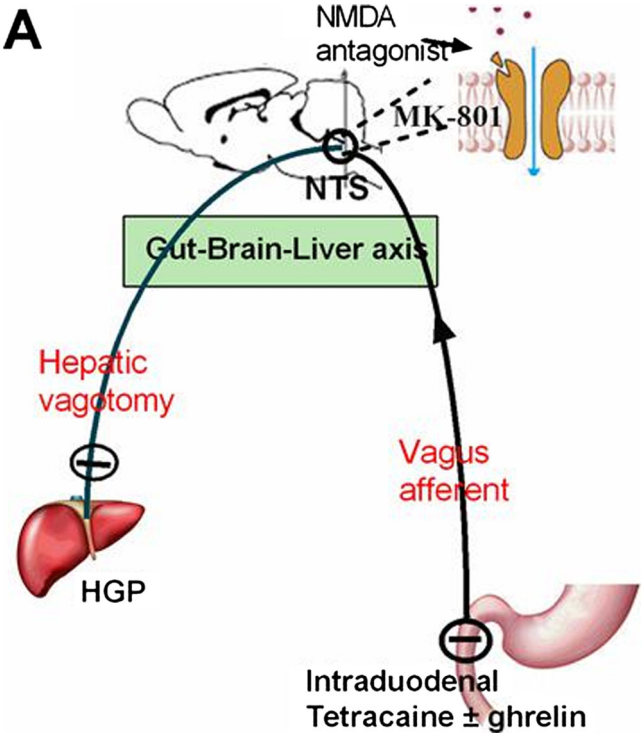
Background and aims: Ghrelin, a 28-amino acid peptide originally isolated from human and rat stomach, it has been demonstrated to regulate a variety of physiological processes such as stimulating food intake and fat deposition. Although ghrelin's involvement in glucose metabolism in peripheral tissues and the central nervous system (CNS) has been reported, the effects of gut ghrelin on glucose homeostasis and insulin signaling remain unknown. In this study, we clarified a novel role of gut ghrelin in the regulation of hepatic glucose production (HGP) via neuronal network.

Materials and methods: We established the system of intraduodenal infusion and intracerebral micro infusion into the nucleus of the solitary tract (NTS) in normal chow-diet rats. Ghrelin and the local anesthetic tetracaine were co-infused into duodenum. MK-801, a NMDA receptor blocker to inhibited the NMDA receptors-mediated neuronal transmission in the NTS, was administrated into the dorsal vagal complex (DVC) targeting the NTS though bilateral catheters. The hepatic vagus nerve was transected by surgery. And the changes in glucose kinetics and hepatic insulin signaling were evaluated with the pancreatic-euglycemic clamp (PECs) technique combined with [3-^{3}H]glucose as a tracer. Gut nutrition sensing was assessed by co-infused lipid with ghrelin into duodenum during PECs. We further activated duodenal mucosal Adenosine Monophosphate Activated Protein Kinase(AMPK) signaling via intraduodenal co-infusion of ghrelin with the AMPK activator(AICAR) in vivo.

Results: Intraduodenal infusion of ghrelin induced a significant increase of HGP with inhibited hepatic insulin signaling which suppressed phosphorylation of insulin receptors(InsR) and Akt kinase during the clamps. These changes were associated with increased hepatic expression of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase). The metabolic and molecular signal effects of duodenal ghrelin were negated by co-infusion with tetracaine, MK-801 or hepatic vagotomy in rats. Iintraduodenal infused of lipid with ghrelin attenuated the ability of duodenal lipid to increase the glucose infusion rate(GIR) and lower HGP. In addition, duodenal ghrelin decreased AMPK phosphorylation in the duodenal mucosa and the role of ghrelin to increase HGP was abolished by AMPK activator.

Conclusion: Gut ghrelin plays an important role in glucose homeostasis by the regulation of hepatic glucose metabolism and insulin sensitivity, which is associated with the activation of the Gut-Brain-Liver Pathway.



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OP 05 Beta cell development: genetic and epigenetic regulation

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H3K4 methylation is essential for endocrine progenitor cell specification and migration

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Background and aims: Following induction of *Neurog3* in bipotent pancreas progenitors, endocrine progenitors begin specification into the islet cell types through a transcription factor cascade. For example, beta-cell specification requires induction of Pdx1, Nkx6-1, Mafa, and Pax4. However, exactly how these factors are activated during pancreas development is largely unknown. The Trithorax group (TrxG) complexes are chromatin remodelers that promote gene activation by catalyzing histone H3 lysine 4 methylation. We previously observed that loss of the TrxG core protein Dpy30 from pancreas progenitors (*Pdx1*-Cre; *Dpy30* knockout mice) results in decreased H3K4 methylation, which prevents reliable gene induction (e.g. *Neurog3*, *Cpa1*, etc.) and appropriate specification into endocrine and acinar cells. Similarly, we hypothesize that the TrxG complexes are a critical player in the appropriate induction of factors that control Neurog3-induced endocrine cell specification and maturation.

Materials and methods: We generated endocrine-specific Dpy30 knockout mice (*Neurog3*-Cre; $Dpy30^{flox/flox}$; *Dpy30ΔN*) and examined the role of Dpy30 in endocrine progenitor development.

Results: To investigate the formation of $Neurog3^+$ endocrine progenitors, we quantified the number of $Neurog3^+$ cells at embryonic day 14.5 (E14.5), but found no apparent difference between control and *Dpy30ΔN* pancreata. This suggests that endocrine progenitors are formed correctly in Dpy30 knockout mice. To examine loss of H3K4 methylation in *Dpy30ΔN* mice, we stained for trimethylation of histone 3 lysine 4 (H3K4me3). We found that H3K4 methylation was maintained until E18.5, but was lost in $insulin^+$ beta-cells at 4 weeks of age. This data implies that loss of H3K4 methylation is only observed after post-natal expansion of endocrine cells. To determine whether endocrine progenitors specify into the correct proportions of alpha-, beta-, and delta-cells, we quantified the number of $glucagon^+$, $insulin^+$, and $somatostatin^+$ cells relative to the entire pancreas at 4 weeks. Compared to control, we found no difference in the number of delta-cells, a decrease in the number of beta-cells generated and an increase in the number of alpha-cells in *Dpy30ΔN* mice. Our data suggests that H3K4 methylation is required to activate the beta-cell lineage and repress the alpha-cell lineage during endocrine progenitor cell fate decisions. Interestingly, the islets in *Dpy30ΔN* pancreas at 4 weeks are more often found closely associated with the ducts in elongated endocrine cell structures (as observed in embryonic pancreas), rather than throughout the pancreas in adult islet cell clusters. This implies that endocrine cells fail to migrate into islets from the progenitor epithelium during specification in the absence of H3K4 methylation. To assess islet function, we measured random blood glucose and found that *Dpy30ΔN* mice become diabetic (2 blood glucose readings $\geq$ 20 mM) by 4 weeks of age ($n=3$, $p<0.05$), suggesting their islet cells have failed to reach functional maturity. Consistent with this data, we observed a decrease in fraction of $insulin^+$ beta-cells that express the mature beta-cell marker, Nkx2-2, in *Dpy30ΔN* mice ($n=2$, $p<0.01$).

Conclusion: Overall, these results suggest that the core TrxG complex protein Dpy30 is essential for differentiation of endocrine progenitors and islet cell migration.

Disclosure: **S.A. Campbell:** None.

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p300 is an important cofactor for beta cell development and function

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Background and aims: We have previously shown that the expression of p300, a transcriptional coactivator, in pancreatic islets maintains whole body glucose homeostasis in mice due to defects in various islet endocrine cell types. We hypothesized that such glucose metabolism defects are primarily due to the loss of p300 in beta cells.

Materials and methods: To determine the direct cause of the glucose intolerance in mice lacking p300 in pancreatic islets, we generated and studied mice lacking p300 specifically in beta cells using *Ins1*-Cre knockin line ($p300^{BetaKO}$ mice) and inducible *Pdx1*-CreER line ($p300^{PKO}$ mice). We had also acquired a cohort of patients with p300 mutations that also had MODY-like phenotypes.

Results: $p300^{BetaKO}$ mice suffered from glucose intolerance by eight weeks of age with impaired plasma insulin responses resembling our *Neurog3*-Cre model. These mice also had reduced beta cell area but normal alpha cell area. Thus, the expression of p300 specifically in beta cells is required for glucose homeostasis. Interestingly, although islets derived from $p300^{BetaKO}$ mice had lower insulin content in part due to a skewed beta-to-alpha ratio, transmission electron microscopy revealed that p300-null beta cells had significantly smaller mature insulin granules. As insulin granule defect is likely a mature beta cell phenotype, we hypothesized that the postnatal expression of p300 in beta cells is required to maintain beta cell function. Using $p300^{PKO}$ mice, we found that they developed glucose intolerance nine weeks after tamoxifen administration without loss of beta cell area. Archived case reports of Rubinstein-Taybi syndrome (caused by mutations in CBP/p300) and phenotyping of a subset of Rubinstein-Taybi syndrome patients with p300 mutations showed association between p300 mutations and MODY-like phenotypes ranging from early-onset diabetes to neonatal hyperinsulinemic hypoglycaemia.

Conclusion: p300 appeared to be not only required for establishing normal beta cell population but also for insulin granule synthesis. p300 is known to coactivate MODY transcription factors such as HNF4a and HNF1a, and mutations in these proteins had been shown to cause similar phenotypes observed in our cohort of patients with p300 mutations. We argue that p300 is a crucial cofactor for beta cell development and maintenance of beta cell function.

Supported by: CIHR

Disclosure: **C. Wong:** None.

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Suppression of Stat3 signalling promotes acinar-to-beta reprogramming

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Background and aims: Uncovering pancreas development and β-cell differentiation has helped us to explore efficient methods to generate surrogate β cells to cure diabetes. Since it has been demonstrated that Stat3 plays a role in the plasticity of pancreatic acinar cells, we investigated how Stat3 signaling affects the cellular reprogramming of pancreatic exocrine cells into β cells.

Materials and methods: The combined expression of the defined transcription factors Pdx1, Neurog3, and Mafa was induced in the pancreatic progenitor-like cells *in vitro* and in the mouse models *in vivo*.

Results: Whereas phosphorylation of Stat3 (pStat3) was induced by adenoviral vectors expressing Pdx1 or Mafa, most of the reprogrammed β cells induced by an adenoviral vector carrying Pdx1- Neurog3- Mafa polycistronic cassette (Ad-PNM) were negative for pStat3. Furthermore, suppression of pStat3, using small molecules or dominant-negative form of Stat3, significantly enhanced β-cell neogenesis by Ad-PNM, while a constitutively active form of Stat3 inhibited the reprogramming efficiency into β cells. In order to confirm the role of Stat3 *in vivo* as well as *in vitro*, we generated a transgenic mouse line "*acinar-PNM*", which ectopically expressed Pdx1, Neuro3, and Mafa in acinar cells to induce acinar-to-β reprogramming, and found that Stat3 was activated in the acinar cells surrounding the reprogrammed β cells. In Stat3-deficient *acinar-PNM* mice, the number of newly differentiated β cells was significantly higher than that in control mice. Furthermore, the newborn β cells in *acinar-PNM; Stat3KO* pancreata formed islet-like clusters.

Conclusion: Both *in vitro* and *in vivo* experiments suggest that Stat3 signaling negatively regulates acinar-to-β reprogramming induced by distinct transcription factors, and that Stat3 inhibition promotes the reprogramming efficiency into β cells, which could lead to future cell therapy for cure of diabetes.

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Disclosure: **T. Miyatsuka:** Grants; JSPS KAKENHI (No. 25461348), Takeda Science Foundation.

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Glucokinase- and insulin receptor substrate-2 independent pathway was involved in pancreatic beta cell replication induced by short-term high-fat diet feeding in mice

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Background and aims: Glucokinase and insulin receptor substrate-2 (Irs2) play an important role in compensatory beta cell hyperplasia in response to insulin resistance induced by longitudinal high-fat (HF) diet feeding. In this study, we investigated whether glucokinase and Irs2 were required for beta cell replication induced by short-term HF diet feeding.

Materials and methods: Eight-week-old C57BL/6J mice were exposed to either standard chow (SC) or a HF diet. After 1 week on the diet, histopathological beta cell proliferation was compared. In addition, 8-week-old beta cell-specific glucokinase haploinsufficient ($Gck^{+/-}$) and Irs2 knockout ($Irs2^{-/-}$) mice were exposed to either a SC or HF diet. To identify key genes involved in beta cell proliferation following short-term HF diet consumption, gene expression in isolated islets from 8-week-old C57BL/6J mice fed either diet for 1 week was analyzed using cDNA microarray and real-time quantitative PCR. Moreover, we orally administered a placebo or glucokinase activator to SC- and HF-fed mice during the final 3 days of the diet and compared beta cell proliferation.

Results: Immunohistochemical analysis revealed that short-term HF diet feeding resulted in a significant increase in the BrdU incorporation rate compared with SC consumption. Western blot analysis demonstrated that Irs2 expression levels did not differ between the two groups. There was a significant increase in the BrdU incorporation rate in the HF diet group compared with the SC group in both $Gck^{+/-}$ and $Irs2^{-/-}$ mice. By cDNA microarray analysis, we identified 62 genes expressed differentially (fold change ≥ 1.5) in HF diet-fed mice compared with SC-fed mice. When we clustered placentas according to the gene expression profiles for those 62 genes, three genes, cyclin A2, cyclin B1 and centromere protein A, were relatively close to each other. These three genes are located downstream of Foxm1. Real-time quantitative PCR revealed that Foxm1, cyclin A2, cyclin B1 and centromere protein A expression levels were significantly increased in HF diet mice compared with those of SC mice. Interestingly, glucokinase activator treatment resulted in an additive effect on proliferation induced by HF diet feeding for 1 week.

Conclusion: The pancreatic beta cell replication mechanism induced by short-term HF diet feeding in mice involved a glucokinase- and Irs2-independent pathway. Our results suggest that the beta cell replication pathways involved in short-term HF diet feeding may differ from those involved in chronic HF diet feeding.

Disclosure: **N. Kitao:** None.

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Pancreas heterotopia in Gata4-deficient mice

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Background and aims: Mutations in *GATA4* have been linked to pancreas agenesis in humans. In mice, while single inactivation of *Gata4* does not have a major impact in pancreas development, simultaneous inactivation of *Gata4* and *Gata6* results in pancreas agenesis, indicating redundancy between the two GATA factors. Adult *Gata4* conditional knockout mice (Gata4 KO) are normoglycemic, fertile and the body weight is similar to control littermates. Interestingly, adult mice lacking GATA4 display heterotopic pancreas, which localizes in the serosa layer of the antrum stomach. The aim of this work is to analyze the molecular mechanism underlying the heterotopic pancreas in Gata4 KO mice.

Materials and methods: To analyze the ectopic pancreas we are using conditional *Gata4* Knockout mice where the Cre recombinase transgene is under the control of the *pancreatic and duodenal homeobox gene* (*Pdx1*). Immunohistochemistry and immunofluorescence analyses of embryonic and adult stomach were performed.

Results: Analysis of *Gata4* KO stomach through embryonic development shows ectopic pancreas from E17.5 onwards. Immnuohistochemical and immunofluorescent analyses of adult heterotopic pancreas reveal complete differentiation of exocrine cells. Moreover, small cluster of insulin and glucagon expressing-cells are also observed. Histological analyses show an ingression of the glandular epithelial cells into the serosa, suggesting a stomach epithelial origin of heterotopic pancreatic cells. In agreement with these results, we found lack expression of glandular stomach differentiation markers, including H^+/K^+ ATPase, in the near proximity of the heterotopic pancreatic cells.

Conclusion: Inactivation of *Gata4* in the glandular stomach leads to changes in the cellular phenotype towards a pancreatic fate. Heterotopic pancreatic cells are able to fully differentiate into acinar and endocrine pancreatic cells.

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Disclosure: **E. Rodríguez-Seguel:** None.

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A novel, highly proliferative population of islet endocrine cells in adolescent and adult human pancreata

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Background and aims: Although previous studies have focused on turnover of human β-cells from cadaveric pancreata, little is known regarding proliferation of other islet endocrine cell types in control and T1D states.

Materials and methods: We studied a large collection of pancreata from JDRF nPOD across a wide range of ages, quantifying islet endocrine cell turnover by high throughput imaging.

Results: In a previous study we found no evidence of ongoing β-cell regeneration in T1D pancreata compared to controls, regardless of diabetes duration. Surprisingly, we observed plentiful islet endocrine cell proliferation in many of the sample samples derived from adolescent and young adult T1Ds and controls. However, the increased islet endocrine cell proliferation was not within insulin, PP, somatostatin, or ghrelin expressing cells. Glucagon-expressing α-cells accounted for a large fraction (~30%) of islet cell proliferation in those samples from adolescents and young adults that exhibited high proliferation. To further characterize the proliferative cells we tested for a variety of islet endocrine markers in proliferative samples from adolescents and young adults. The highly proliferative cells represent a novel population hormone negative islet endocrine cells, with ARX (known to determine α-cell identity), INSM1 (present in human α- and β-cells), and cytoplasmic Sox9 cytoplasmic-immunoreactivity. These highly proliferative cells represented the majority of islet endocrine Ki67+ cells in adolescent and young adult pancreata with high turnover.

Conclusion: We have identified a novel population of highly proliferative, α-related cells in adolescent and young adult pancreata. These α-related islet endocrine cells suggest the possibility of previously unrecognized ongoing islet development and/or lineage plasticity within adolescent and adult human pancreata.

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Disclosure: **J.A. Kushner:** Employment/Consultancy; Lexicon Pharma.

OP 06 Linking adipose tissue to metabolic disease

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Remodelling of adipose tissue transcriptome by diurnal distribution of carbohydrates and fat in humans

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Background and aims: Time of food consumption and meal composition strongly affect metabolic state and gene expression in metabolically active tissues. Here, we for the first time investigated effects of the diurnal distribution of carbohydrates and fat consumption on the subcutaneous adipose tissue (SAT) transcriptome in humans.

Materials and methods: 29 non-obese men without diabetes (age 45.9 ± 2.5 years, BMI 27.1 ± 0.8 kg/m^2) participated in the cross-over study. They consumed two 4-week isocaloric diets - (1) HC/HF diet consisted of a carbohydrate-rich breakfast and lunch (65 EN% carbohydrates, 20 EN% fat, 15 EN% protein) and a fat-rich snack and dinner (35 EN% carbohydrates, 50 EN% fat, 15 EN% protein) and (2) HF/HC diet consisted of a fat-rich breakfast and lunch and a carbohydrate-rich snack and dinner. At the end of each intervention period, SAT samples were collected three times during the investigation day (at 8.40, 12.20 and 19.00), and saliva samples were collected every 4 h during 24 h. Microarray analysis of SAT samples from 15 subjects was performed using GeneChip® Human Gene 2.0ST Arrays (Affymetrix) and validated by real-time PCR. Parameters of circadian oscillations were estimated by a 24 h rhythm prediction method established previously.

Results: From 2372 oscillating SAT transcripts, 644 (27.2%) transcripts were rhythmic on both HC/HF and HF/HC diets, whereas 531 (22.4%) and 1197 (50.5%) transcripts showed circadian oscillations only on the HC/HF and HF/HC diet, respectively. A number of genes rhythmic in both dietary conditions demonstrated alterations of acrophase (37), amplitude (22) and mesor (52). Different patterns of meal composition did not affect both oscillations of clock genes in SAT and the salivary cortisol rhythm used as a central clock marker. However, we found dietary effects on numerous oscillating and non-oscillating genes involved in glucose (e.g. IRS1, IRS2, PCK1, PDK4) and lipid metabolism (e.g. ACACA, ACAT1, ACOX2, PPARA) as well as inflammatory response (e.g. CCL5, IL1B, ITGAX, CD3E). Mechanisms underlying this remodeling include the change of activity of the insulin signaling, I-kappa B kinase/NF-kappa B and MAPK pathways. Serum leptin rhythm was delayed on the HF/HC diet, whereas levels of adiponectin, visfatin, IL-6 and C-reactive protein were not changed.

Conclusion: Our study revealed that diurnal distribution of carbohydrates and fat consumption induces deep remodeling of adipose tissue transcriptome in humans including the alteration of glucose and lipid metabolism and inflammatory pathways.

Clinical Trial Registration Number: NCT02487576

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Disclosure: **O. Pivovarova:** None.

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The FAT score, a Fibrosis score of Adipose Tissue: predicting weight loss outcome after gastric bypass

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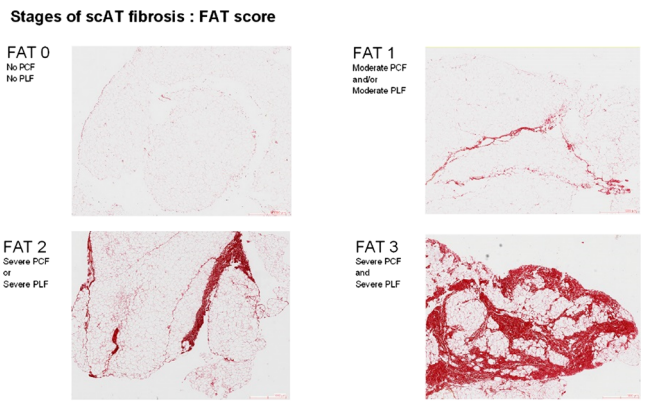
Background and aims: Bariatric surgery (BS) induces major and sustainable weight loss in many but not all patients. Factors predicting poor responders (PR) need to be identified to improve patient care. Quantification of subcutaneous adipose tissue (scAT) fibrosis is negatively associated with post-BS weight loss, but whether it could constitute a predictor applicable in clinical routine remains to be demonstrated. The objective of this work was to create a semi-quantitative score evaluating scAT fibrosis and test its predictive value on weight loss response after Roux-en-Y gastric bypass (RYGB).

Materials and methods: We created a Fibrosis score of Adipose Tissue (FAT score) integrating peri-lobular and peri-cellular fibrosis. Using this score, we characterized 183 peroperative scAT biopsies (stained with picrosirius red) of severely obese patients who underwent RYGB (85 from a training cohort, 98 from a confirmation cohort). Poor Response (PR) to RYGB was defined as < 28% of total weight-loss at one year (lowest tertile). The link between FAT score and PR was tested in univariate and multivariate models.

Results: FAT score was directly associated with increasing scAT fibrosis measured by a standard quantification method (p for trend <0.001). FAT score inter-observer agreement was good (κ =0.76). FAT score ≥2 was significantly associated with PR. The association remained significant after adjustment for age, diabetes status, hypertension, percent fat-mass and IL-6 (adjusted OR =3.6, IC 95% [1.8, 7.2]; p =0.003).

Conclusion: The FAT score is a new simple semi-quantitative evaluation of human scAT fibrosis that may be used to help identifying patients with a potential limited weight loss response to RYGB.



Peroperative scAT biopsies stained with picrosirius red to reveal fibrosis (collagen accumulation)
scAT: subcutaneous adipose tissue; PLF: perilobular fibrosis; PCF: pericellular fibrosis; FAT score: Fibrosis Adipose Tissue score

Clinical Trial Registration Number: NCT01655017

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Disclosure: **P. Bel Lassen:** None.

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Characteristics of differentiated small proliferative adipocytes (SPA), a newly identified adipocyte progenitor

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Background and aims: We reported cells expressing adipocyte specific genes and proliferative activity in adipose tissue last year. We considered these cells as a new population of adipocyte progenitors, and named them small proliferative adipocytes (SPA). We found that SPA expressed several neuronal genes, such as synaptic vesicle, GABA-A receptor, serotonin receptor 1F and glutamate receptor 7 (Grm7). Moreover, SPA, as well as stromal vascular cells (SVC), were found to differentiate into adipocytes. In this study, we examined the difference between adipocytes differentiated from SPA (DSPA) and ones from SVC (DSVC).

Materials and methods: Mouse epididymal adipose tissue was digested with collagenase, and centrifuged at 9g for 1 sec. Cells in the sedimentary fraction were regarded as SVC. Floating cell fraction was separated and further centrifuged at 226g for 3 min. The resultant sedimentary cells were regarded as half-floating cells. Half-floating cells were small but expressed adiponectin, fatty acid binding protein 4 and leptin. In addition, incorporation of EdU was detected in these cells. Therefore, they were considered as SPA. Adipogenic differentiation was induced with differentiation medium containing insulin/dexamethasone/IBMX. Gene expression of DSPA was compared with DSVC with microarray.

Results: Incubation of SPA with differentiation medium for 2 days yielded lipid laden cells expressing increased amount of PPARγ2 and adiponectin mRNA (DSPA). Neuronal genes, such as Grm7 were more abundantly expressed in DSPA than in DSVC. Treatment with dexamethasone or pioglitazone alone, but not insulin, led to lipid accumulation to a less extent than differentiation medium in SPA. In contrast, fewer lipid droplets ware detected in SVC treated with differentiation medium (DSVC). To characterize DSPA, microarray was performed comparing with DSVC. Adipocyte genes including adipsin, β3 adrenergic receptor (Adrb3) and perilipin 4, and neuronal genes, neurofilament light (Nefl), synapsin 3 and adenosine A1 receptor, were more abundantly expressed than adiponectin in DSPA compared with DSVC. Immunocytochemical study confirmed these results. In addition, immunohistochemical study detected two populations of adipocytes, (adipocytes positive for Nefl and Adrb3, and negative for Nefl and Adrb3). Recent study demonstrated that stimulation of Adrb3 induces beige remodeling in white adipose tissue. These data suggested that DSPA may functionally differ from DSVC.

Conclusion: Differentiation of SPA and SVC resulted in functionally distinct mature adipocytes.

Disclosure: **K. Kajita:** None.

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Bone morphogenetic protein 2 is a depot-specific regulator of human adipogenesis

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Background and aims: Body fat distribution is a strong determinant of human metabolic health but the mechanisms underpinning regional deposition of adipose tissue remain poorly understood. Recent genome-wide association studies of body fat distribution suggest that bone morphogenetic protein 2 (BMP2) and Smad signalling regulate this phenotype. The aim of this project was to investigate whether BMP2 modulates adipogenesis in a depot-specific manner.

Materials and methods: BMP2 gene expression was measured in adipose tissue and analysed in combination with genotype data from the Oxford Biobank to identify eQTLs. Depot-specific effects of BMP2 signalling were studied in immortalised human subcutaneous abdominal and gluteal preadipocytes. Smad1/5/8 signalling activity was measured via Western blot; adipogenesis was evaluated via triglyceride (TG) quantification and qPCR. Preadipocytes were treated with BMP2 and/or K02288, an inhibitor of Smad signalling.

Results: BMP2 expression in both male and female abdominal and gluteal adipose tissue was 15% lower in BMP2 rs2145270 CC carriers ($p<0.05$, ANOVA corrected for age and BMI), a recently-identified GWAS locus for body fat distribution. Adipose tissue BMP2 expression did not exhibit any regional variation. Acute treatment with 50ng/ml exogenous BMP2 stimulated the phosphorylation of Smad1/5/8 in preadipocytes from both depots but this was blocked by co-administration of 500nM K02288. Supplementation of differentiation media with BMP2 enhanced adipogenesis specifically in abdominal preadipocytes in a dose-dependent manner: compared to vehicle, 5ng/ml BMP2 stimulated a 2-fold increase in *PPARG* expression ($p<0.05$; post-hoc T-test for ANOVA) and 1.5 fold increase in TG accumulation ($p<0.05$, post-hoc T-test for ANOVA); 50ng/ml BMP2 increased *PPARG* expression 3.5 fold ($p<0.05$, post-hoc T-test for ANOVA) and TAG accumulation 3 fold ($p<0.05$, post-hoc T-test for ANOVA). However BMP2 treatment had no effect on adipogenesis in gluteal cells. The depot-specific, pro-adipogenic action of BMP2 was blocked by co-administration with K02288.

Conclusion: BMP2 represents a novel depot-specific regulator of (subcutaneous) adipogenesis. Its pro-adipogenic effects require activation of Smad1/5/8 signalling but the depot-specific nature of BMP2 action likely manifests through regional variation in Smad1/5/8-regulated gene expression. These functional studies build on recent GWAS meta-analysis results and strongly implicate BMP2-Smad signalling in preadipocyte biology and the determination of human body fat distribution.

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Disclosure: **N. Denton:** None.

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Effect of diet-induced obesity on peripancreatic adipose tissue secretion: TIMP-1

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Background and aims: Besides storing energy, adipose tissue integrates a wide array of physiological processes which are coordinated mainly through the synthesis and release of adipokines which can act either locally or through the blood stream. Using a model of obesity and insulin resistance induced by a hypercaloric diet (representative of the unhealthy so-called western diet), we demonstrated that the peripancreatic adipose tissue is able to secrete some proliferative signals involved in the pancreatic beta cell plasticity, suggesting a cross-talk between the adipose tissue and the beta cell. The objective of this study is to investigate signals emanating from the adipose tissue that potentially may contribute to both beta cell compensation and decompensation processes.

Materials and methods: The study has been carried out along the "Principles of laboratory animal care" and according to the national law. Male Wistar rats were exposed to a high caloric cafeteria (CAF) diet (65% of the energy derived from lipids) (n=29) or standard (STD) chow diet (n=15) for 24 weeks. Body weight was measured weekly. Pancreas was removed to perform morphometric studies. Adipose tissues (AT) from epididymal (eWAT) and peri-pancreatic (pWAT) depots, as well as pancreatic islets, were collected. Conditioned medium (CM) was obtained from AT by culturing in serum-free medium for 24 hours. The expression profile of peri-pancreatic fat derived factors was analyzed by antibody-array. TIMP-1 expression was determined by qRT-PCR,

immunoblotting and TIMP-1 concentration was quantified by ELISA. Proliferation in INS1E cells was evaluated by BrdU incorporation.

Results: Administration of CAF diet induced a rapid increase in the animals' body weight. The CAF diet increased the weight of all adipose depots analyzed (eWAT: 27.17g ±5.34 vs. 14.37g ±2.81, pWAT: 5.76g ±1.33 vs. 2.39g ± 0.80; p<0.001). As expected, the obesity induced by the long term CAF diet led to an impairment of glucose tolerance. Although no significant difference were observed in fasting glycaemia, CAF diet fed rats had higher fasted levels of insulin compared with lean ones (3.9ng/mL ±1.1 vs. 1.8ng/mL ±0.7, p<0.001). Changes in cytokine and chemokine secretion profiles of peripancreatic adipose tissue (pWAT) due to diet-induced obesity have been observed. We are particularly interested by tissue inhibitor of metalloproteinase 1 (TIMP-1). TIMP-1 expression was increased after 4 weeks of CAF diet (4-fold, p<0.01), but decreased by half after 24 weeks (p<0.01), suggesting an effect of age and diet. The decrease in the expression of Timp-1 transcript was observed in pWAT relative to lean animals. Western blot analysis confirmed that long-term CAF diet decreased TIMP-1 expression in peripancreatic adipose tissue (pWAT) and in CM from pWAT. TIMP-1 concentration was quantified precisely in CM from pWAT by ELISA (STD: 3.2 ng/mL ±0.1 and CAF: 0.6ng/mL ±0.4, p<0.001) and these concentration was tested on INS1E cells proliferation.

Conclusion: TIMP-1 is known to exhibit a broad range of biological activities, including inhibition of MMP activity, regulation of proliferation and apoptosis of a variety of cell types. Here, we suggested that TIMP-1, secreted by peripancreatic adipose tissue, might have a survival effect on pancreatic β-cells.

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The crucial role of Fibrinogen-related protein 1 in obesity

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Background and aims: The prevalence of obesity has significantly increased in the past few decades, and the excessive body fat is associated with many metabolic disorders. Changes of adipokines release in obesity were the key mechanism that led to the development of insulin resistance. Fibrinogen-like protein 1 (FGL1) was previously discovered as a novel adipokine that led to insulin resistance. However, the physiological functions in adipogenesis and the role of FGL1 in obesity are still obscure.

Materials and methods: A total of 359 subjects (included 290 women and 129 men) were enrolled in the present study. Among these subjects, 224 participants were normal weight (body mass index <25) and 135 participants were overweight/obesity (body mass index ≥25). Body fat composition was determined using computed tomography. Serum FGL1 concentrations were measured by enzyme-linked immunosorbent assay. Overexpression or knockdown of FGL1 in the adipose tissue of high fat diet- fed mice was achieved by lenti-viral vector transfection to evaluate the effects of FGL1 in fat pad. In addition, 3T3-L1 adipocytes were used to investigate the possible mechanisms in FGL1-induced adipogenesis.

Results: Participants with overweight/obesity had higher fat composition, fasting plasma glucose, HbA1C, blood pressure, triglyceride levels, glutamate oxalate transaminase, glutamate pyruvate transaminase levels, and lower high-density lipoprotein levels. The fasting plasma FGL1 concentration was 689 ng/ml in normal weight group and 743 ng/ml in overweight/obesity group (p=0.003). In univariate analyses, we found that plasma FGL1 level was associated with body mass index (BMI), waist circumference, total fat area, visceral fat area and subcutaneous fat area (p<0.01). In multivariate logistic regression analysis, plasma FGL1 was significantly associated with BMI, waist circumference, log-transformed total fat area, and log transformed subcutaneous fat area (p<0.05) after the adjustment of age. Overexpression of FGL1 in epididymal adipose tissue significantly increased the size of the fat pad in mice. On the other hand, knockdown of FGL1 in the fat pad significantly decreased the size of the fat pad in high fat diet-fed mice. Furthermore, we found FGL1 facilitated 3T3-L1 pre-adipocyte proliferation through an ERK1/2-dependent pathway.

Conclusion: FGL1 increased the proliferation of pre-adipocytes and contributed to the development of obesity. In this study, we clarified the physiological function of FGL1 in adipose tissue and provided a novel mechanism for the development of obesity.

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Supported by: MOST

Disclosure: **H. Wu:** None.

OP 07 SGLT2 inhibitors: clinical utility

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Time to next therapy for patients with type 2 diabetes in the UK: canagliflozin compared with other antihyperglycaemic agents

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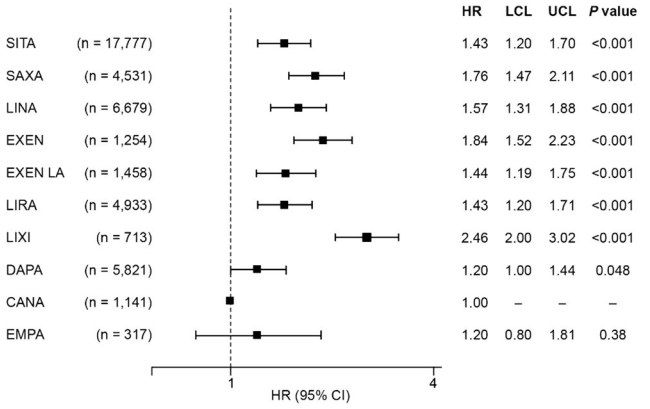
Background and aims: This study describes the pattern of changes in therapy for patients with type 2 diabetes mellitus (T2DM) treated with canagliflozin (CANA), a sodium glucose co-transporter 2 inhibitor (SGLT2i), versus other antihyperglycaemic agents (AHAs) using retrospective data in the UK.

Materials and methods: Patients with T2DM who were initiated on glucagon-like protein-1 receptor agonists (GLP-1 RA), dipeptidyl peptidase-4 inhibitor (DPP-4i), or SGLT2i between 2012 and 2015 were extracted from Clinical Practice Research Datalink (N = 46,349). Time to next therapy was analysed using Kaplan-Meier analysis and multivariate Cox proportional hazards regression, with age, sex, HbA1c, systolic blood pressure, and body mass index (BMI) at baseline, and background therapy as covariates.

Results: Time to next therapy was longer for CANA (n = 1,143) versus GLP-1 RA, DPP-4i, and other SGLT2i (Figure). Adjusted hazard ratios (HRs; 95% confidence interval [CI]) versus CANA ranged from 1.43 [1.20, 1.70] (sitagliptin; n = 17,777) and 1.76 [1.47, 2.11] (saxagliptin; n = 4,531) for DPP-4i (all P <0.001); from 1.43 [1.20, 1.71] (liraglutide; n = 4,933) and 1.84 [1.52, 2.23] (exenatide; n = 1,254) for GLP-1 RA (all P <0.001); and from 1.20 [1.00, 1.44] (P = 0.048; dapagliflozin; n = 5,821) and 1.20 [0.80, 1.81] (P = 0.38; empagliflozin; n = 317) for SGLT2i.

Conclusion: These analyses indicate that patients receiving CANA versus other AHAs had a longer time on treatment before a change in therapy, which may reflect better effectiveness and/or tolerability.

Figure. Adjusted HRs (95% CI) for time to next therapy for selected GLP-1 RA, DPP-4i, and other SGLT2i versus CANA based on the multivariate Cox proportional hazards regression.



Estimated HR by Cox model.
LCL, lower confidence level; UCL, upper confidence level; SITA, sitagliptin; SAXA, saxagliptin; LINA, linagliptin; EXEN, exenatide; LA, long acting; LIRA, liraglutide; LIXI, lixisenatide; DAPA, dapagliflozin; EMPA, empagliflozin.

Supported by: Janssen Pharmaceutica NV

Disclosure: **J. Diels:** Employment/Consultancy; Janssen Research & Development.

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Safety and efficacy of ertugliflozin compared to glimepiride in patients with type 2 diabetes inadequately controlled on metformin: the VERTIS SU trial

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Background and aims: Ertugliflozin (ERTU) is an oral sodium/glucose cotransporter 2 (SGLT2) inhibitor in development for treatment of type 2 diabetes mellitus (T2DM). This study assessed the safety and efficacy of once-daily ERTU 15 mg (E15) or 5 mg (E5), compared with glimepiride (G), over 52 weeks in patients with T2DM inadequately controlled on metformin.

Materials and methods: In a double-blind, randomised Phase 3 trial, patients with HbA1c 7.0-9.0% on stable metformin ≥1500 mg/day were randomised 1:1:1 to E15, E5, or G (initiated at 1 mg QD and uptitrated to a maximum of 6 or 8 mg/day according to local label or maximum tolerated dose; appropriateness of up titration was assessed at study visits or more frequently as per investigator discretion, and was guided by an algorithm that considered glycaemic control and hypoglycaemia). Endpoints were analysed at Week 52. Primary hypothesis was that E15 was non-inferior to G in reducing HbA1c; non-inferiority was to be declared if the upper bound of the two-sided 95% CI of the mean treatment difference was <0.3%. Key secondary endpoints included change from baseline in HbA1c (E5 vs G), incidence of symptomatic hypoglycaemia, and change from baseline in body weight and systolic BP (SBP). Efficacy endpoints were analysed with a longitudinal model using a pre-specified testing sequence to control for multiplicity.

Results: In total, 1326 patients were randomised. Baseline characteristics were generally comparable between arms (mean age 58.2 yrs, HbA1c 7.8%, eGFR 87.2 mL/min/1.73m^2, weight 86.8 kg). The mean dose of G was 3.0 mg. After 52 weeks, E15 was non-inferior to G in reducing HbA1c (Table). Significantly less symptomatic hypoglycaemia (p<0.001) and greater reductions in body weight (p<0.001) were observed with E15 vs G. E5 was not non-inferior to G in reducing HbA1c. Less symptomatic hypoglycaemia (nominal p<0.001) and greater reductions in body weight (nominal p<0.001) were observed with E5 vs G. Greater reductions in SBP were observed in the E15 and E5 arms vs G (nominal p<0.001). ERTU was generally well-tolerated. AE incidence was similar across arms, with a low frequency of serious AEs (SAEs), drug-related SAEs and AEs leading to discontinuation. One (0.2%) and 5 (1.1%) patients in the E15 and E5 arms, respectively, died during the treatment period; 1 death occurred in the G group due to an AE that started after the last treatment dose. Higher rates of genital mycotic infections (GMI) were reported in the E15 and E5 arms vs G. Comparable rates of urinary tract infection and hypovolaemia AEs were observed.

Conclusion: E15 was non-inferior to G in reducing HbA1c and resulted in greater reductions in body weight than G. ERTU was well tolerated and resulted in less hypoglycaemia, but more GMI, than G.

Table. Summary of efficacy and safety analyses at Week 52

		E15 (n=440)	E5 (n=448)	G (n=437)	Difference (95% CI)[b] E15 vs. G	Difference (95% CI)[b] E5 vs. G
Efficacy analysis LS mean change from baseline[a]	HbA1c, %	−0.6	−0.6	−0.7	0.1 (−0.0, 0.2)[c]	0.2 (0.1, 0.3)
	Body weight, kg	−3.4	−3.0	0.9	−4.3 (−4.8, −3.8)*	−3.9 (−4.4, −3.4)**
	SBP, mmHg	−3.8	−2.3	1.0	−4.8 (−6.3, −3.3)**	−3.2 (−4.7, −1.7)**
Safety analysis Number of patients, n (%)	AEs [drug-related]	262 (59.5) [95 (21.6)]	263 (58.7) [82 (18.3)]	269 (61.6) [78 (17.8)]	−2.0 (−8.5, 4.5) [3.7 (−1.5, 9.0)]	−2.9 (−9.3, 3.6) [0.5 (−4.6, 5.5)]
	SAEs	17 (3.9)	27 (6.0)	12 (2.7)	1.1 (−1.3, 3.7)	3.3 (0.6, 6.2)
	AEs leading to discontinuation	25 (5.7)	18 (4.0)	17 (3.9)	1.8 (−1.1, 4.8)	−0.1 (−2.6, 2.8)
	Symptomatic hypoglycaemia	23 (5.2)	14 (3.1)	84 (19.2)	−14.0 (−18.4, −9.8)*	−16.1 (−20.3, −12.2)**
	GMI, male / female	4 (2.1) / 25 (10.0)	10 (4.4) / 17 (7.7)	0 (0) / 3 (1.4)	2.1 (0.4, 5.3)§ / 8.6 (4.8, 13.2)*	4.4 (2.4, 7.9)# / 6.3 (2.6, 10.7)#
	Urinary tract infection	28 (6.4)	30 (6.7)	30 (6.9)	−0.5 (−3.9, 2.9)	−0.2 (−3.6, 3.2)
	Hypovolaemia	3 (0.7)	6 (1.3)	3 (0.7)	−0.0 (−1.4, 1.4)	0.7 (−0.8, 2.3)

[a]Longitudinal model with fixed effects for treatment, time, prior antihyperglycaemic medication (monotherapy or dual therapy), baseline eGFR (continuous) and the interaction of time by treatment.
[b]The difference between E15 or E5 and G for the safety endpoints is given in %
[c]Non-inferiority was declared when the upper bound of the two-sided 95% CI for the mean difference was <0.3%.
*p<0.001; **nominal p<0.001 (non-inferiority for HbA1c was not demonstrated for E5 and the multiplicity strategy did not permit testing of any further secondary hypotheses; p-values provided for descriptive purposes only); §p=0.03; #p=0.002.
LS, least squares

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Long-term efficacy and safety of canagliflozin in combination with insulin in Japanese type 2 diabetes patients

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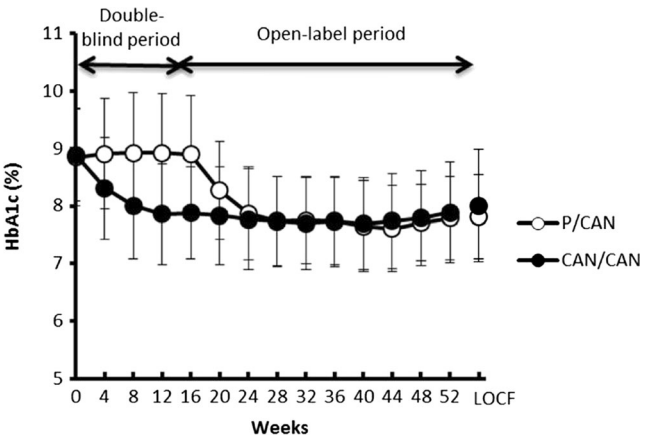
Background and aims: This study investigated the efficacy and safety of canagliflozin (CAN) administration in Japanese type 2 diabetes mellitus patients with inadequate glycemic control on insulin monotherapy.

Materials and methods: The study comprised a 16-week double-blind period in which patients were randomized to either CAN 100 mg (N=76) or placebo (P; N=70), followed by a 36-week open-label period in which all patients received CAN 100 mg.

Results: Placebo-adjusted changes (least squares mean, last observation carried forward [LOCF]) in HbA1c, body weight, and homeostatic model assessment (HOMA) 2-%B were −1.10% (95% CI −1.33, −0.87), −2.37% (95% CI −3.09, −1.65), and 9.27% (95% CI 5.35, 13.19), respectively (all p<0.001). These effects were sustained throughout the open-label period: the changes from baseline (LOCF) in the P/CAN (36-week treatment, N=67) and CAN/CAN groups (52-week treatment, N=76), respectively, were −1.09% (95% CI −1.29, −0.88; p<0.001) and −0.88% (95% CI −1.08, −0.68; p<0.001) for HbA1c; −1.40% (95% CI −2.03, −0.77; p<0.001) and −2.14% (95% CI −2.78, −1.51; p<0.001) for body weight; and 7.84% (95% CI 4.25, 11.43 ; p<0.001) and 8.91% (95% CI 6.41, 11.41; p<0.001) for HOMA2-%B. The incidence rates of adverse events were 64.8% and 68.0% in the P and CAN groups, respectively, during the double-blind period, and 85.1% and 92.0% in the P/CAN and CAN/CAN groups, respectively, during all treatment period of CAN (P/CAN:36-week treatment, CAN/CAN:52-week treatment). The incidence rates of hypoglycemia per subject-year exposure were 4.51 and 7.97 in the P and CAN groups, respectively, during the double-blind period, and 4.85 during all treatment period of CAN. Hypoglycemic events in both groups were mild in severity and insulin dose reduction decreased the incidence rate of hypoglycemic events per subject-year exposure (14.76 before dose reduction, 9.30 after dose reduction; N=38). Cumulative logistic model obtained by the number of hypoglycemic events was applied to investigate risk factors for hypoglycemia and suggested that lower baseline plasma C-peptide was the risk factor for hypoglycemia in both the P and CAN groups in the double-blind period as well as in all treatment period of CAN.

Conclusion: This study demonstrates the long-term efficacy and safety of CAN in combination with insulin in Japanese patients.



Clinical Trial Registration Number: NCT02220920/NCT02622113
Supported by: Mitsubishi Tanabe Pharma Corporation

Disclosure: **S. Harashima:** Employment/Consultancy; Novo Nordisk Inc, Mitsubishi Tanabe Pharma Corporation. Other; AstraZeneca, Mitsubishi Tanabe Pharma, Eli Lilly Japan K.K, Novo Nordisk Inc, Sanofi K.K.

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Dapagliflozin + saxagliptin + metformin triple therapy vs sitagliptin add-on to metformin dual therapy in subgroups of patients with uncontrolled type 2 diabetes

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Background and aims: Stepped up therapy adding a single antidiabetes agent often fails to improve glycaemic control meaningfully in patients with significant hyperglycaemia and metformin-treated type 2 diabetes mellitus (T2DM). The efficacy and safety of triple therapy achieved with add-on of saxagliptin (SAXA) plus dapagliflozin (DAPA) to metformin (MET) vs dual therapy with a single addition of sitagliptin (SITA) to MET were evaluated in this randomized, double-blind, double-dummy, 26-week trial in patients with T2DM.

Materials and methods: Patients with T2DM and glycated haemoglobin (HbA_{1c}) levels 8.0%—10.5% on stable MET therapy (≥1500 mg/day) were randomized (1:1) to add-on oral therapy with DAPA 10 mg/day plus SAXA 5 mg/day or SITA 100 mg/day. The primary efficacy end point was change in HbA_{1c} from baseline to week 26; secondary end points were the proportion of patients achieving a therapeutic glycaemic response (HbA_{1c} <7.0%) and changes in body weight and fasting plasma glucose (FPG) at week 26. The primary end point was analysed for predefined subpopulations stratified by baseline HbA_{1c} category, sex, age, race, and region using a longitudinal repeated-measures model. The incidences of adverse events and hypoglycaemia were also assessed.

Results: The analysis included 461 patients (45.6% men; mean±SD age, 55.9±9.2 years; BMI, 33.1±6.2 kg/m^2; duration of T2DM, 8.0±5.4 years; HbA_{1c}, 8.8±0.9%). The reduction from baseline to week 26 in HbA_{1c} was greater with DAPA+SAXA+MET vs SITA+MET (−1.41% vs −1.07%; *P*=0.0008) irrespective of baseline HbA_{1c} (<8.0% vs 8.0%—9.0% vs ≥9.0%), sex, age, race, or region (Table). Reductions in total body weight (−1.9 kg vs −0.5 kg; *P*<0.0001) and FPG (−32 mg/dL [1.8 mmol/L] vs −11 mg/dL [0.6 mmol/L]; *P*<0.0001) were greater, and significantly more patients achieved HbA_{1c} <7.0% (37% vs 25%; *P*=0.0034), with DAPA+SAXA+MET vs SITA+MET. Both treatments were well tolerated, and the incidence of confirmed hypoglycaemia was low (<5%).

Conclusion: Add-on of DAPA+SAXA to MET provided significantly greater improvements in HbA_{1c} and other glycaemic parameters, weight, and the proportion of patients achieving target HbA_{1c} <7.0%, compared with SITA+MET, across diverse predefined subpopulations with poorly controlled T2DM.

Table. Adjusted mean HbA_{1c} change by baseline characteristics

Treatment	Interaction tested for adjusted mean HbA_{1c} change from baseline												
	Baseline HbA_{1c} (%)			Age (years)		Sex		Race			Region		
	<8.0%	8.0% to <9.0%	≥9.0%	<65	≥65	Male	Female	White	Black or African-American	Other	North America	Latin America	EU
DAPA+SAXA+MET (N=232)	n=32	n=95	n=79	n=173	n=33	n=88	n=118	n=143	n=24	n=33	n=99	n=44	n=63
	−0.85	−1.29	−1.74	−1.41	−1.42	−1.39	−1.42	−1.34	−1.46	−1.70	−1.21	−1.70	−1.53
SITA+MET (N=229)	n=24	n=77	n=83	n=148	n=36	n=87	n=97	n=122	n=24	n=32	n=84	n=42	n=58
	−0.65	−0.79	−1.48	−1.01	−1.32	−1.18	−0.97	−1.13	−0.61	−1.28	−1.01	−1.20	−1.11
Treatment comparison: difference (95% CI)													
DAPA+SAXA+MET vs SITA+MET	−0.20 (−0.74, 0.34)	−0.50 (−0.80, −0.20)	−0.27 (−0.58, 0.04)	−0.39 (−0.61, −0.18)	−0.11 (−0.58, 0.37)	−0.21 (−0.50, 0.08)	−0.45 (−0.72, −0.18)	−0.22 (−0.46, 0.02)	−0.85 (−1.39, −0.32)	−0.42 (0.91, 0.08)	−0.21 (−0.49, 0.08)	−0.50 (−0.92, −0.07)	−0.42 (−0.77, −0.07)
P value	0.7245			0.2771		0.2309		0.0971			0.4629		

A mixed statistical model was used to analyse between-group differences.
n is the number of randomized patients with non-missing baseline and week 26 values (last observation carried forward). Data are included up to 8 days after the last dose of short-term double-blind treatment.
The race subgroup of 'other' includes American Indian/Alaska Native, Native Hawaiian/Other Pacific Islander, or other. Categorical subgroups with <10 patients in any treatment group are not included in the interaction model.
The age ≥75 years subgroup (n=3) and the Asian patient subgroup (n=6) were excluded from the interaction owing to small numbers.
CI, confidence interval; DAPA, dapagliflozin; EU, European Union; HbA_{1c}, glycated haemoglobin; MET, metformin; SAXA, saxagliptin; SITA, sitagliptin.

Clinical Trial Registration Number: NCT02284893

Disclosure: **S. Del Prato:** Grants; Merck Sharpe & Dohme, Novartis Pharmaceuticals, Novo Nordisk. Honorarium; AstraZeneca, Boehringer Ingelheim, Eli Lilly & Company, GlaxoSmithKline, Hanmi Pharmaceuticals, Intarcia Therapeutics, Janssen Pharmaceutics, Merck Sharpe & Dohme, Novartis Pharmaceuticals, Novo Nordisk, Sanofi, Servier, Takeda Pharmaceuticals.

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Safety and efficacy of ertugliflozin after 52 weeks in patients with type 2 diabetes inadequately controlled on metformin and sitagliptin: VERTIS SITA2 trial extension

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Background and aims: Ertugliflozin (ERTU) is an oral sodium/glucose cotransporter 2 (SGLT2) inhibitor in development for treatment of type 2 diabetes mellitus (T2DM). This study assessed the safety and efficacy of adding ERTU 5 mg or 15 mg compared with placebo (PBO) to the dual combination of metformin and sitagliptin over 52 weeks of treatment.

Materials and methods: Patients (n=464) with HbA1c 7.0-10.5% on stable metformin ≥1500 mg/day and sitagliptin 100 mg/day were randomised to ERTU 5 mg, ERTU 15 mg, or PBO in a double-blind Phase 3 trial. The primary outcome was at Week 26 and treatment was continued until Week 52 in a double-blind extension phase.

Results: Baseline characteristics were generally comparable between groups (mean age 59.1 years, mean HbA1c 8.0%, mean body weight 86.9 kg, mean eGFR 87.9 mL/min/1.73m^2). After 52 weeks, greater reductions in HbA1c, fasting plasma glucose (FPG), body weight and systolic BP and a greater proportion of patients with an HbA1c <7.0% were observed in the ERTU groups versus PBO (Table), consistent with previously reported Week 26 findings. Rates of genital mycotic infections were higher in patients receiving ERTU compared with PBO at Week 52 (males: 4.9% [ERTU 5 mg], 3.7% [ERTU 15 mg], 0 [PBO]; females: 12.0% [ERTU 5 mg], 14.1% [ERTU 15 mg], 1.9% [PBO]; all p<0.05 vs PBO except ERTU 15 mg males). The incidences of urinary tract infections, symptomatic hypoglycaemia and hypovolaemia adverse events were not meaningfully different across groups.

Conclusion: Addition of ERTU 5 mg or 15 mg to metformin and sitagliptin provided clinically meaningful glycaemic control over 52 weeks and was well-tolerated.

Table. Summary of key efficacy endpoints at Week 52 (excluding rescue approach)

		PBO (n=153)	ERTU 5 mg (n=156)	ERTU 15 mg (n=153)[a]	Pairwise comparison:[b] ERTU 5 mg vs PBO	Pairwise comparison:[b] ERTU 15 mg vs PBO
LS mean change from baseline (95% CI)[c]	HbA1c, %	0.0 (−0.2, 0.2)	−0.7 (−0.9, −0.6)	−0.8 (−1.0, −0.7)	−0.8 (−1.0, −0.5)	−0.8 (−1.1, −0.6)
	FPG, mmol/L	0.2 (−0.2, 0.5)	−1.4 (−1.7, −1.1)	−1.5 (−1.8, −1.2)	−1.6 (−2.0, −1.2)	−1.6 (−2.1, −1.2)
	mg/dL	*3.2 (−3.1, 9.5)*	*−25.6 (−30.9, −20.2)*	*−26.4 (−31.8, −21.0)*	*−28.8 (−36.4, −21.1)*	*−29.6 (−37.3, −21.9)*
	Body weight, kg	−1.0 (−1.7, −0.3)	−3.5 (−4.1, −2.9)	−2.8 (−3.4, −2.2)	−2.5 (−3.4, −1.6)	−1.9 (−2.8, −1.0)
	Systolic BP, mmHg	0.8 (−1.4, 3.1)	−4.2 (−6.0, −2.3)	−4.1 (−6.0, −2.2)	−5.0 (−7.8, −2.2)	−4.9 (−7.8, −2.1)
Patients with an HbA1c <7.0%, n (%)		21 (13.7)	52 (33.3)	50 (32.7)	OR:[d] 3.6 (2.0, 6.6)	OR:[d] 4.0 (2.2, 7.3)

[a]Two patients randomised to ERTU 15 mg did not receive study medication and were excluded from the analysis
[b]As the primary outcome was at Week 26, no formal statistical inference was performed to compare treatment groups for efficacy endpoints at Week 52
[c]Constrained longitudinal (cLDA) model with fixed effects for treatment, time, prior antihyperglycaemic medication (metformin + dipeptidyl peptidase-4 inhibitor / metformin + sulfonylurea), baseline eGFR (continuous) and the interaction of time by treatment
[d]Adjusted OR (95% CI) based on a logistic regression model fitted with fixed effects for treatment, prior antihyperglycaemic medication, baseline HbA1c and baseline eGFR (continuous), with missing data imputed using the cLDA model
LS, least squares

Clinical Trial Regsitration Number: NCT02036515
Supported by: Merck & Co., Inc., Kenilworth, NJ USA, in collaboration with Pfizer.
Disclosure: **R. Eldor:** None.

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Empagliflozin (EMPA) reduces mortality in analyses adjusted for control of blood pressure (BP), low density lipoprotein cholesterol (LDL-C) and HbA_{1c} over time

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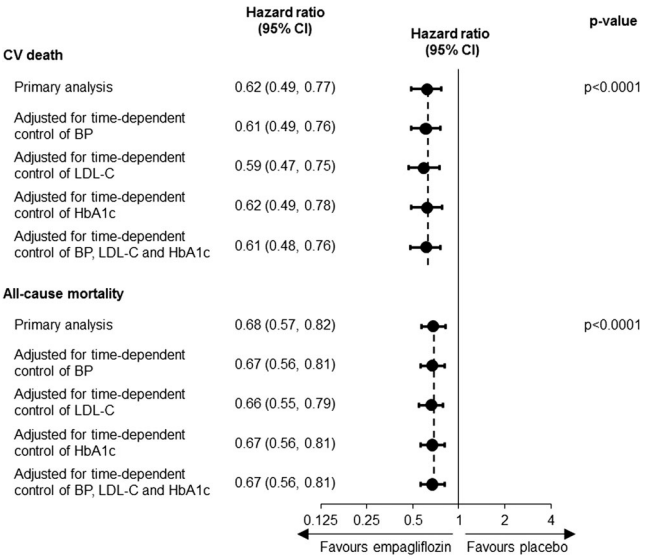
Background and aims: In EMPA-REG OUTCOME, EMPA given in addition to standard of care significantly reduced the risk of cardiovascular (CV) (HR 0.62 [95% CI 0.49, 0.77]) and all-cause (0.68 [0.57, 0.82]) mortality vs placebo (PBO) in patients with type 2 diabetes (T2DM) and established CV disease. We investigated the effects of controlling BP, LDL-C and HbA1c on the treatment difference in mortality.

Materials and methods: Patients were randomised to EMPA 10 mg, EMPA 25 mg, or PBO. CV and all-cause mortality were assessed in the pooled EMPA group vs PBO adjusting for control of BP, LDL-C and HbA1c at baseline and during the study as time-dependent covariates. Control was defined as systolic BP <140 mmHg and diastolic BP <90 mmHg, LDL-C <100 mg/dL, and HbA1c <7.5%.

Results: Adjusting for control of BP, LDL-C, HbA1c and all these covariates at baseline and during the study, HRs for CV death with EMPA vs PBO were 0.61 (0.49, 0.76), 0.59 (0.47, 0.75), 0.62 (0.49, 0.78) and 0.61 (0.48, 0.76), and for all-cause mortality were 0.67 (0.56, 0.81), 0.66 (0.55, 0.79), 0.67 (0.56, 0.81) and 0.67 (0.56, 0.81), respectively (Figure).

Conclusion: EMPA reduced CV and all-cause mortality to the same extent when analyses were adjusted for control of BP, LDL-C and HbA1c over time, suggesting that the mortality reductions were not driven by controlling or not controlling these CV risk factors during the study.



Cox regression analysis in patients treated with ≥1 dose of study drug.
Control of BP, LDL-C and HbA1c were defined as systolic BP <140 mmHg and diastolic BP <90 mmHg, LDL-C <100 mg/dL, and HbA1c <7.5%.

Clinical Trial Registration Number: NCT01131676
Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance
Disclosure: **D. Fitchett:** Honorarium; Sanofi, Merck & Co., Amgen, AstraZeneca, Eli Lilly and Company and Boehringer Ingelheim.

OP 08 Insulin synthesis and turnover at the cellular level

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Longitudinal in vivo imaging of glucose-induced gene transcription in pancreatic beta cells

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Background and aims: Glucose induced gene-transcription represents an important functional parameter of the pancreatic β-cell. It was successfully analyzed in vitro using microscopic detection of fluorescent protein expression controlled by either the rat insulin 1 or the rat β-cell glucokinase gene promoter. To monitor this process in vivo longitudinally and non-invasively during the development of β-cell dysfunction could provide new insights into the etiology of type 2 diabetes. We therefore created an expression construct that facilitates in vivo imaging of glucose-induced gene transcription with cellular resolution. Furthermore, we wanted to study whether a high-fat high-sucrose diet (HFHSD) affected β-cell specific glucose-induced gene transcription.

Materials and methods: GFP under the control of a -278/+123 bp glucokinase (rβGKGFP) and DsRed2 under a -410 bp insulin promoter (rIns1DsRed) were combined with cerulean under the non-glucose responsive CMV promoter as a reference into a single adenoviral construct to enable expression of all three reporters in transduced β-cells. Transduced C57B/6J islets were transplanted into the anterior chamber of the eye of littermates. Islets were allowed to engraft for 4 weeks. In vivo monitoring of glucose induced reporter gene expression was performed after an overnight fast. Mice were anesthetized and cerulean-, GFP and DsRed2 fluorescence were imaged using confocal microscopy. The awake mouse was then injected with 2g/kg glucose (or saline), followed by a glucose tolerance test. 4h after injection, imaging was repeated with identical settings. GFP and DsRed2 fluorescence were normalized to cerulean fluorescence. The ratio of normalized GFP and DsRed2 fluorescence obtained before and 4 h after glucose stimulation was calculated as increase in normalized fluorescence intensity (INFI) and used as a measurement of glucose-induced gene transcription. Mice were put on either a control diet or on HFHSD (60% calories fat, 32% sucrose in drinking water) for 8 weeks. Imaging was performed after 4 and 8 weeks of diet and following a 4 week refeeding with normal chow. Glucose tolerance and bodyweight were measured during the imaging experiments. Additionally, insulin tolerance was determined during the diet treatment.

Results: Glucose, but not saline injection resulted in a significant increase of both rIns1DsRed (INFI=1.58±0.12, $p<0.001$ vs. saline) and rβGKGFP expression (1.54±0.08, $p<0.001$). Glucose induced gene transcription in control animals was detectable in vivo until the end of the 16 week experiment. Mice receiving the HFHSD for 8 weeks showed increased bodyweight and impaired glucose- and insulin tolerance. These animals also demonstrated reduced glucose-stimulated expression of both rIns1DsRed (HFHSD INFI=0.99 ±0.05 vs control 1.53±0.06, $p<0.001$) and rβGKGFP (HFDHSD 0.94±0.05 vs 1.48±0.05, $p<0.001$). All parameters were restored after 4 weeks of normal diet.

Conclusion: A construct expressing rIns1DsRed2, rβGKGFP and CMVCerulean enables non-invasive longitudinal in vivo monitoring of glucose stimulated gene transcription in pancreatic β-cells. This process is reversibly disturbed when the mice are overweight, glucose- and insulin-intolerant after receiving a high-fat high-sucrose diet for 8 weeks. Thus, using our in vivo imaging approach will allow us to determine key-events in β-cell failure also at the gene transcription level during the progression of type 2 diabetes.

Supported by: ERC, KI, VR, Novo Nordisk Foundation, Family Erling-Persson Foundation

Disclosure: **T. Moede:** None.

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Long-term live-cell imaging of endogenous Ins2 gene activity reveals distinct beta cell states

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Background and aims: For regenerative therapies targeting type 1 diabetes (T1D), it is important to understand islet cell heterogeneity and plasticity. To address these questions, we have begun to extend our previous studies using more sophisticated mouse models, including an *Ins2*^{GFP} knock-in/knockout line. Here, we leverage our live-cell imaging tools and expertise, as well as complementary fluorescent reporter technologies, to capture β-cell heterogeneity and transient state-switching events as they happen. Our aim was to catalogue and quantify state transitions of β-cell maturation, differentiation, dedifferentiation *in vitro* and *in vivo* as well as to screen for factors that can modulate these states.

Materials and methods: We have combined *Ins2*^{GFP} knock-in and *Ins1*^{mCherry}:*Ins2*$^{GFP/wt}$ genetically modified mouse alleles such that transcription at the endogenous *Ins2* locus can be recorded using ImageXpress^{MICRO} live-cell, high-throughput imaging systems. Multiple days live cell imaging of dispersed islets were complemented with FACS analysis, histology of pancreatic sections, and functional studies of insulin secretion using a perifusion system. cDNA libraries were prepared from FACS purified GFP-positive β-cells using the SmartSeq2 protocol.

Results: Analysis of pancreatic tissue sections from *Ins2*^{GFP} knock-in mice showed that, at any given time, only about half of all β-cells were robustly GFP-positive, suggesting that not all β-cells have active transcription at the *Ins2* locus. To determine whether states of high versus low *Ins2* gene activity were stable, we conducted long-term robotic imaging studies of dispersed primary islets. We added a transgenic allele wherein H2B-mCherry is driven by an *Ins1* promoter to image virtually all β-cells. In these 3 day long imaging studies, we were surprised to see GFP fluorescence flash on and off in a few individual β-cells, suggesting bursts of transcription at the *Ins2* gene locus. To the best of our knowledge, this represents a previously uncharacterized form of β-cell plasticity/heterogeneity. FACS analysis confirmed *Ins2* mRNA and pre-mRNA were increased in GFP-positive cells compared to negative cells (861.9 ±110.5 vs 97.2±16.3, 2.06±0.45 vs 1.01±0.04 respectively). RNAseq analysis on GFP-positive cells further elucidated the molecular features of this β-cell state. Interestingly, we observed that GFP-positive cells with active transcription at the endogenous *Ins2* locus were more susceptible to apoptosis under a broad range of conditions than islet cells lacking GFP (including both non-β-cells and β-cells without GFP). Expectedly, *in vitro* perifusion of islets isolated from *Ins2*$^{GFP/GFP}$ knock-in/knockout mice showed reduced insulin secretion at 20 mM glucose (AUC = 44.63 ±4.7) compared to heterozygous *Ins2*$^{GFP/Wt}$ knock-in/knockout mice (AUC = 82.5 ±6.3) and control *Ins2*$^{Wt/Wt}$ mice (AUC = 71.7 ±10.2).

Conclusion: Collectively, our results demonstrate the feasibility of long-term imaging of primary islet cells and establish *Ins2*^{GFP} knock-in mice as a new tool for studying β-cell heterogeneity, state transitions and plasticity. We will continue to combine this model with other tools including Gcg-Venus transgenic lines to examine transdifferentiation.

Supported by: JDRF

Disclosure: **H. Modi:** None.

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Glucotoxicity-induced upregulation of lincRNA GAS5 in the pancreatic islets of type 2 diabetes donors and in Goto-Kakizaki rats

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Background and aims: We have previously shown Growth Arrest-Specific 5 (GAS5) long intergenic non-coding RNA (lincRNA) to be abundantly expressed in human pancreatic islets. GAS5 is a known tumour-suppressor that is dysregulated in many types of cancer. However, the role of GAS5 in pancreatic islet dysfunction caused by hyperglycaemia is not known. Here, we aimed to determine whether the expression of GAS5 in the pancreatic islets is

regulated under glucotoxic conditions in diabetic donors and in the type-2 diabetes (T2D) model GK rats. We also aimed to dissect the glucose-dependent regulation of GAS5 using the insulin-secreting human beta cell line, EndoC-BH1.

Materials and methods: Human islets RNA-seq data (N=195) were used in global co-expression analysis and correlation of GAS5 expression with donor phenotypes. A separate cohort of 19 human islet preparations from donors with T2D (N=9) or controls (N=10) were used for qPCR validation. Isolated islets from Goto-Kakizaki rats (N=4) or Wistar controls (N=4) were also used. RNA or protein expression levels were determined by qPCR or western blot, respectively. The human beta cell line, EndoC-BH1 was incubated in 5 mM glucose or 20 mM glucose for 1 h, 6 h, or 24 h. An F-test for simple linear regression was employed to determine significant correlations among gene expression levels and versus donor phenotypes, e.g. HbA1c. Significant differences between two samples were determined using the Student's t-test or using ANOVA for multiple testing.

Results: Using RNA-seq data from 175 human islet samples, we found GAS5 expression to be positively correlated with the donor's long-term glycaemic indicator, HbA1c (F-test, $p<0.05$). We validated this result by qPCR using19 human islet samples (F-test, $p<0.001$). Furthermore, we found GAS5 expression to be elevated in the islets from T2D donors (N=9) than those from NGT donors (N=10) (Student's t-test, unpaired, two-sided, $p<0.01$). Co-expression analysis strengthened our previous findings regarding the significant negative correlations of *GAS5* with beta cell transcription factors *PDX1* and *NKX6-1*, and the exocytotic gene *SYT13*. In the GK islets, GAS5 was found to be ~50% upregulated compared to the controls (N=4, $p<0.05$), with profound downregulation of PDX1, NKX6-1 and SYT13 proteins. In the human EndoC-BH1 beta cell line, GAS5 was found to be ~50% more expressed in 20 mM glucose than in 5 mM glucose at 6 hours and 24 hours incubation (N=3, ANOVA, $p<0.05$).

Conclusion: The lincRNA GAS5 is highly upregulated under glucotoxic conditions in the islets from humans with T2D or in the GK rats. Moreover, upregulation of GAS5 in the human EndoC-BH1 cells during exposure to high glucose levels indicate potential involvement of GAS5 lincRNA in beta cell adaptations to hyperglycaemic environment.

Supported by: Swedish RC, T2DSys ERC, Diabetesfonden, Påhlssson, Crafoord, Bergvall

Disclosure: **J.L.S. Esguerra:** None.

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A novel method for the background-free purification of age-distinct insulin secretory granules for proteomic and lipidomic analyses

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Background and aims: The hormone insulin is stored in dense-core secretory granules (SGs) of pancreatic beta cells and is released in two distinct phases upon glucose stimulation. While the proximity of SGs to the plasma membrane (PM) likely influences their secretion probability, a temporal component is also important. In particular, upon glucose stimulation the beta cell favors the secretion of newly synthesized insulin SGs, or young SGs, over their older counterparts. Based on image analysis, we have previously found that younger SGs are more motile and their luminal pH more acidic relative to their older counterpart. However, the molecular changes accounting for the characteristic of age-distinct SG pools remain unknown. A plausible approach to identify potential mediators for the ageing of SGs is to investigate their proteomic and lipidomic composition. In order to achieve this ambitious goal, we report the development of the first protocol for the purification of age-distinct SGs.

Materials and methods: The SG-specific transmembrane protein phogrin was tagged with a cytosolic CLIP-tag to allow for the age-defined pulse-chase labeling of SGs. Specific antibodies for the CLIP substrates were used to immunopurify the labeled SGs. A protease cleavage site between the CLIP-tag and phogrin allowe the elution of intact SGs from magnetic beads.

Results: A stable INS-1 cell line for expression of phogrin-CLIP was established as a surrogate model of primary beta cells. Super-resolution microscopy validated the restricted localization of TMR-labeled phogrin-CLIP on insulin SGs. The effective immunoisolation of SGs with antibodies specific for the CLIP substrate TMR was confirmed by electron microscopy (EM) on the magnetic beads. EM analysis further validated the elution of intact SGs upon incubation of the beads with a protease specific for the linker region. SDS electrophoresis and silver staining of the SGs immunoisolated from TMR-labelled phogrin-CLIP INS-1 cells revealed a very distinct protein profile, while the corresponding control sample from non-labeled cells was virtually void of proteins, except for the protease. Further evidence for the high purity of the SGs eluted from the beads were obtained by western blotting, which showed the virtual lack of contaminating protein markers of other cellular compartments, such as ER, endosomes, lysosomes or the Golgi complex.

Conclusion: Here we report the first protocol for the immunoisolation of intact insulin SGs of distinct age, without significant contamination by other organelles. This approach shall enable proteomic and lipidomic analyses of age-distinct SG pools.

Supported by: BMBF-DZD, DIGS-BB

Disclosure: **M. Neukam:** None.

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Glucose but not KCl diminishes submembrane granule turnover in mouse beta cells

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Background and aims: The conventional view that insulin granules sequentially approach at the plasma membrane, then bind and finally fuse when the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) increases has been challenged by the recognition that granules continually arrive at and depart from the submembrane space. Here it was tested whether KCl depolarization elicits the same functional consequences on insulin secretion and granule turnover as high glucose.

Materials and methods: All parameters were measured in perifused single mouse beta-cells. $[Ca^{2+}]_i$ was measured by the Fura technique, the mobility of the granules in the submembrane space and the actin cytoskeleton were imaged by TIRF microscopy. Actin was visualized by mTagRFP-Lifeact, the insulin granules were visualized by two cargo-directed labels, namely insulin-EGFP and C-peptide-emGFP. The granule behavior, common to both, was used to compare the effect of sequential stimulation with 40 mM KCl and 30 mM glucose and sequential stimulation in reversed order. The same protocol was used to measure the insulin secretion of perifused cultured islets.

Results: At 32 °C but not at 37 °C, the control of $[Ca^{2+}]_i$ in single adenovirally transduced beta cells was unimpaired for the entire length (90 min) of continuous perifusion experiments. Thus the measurement of insulin secretion by perifused islets was performed at 37 °C and at 32 °C to ascertain the relevance of the single cell TIRF measurements. The sequential pulse protocol revealed clear differences between glucose and KCl depending on the order of application. KCl produced higher maximal secretion rates and diminished the response to the subsequent glucose stimulus whereas glucose enhanced the response to the subsequent KCl stimulus. The overall secretion rates were lower at 32 °C, but the pattern was the same and the differences between the stimuli were even more marked. To specifically measure the effect of the stimuli on the behaviour of insulin granules by TIRF microscopy net values were calculated by subtracting the values obtained during control perifusions (5 mM glucose throughout) from those of the test perifusions. At the level of granule behaviour a difference between glucose and KCl developed during the first stimulation phase in that the total number of granules, the short term resident granules, and the arriving granules, which are all parameters of granule turnover in the submembrane space, were significantly smaller for glucose. The frequency of exocytotic events

was not increased during the phases of stimulation. Single beta cells, as studied here, had a remarkably thick cortical actin web as compared with beta cells within clusters. Actin was particularly dense in the area on which the perifusion solution impinged.

Conclusion: Glucose and KCl did not only elicit different response patterns at the level of islet secretion but also at the level of insulin granule turnover in the submembrane space. This argues against the hypothesis that KCl elicits the same initial response mechanisms as glucose. Exocytotic rates of single primary beta cells may not reflect the secretion pattern of intact islets, possibly because of cytoskeletal reorganization after dissociation.

Supported by: DDG, DFG

Disclosure: **D. Brüning:** None.

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Modulation of endoplasmic reticulum/mitochondrial interface and mitochondrial dynamic by glucotoxicity in pancreatic beta cells

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Background and aims: Mitochondria network plays a pivotal role in insulin release in pancreatic beta cells by its ability to product ATP, and is controlled by calcium, a key regulator of fission/fusion event and oxidative energy production. Alterations of mitochondria and endoplasmic reticulum (ER) have been shown to contribute to metabolic disorders such as type 2 diabetes (TD2). To determine how glucotoxicity alters insulin secretion we focus on mitochondria fusion and fission dynamics and on ER/mitochondria interactions in studying MAMs (Mitochondria Associated endoplasmic reticulum Membrane) in beta cells. MAMs are conserved structures with crucial role for cell survival and death through the transfer of calcium, lipid and metabolite exchange. Increasing evidence in liver tissue indicates that dysfunctional MAMs can be an important precursor of disease in TD2.

Materials and methods: Human islets and INS-1 rat beta cells were exposed during 72h to moderate glucotoxicity (16.5 mM and 22.5mM Glucose). ER stress was measured by qPCR, mitochondrial respiration by oxygraphy and insulin secretion in response to glucose (GSIS) by ELISA. Mitochondrial and reticulum calcium contents were respectively analyzed using 4mtD3CPV and ERGAP1 probes, and mitochondrial dynamic using mitotracker Green. MAMs were quantified using the In situ Proximity Ligation Assay (PLA) between IP3R2 and VDAC1, respectively ER and mitochondria calcium channels implicated in MAM tethering. All statistical tests have been done with unpaired Mann-Whitney test and paired Student t-test.

Results: Chronic exposure of human islets and INS-1 cells to moderate glucotoxicity induced an increase in ATF4 and spliced-XBP1 mRNA expression ($P<0.01$) without modification of CHOP mRNA expression, an ER stress marker of apoptosis way, reflecting a mild ER stress as in TD2, and a decrease in ER calcium content ($P<0.0001$). A decrease in mitochondrial respiration ($p<0.0001$) and insulin secretion ($P<0.001$) was parallely observed. Mitochondria fusion/fission dynamics were disrupted in favor of a fission state ($P<0.0001$), in synergy with a decrease in mitochondrial calcium content ($P<0.0001$) in INS-1 cells. In the same conditions, we showed an up-regulation of the contacts between ER and mitochondria highlighted by the increase of IP3R2-VDAC1 interactions ($P<0.05$) in INS-1 cells.

Conclusion: We confirmed that glucotoxicity induces a metabolic stress in both ER and mitochondria in human islets and rat beta cells. The decrease in mitochondrial calcium content associated with mitochondrial fission were consistent with an alteration of beta cell function as respiration, calcium homeostasis and insulin secretion. Interestingly we observed for the first time an increase in ER/mitochondria tethering in beta cells in response to glucotoxic conditions. The modulation of these interactions could help us to clarify the role of MAMs in pancreatic beta cells and their dysfunction in TD2 and may open a new potential way of treatments in TD2.

Disclosure: **F. Dingreville:** None.

OP 09 Genetics of type 1 diabetes and MODY

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New insights into HLA in type 1 diabetes from population analysis: DR4 homozygosity specifically predisposes to type 1 diabetes after 30 years

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Background and aims: The major genetic determinants of Type 1 diabetes (TID) are the DR3, DR4 polymorphisms of class II HLA genes encoding DQ and DR. DR3/DR4 carriers show increased risk of T1D relative to DR3/DR3 and DR4/DR4 carriers in children and young adults but it is not known if this relative risk persists in individuals diagnosed with T1D later in life.

Materials and methods: We analysed the development of diabetes (aged 0-60 years) in subjects with highest risk HLA groups DR3/DR3, DR3/DR4 and DR4/DR4 (allele frequencies 2.4%, 2.9% 1.1%) in 120,192 UK individuals from the UK Biobank. We used the Exeter susceptibility-exclusion method with HLA group X/X being assumed to have no T1D.

Results: The highest risk genotypes represent 6.4% (7,676/120,192) of the population but contribute 61% (228/376) of all cases of T1D. In high risk HLA groups DR3/DR3, DR3/DR4 and DR4/DR4 there were marked differences in likelihood of developing T1D (1.2%, 4.2%, 3.5%, $p<0.001$) and in the mean age of diagnosis (17, 28, 38 years $p<0.001$). The majority 71% (32/45) of T1D cases associated with DR4/DR4 are diagnosed over 30 years of age unlike 26%(9/34) DR3/DR3 and 40%(59/149) DR3/DR4 $p<0.001$. (Fig.)

Conclusion: Whilst all three major genotypes greatly increase risk of T1D throughout life, population analysis has shown for the first time that DR4/DR4 specifically predisposes to T1D over 30 years of age and carriers of this genotype have the highest risk for development of late-onset T1D. This result is consistent with heterogeneity in age of diagnosis reflecting heterogeneity in immune mechanisms in T1D.

Figure 1

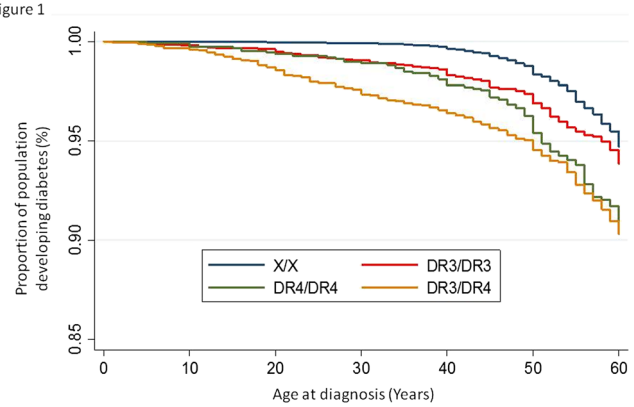

Figure 1:- DR4/DR4 predominately presents after 30 years of age
The majority 71% (32/45) of T1D cases associated with DR4/DR4 are diagnosed over 30 years of age unlike 26%(9/34) DR3/DR3 and 40%(59/149) DR3/DR4 ($p<0.001$).

Disclosure: **N.J.M. Thomas:** None.

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Whole-exome sequencing in rare families identified novel genetic variants for familial type 1 diabetes

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Background and aims: The prevalence of type 1 diabetes is much lower in Japanese than in populations of European descent (0.014% vs. 0.4%). The prevalence in siblings of proband with type 1 diabetes, however, is comparable between them (Japanese 3.8% vs. European 6.0%). Therefore, markedly high λs in Japanese was observed in comparison with European populations (271 vs. 15), indicating strong familial clustering and the involvement of rare genetic variants for the development of type 1 diabetes in Japanese population. Although very rare in Japan, we have previously reported two rare families clustered with type 1 diabetes. Present study aimed to identify the genetic causality of familial type 1 diabetes and study association of the identified variants with susceptibility to sporadic type 1 diabetes in Japanese population.

Materials and methods: Twenty one individuals from four families (family A to D: twelve patients with type 1 diabetes and nine unaffected individuals) were subjected to whole exome sequencing (Illumina HiSeq). After variants were detected, genome-wide linkage analysis was performed for all families to scan high LOD score region. Linkage was estimated using non-parametric linkage analysis assuming a dominant mode of inheritance. The exome variants were filtered on the basis of variant annotation, functional expectation, allele frequency and LOD score, to narrow down the candidate causal variants as exome variant analysis.

Results: All subjects except an unaffected individual of family B possess at least one of the susceptible HLA haplotypes (DR4, DR8 or DR9). Combined linkage analysis of four families identified maximal LOD score of 3.557 with single peak on chromosome 9, which was not overlapped with *GLIS3* locus on 9p24.2, suggesting a novel risk locus of familial type 1 diabetes specific to Japanese population. Two of non-synonymous variants of a gene X on the locus with high LOD score were shared by families A and B, suggesting the gene as a candidate causal gene for familial type 1 diabetes. To identify alternative causal variants specific to each family, exome variant analysis was also performed. As for family A, 29 rare variants from the total of 9,208 exome variants were co-segregated with affected patients with type 1 diabetes. Among these, a non-synonymous variant (SNV_04) with high damage scores (SIFT: 1.00, PolyPhen2: 0.999) was overlapped with genetic loci with high LOD score (max. LOD score 1.2 on chromosome 6) by linkage analysis for family A alone. Association study of the variant (SNV_04) revealed that the variants were absent in the European population (225 controls and 229 cases), but significantly associated with susceptibility to sporadic type 1 diabetes in the Japanese population (minor allele frequency, control: 0.7% vs. case: 6.1%, odds ratio [95%CI]: 9.6 [3.1-26.9], p<0.0003).

Conclusion: These data indicated that linkage analysis combined with whole exome sequencing is a useful tool to identify genetic variants linked to familial type 1 diabetes, particularly in low incidence populations.

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A type 1 diabetes genetic risk score predicts progression of islet autoimmunity and development of type 1 diabetes in individuals at risk

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Background and aims: Current prediction models for type 1 diabetes (T1D) include immunologic and metabolic markers but these change during disease progression and reflect advanced stages in the autoimmune process. Genetic associations to T1D are strong and time-independent. Genotyping can be affordably measured with single nucleotide polymorphism (SNP) analysis. We recently developed and validated a T1D genetic risk score (T1D GRS) that incorporates HLA and non-HLA T1D-associated SNPs. The T1D GRS is highly discriminative of T1D from controls, T2D and monogenic diabetes. We tested the ability of the T1D GRS to predict progression of islet autoimmunity and development of T1D in non-diabetic, autoantibody-positive (Ab+) individuals in the TrialNet Pathway To Prevention (PTP) study.

Materials and methods: We studied the 1244 Ab+ TrialNet PTP participants who were genotyped using the Illumina Immunochip SNP microarray. Median [range] age at Ab+ determination was 11.1 years [1.22-51.8], median follow up 5.5 years [0.2 to 11.9], 47.7% males, 80.5% non-Hispanic White. Participants were monitored with autoantibody testing, HbA1c and OGTTs at 6- or 12-month intervals depending on risk (e.g. single vs multiple Ab+). Of 291 participants with single Ab+ at screening, 156 progressed to multiple Ab+ and 55 developed T1D. Of 953 participants with multiple Ab+ at screening, 421 developed T1D. Time to progression was defined as time from Ab+ determination to T1D diagnosis (for multiple Ab+ subjects) or to development of new autoantibodies (for single confirmed Ab+ subjects). The T1D GRS was calculated as previously described. All results reflect analyses using multivariable Cox regression models, adjusting for age, sex and impaired OGTT at Ab+ determination. Recursive partitioning analyses were used to identify cutpoints.

Results: The T1D GRS ranged from 0.14 to 0.33 in Ab+ subjects (median=0.27). A score increase of 0.05 (e.g. from 0.225 to 0.275) significantly increased by 40% the risk of progression from single to multiple Ab+ (HR=1.4, 95% CI=1.04-1.88, p=0.028) as well as from multiple Ab+ to T1D (HR=1.4, 95% CI=1.11-1.74, p=0.004). Dichotomization of the T1D GRS also was significant in identifying those at greatest risk of conversion from single to multiple Ab+ (T1D GRS≥0.275, HR=1.68, 95% CI=1.21-2.34, p=0.002). Similarly, multiple Ab+ subjects with T1D GRS ≥0.25 were at significantly higher risk of progression to T1D (HR=1.69, 95% CI=1.2-2.4, p=0.004). A low T1D GRS was also effective at identifying subjects who were significantly less likely to progress to T1D (T1D GRS cutoff<0.226, HR 0.39, 95% CI=0.17-0.87, p=0.02).

Conclusion: The T1D GRS is an independent predictor of the risk and rate of progression of islet autoimmunity and development of T1D in non-diabetic, Ab+ individuals in TrialNet. These findings warrant further investigations on the use of the T1D GRS for early assessment of T1D risk, particularly in longitudinal studies.

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Disclosure: **M.J. Redondo:** None.

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A type 1 diabetes genetic risk score discriminates monogenic autoimmune diabetes from polygenic clustering of diabetes and autoimmunity

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Background and aims: Autoimmune diabetes can occur with other autoimmune disease in a patient as a result of either overlapping polygenic predisposition or due to monogenic autoimmunity**.** Diagnosing monogenic autoimmune diabetes is important as it will determine prognosis and optimal treatment. However differentiating monogenic autoimmune diabetes from polygenic type 1 diabetes (T1D) is difficult due to overlapping clinical features and biomarkers (islet autoantibodies). We aimed to test if a T1D genetic risk score (T1D-GRS) could assist the identification of patients with monogenic autoimmune diabetes.

Materials and methods: We studied 79 patients with young-onset diabetes (diagnosed ≤1 year without non-autoimmune monogenic diabetes) and ≥1 autoimmune disorder diagnosed <5 years. We measured the T1D-GRS (based on the top 10 HLA and non-HLA risk alleles) and sequenced all 7 genes known to cause diabetes and monogenic autoimmunity using targeted capture and next generation sequencing. The T1D-GRS centiles were calculated from 1963 T1D patients from the Wellcome Trust Case Control Consortium.

Results: A mutation in a known autoimmunity gene was identified in 47% (37/79) of individuals (25 FOXP3, 8 LRBA, 2 STAT3 and 2 IL2RA). Patients with known monogenic autoimmunity were diagnosed younger than patients were a monogenic cause was not found (median 5.5 v 36 wks, p<0.001). Other clinical features such as the number of additional autoimmune features, insulin dose and the proportion of islet autoantibody-positive patients (47% v. 48%,

p=1.00) were similar in both groups. In the confirmed monogenic autoimmune patients median T1D-GRS was markedly lower than the patients without a defined monogenic cause; 9th v 50th centile (p<0.001). The T1D-GRS was highly discriminatory for identifying those with monogenic autoimmunity (ROC-AUC 0.82 (95% CI 0.72-0.91)). 64% (29/45) patients with a T1D-GRS in the lowest quartile had monogenic autoimmunity v 0% (n=11) in the highest T1D-GRS quartile (p<0.001). 19% of patients without a monogenic cause of autoimmunity had a T1D-GRS below the 10th centile suggesting there are more causes of monogenic autoimmunity to be discovered.

Conclusion: We have shown that the polygenic predisposition to T1D does not determine if a patient with monogenic autoimmunity develops diabetes. The T1D-GRS is a strong predictor of whether monogenic autoimmune diabetes is likely in patients with young-onset diabetes and autoimmune disease and can be used to guide clinical testing. A low T1D-GRS will be used to prioritise patients with early-onset diabetes and autoimmunity for gene discovery studies.

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Disclosure: **M.B. Johnson:** None.

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Targeted next-generation sequencing demonstrates high frequency of monogenic forms in gestational diabetes

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Background and aims: Monogenic forms of diabetes can manifest during pregnancy and misclassified as type 1, type 2 or gestational diabetes (GDM). About 10% of cases of gestational diabetes are shown to be due to the most common forms of monogenic diabetes, such as glucokinase (*GCK*) and hepatocyte nuclear factor 1 alpha (*HNF1A*) defects. We suggest that using a next generation sequencing (NGS) approach may increase the mutation yield in GDM.To define molecular basis of GDM using a targeted NGS.

Materials and methods: 120 patients with GDM were studied according to the inclusion criteria. 'Diabetes panel' genes were sequenced using a custom Ion Ampliseq gene panel and PGM semiconductor sequencer (Ion Torrent). Bioinformatic analysis was carried out using Torrent Suite 4.2.1 and ANNOVAR software packages. Assessment of the pathogenicity of sequence variants was performed according to international guidelines.

Results: In 46 patients (38%) 45 different sequence variants (all heterozygous) were identified that were classified as pathogenic, likely pathogenic or variants of uncertain significance. The majority of variants were detected in *GCK* gene (27%, n=33), including missense mutations (n=27), deletions with frameshifts (n=2), deletions without frameshifts (n=1) and splicing mutations (n=1). Missense variants were also detected in *HNF4A* (n=1), *HNF1A* (n=1), *HNF1B* (n=1), *INSR* (n=3), *GLIS3* (n=2), *KCNJ11* (n=1), *PAX4* (n=1) and *PTF1* (n=2). Digenic or oligogenic variants were detected in 4 patients.

Conclusion: The results demonstrate high frequency of mutations in patients with GDM. The molecular findings were consistent with the phenotype only in the *GCK*/GDM cases. Other mutations/factors may also play a role in development of GDM.

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Modelling of RFX6 associated neonatal diabetes disease modelling using CRISPR/Cas9 genome edited patient derived iPSCs

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Background and aims: Neonatal diabetes mellitus is a monogenic disorder caused by mutations affecting genes involved in pancreatic development and β-cell functionality. One of those is the regulatory factor X 6 (*RFX6*), a transcription factor involved in pancreatic development and β-cell function. *Rfx6* knockout mice show intestinal malformations, hypoplastic pancreas and die soon after birth. Homozygous mutations in *RFX6* causes Mitchell-Riley syndrome (MRS), associated with developmental defects of the pancreas, biliary tract and intestine. The aim of this study was to understand early pancreatic developmental defects in this condition through the use of patient-derived iPS cells. iPSC were derived from a neonatal diabetic patient with MRS caused by a homozygous deletion of the *RFX6* gene (c.878_879delAC). The patient is born with neonatal diabetes and a hypoplastic pancreas as well as intestinal and biliary defects. C-peptide levels were not detected at birth. Although normal levels of elastase in stool indicated normal exocrine functions, the patient manifested chronic diarrhea. As a result, total parenteral nutrition (TPN) was started and still ongoing.

Materials and methods: The mutation was heterozygously corrected using CRISPR/Cas9 mediated genome editing. The mutant and corrected cells were differentiated into pancreatic endocrine lineage using a 6-stage protocol in parallel with iPSCs derived from a healthy donor.

Results: Analysis of the differentiation experiments at different time points showed that the *RFX6*$^{-/-}$ cells progressed similar to control cells until definitive endoderm stage. However, they failed to form confluent PDX1+/NKX6.1+ pancreatic epithelial cell layers in the pancreatic progenitor stage, as was observed in the control cells. The expression level of these critical pancreatic progenitor markers was dramatically decreased (*PDX1* 8-fold decrease, p<0.05; *NKX6.1* 10-fold decrease). *NEUROG3* expression decreased 8-fold at endocrine progenitor stage (p<0.01) in RFX6$^{-/-}$ cells. The *RFX6*$^{-/-}$ cells failed to show expression of endocrine hormones at the latest stages, except for scarce INS+ cells. *INS* expression levels were 52000-fold lower than *CTRL* or *corrected* cells (p<0.01). These differentiation defects were rescued by heterozygous correction of the mutation, including differentiation into all pancreatic endocrine cell lineages.

Conclusion: This study suggests a role for RFX6 already during pancreatic specification stage in humans, earlier to what has been reported in mice. Its expression is essential for pancreatic endocrine development. The genetic correction of only one allele was able to rescue the disease phenotype. Further study of the RFX6 mechanisms of action in pancreatic organogenesis and beta-cell function is currently ongoing using this model.

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OP 10 Non-insulin regulators of fuel metabolism

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Skeletal muscle-specific mitochondrial OxPhos defect mice are protected from obesity and insulin resistance by inducing GDF15 as a myomitokine

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Background and aims: Mitochondria are subject to dynamic processes in order to establish a control system related to survival or cell death and adaptation to changes in the metabolic environment of cells. Reduced mitochondrial OxPhos activity promotes longevity and improves energy homeostasis via cell-autonomous and cell-non-autonomous factors in multiple model systems. This mitohormetic effect is thought to involve the mitochondrial unfolded protein response (UPR^{mt}), an adaptive stress-response pathway activated by mitochondrial proteotoxic stress. To address how tissue-specific activation of the UPR^{mt} in situations of altered mitochondrial proteostasis with reduced OxPhos activity controls systemic energy homeostasis. We developed mice with skeletal muscle-specific Crif1 deficiency, and analyzed skeletal muscle function and metabolic phenotypes

Materials and methods: Skeletal muscle-specific Crif1 knockout (MKO) mice were generated by crossing $Crif1^{f/f}$ mice to transgenic mice expression the Cre under the myosin light chain (MLC) promoter. To identify mitochondrial dysfunction and metabolic phenotypes in MKO and WT mice, we evaluated OxPhos gene and protein expression, mitochondrial activity, mitochondria morphology, metabolic parameters, H&E tissue staining and molecular markers in NCD or HFD conditions.

Results: We show that skeletal muscle-specific deficiency of Crif1, a mitochondrial protein in the large mitoribosomal subunit is sufficient to activate the UPR^{mt} and to produce cell non-autonomous signaling factors, or myomitokines, that regulate systemic energy homeostasis. We identify growth differentiation factor 15 (GDF15), a divergent member of the transforming growth factor family, a critical cell non-autonomous factor enhancing oxidative function in liver and skeletal muscle, and lipolytic action in adipose tissues. Therefore, we propose that increased production of GDF15 in MKO mice was responsible for the marked decrease of fat mass, resistance to obesity, and improved insulin sensitivity. In line with this hypothesis, exogenous administration of GDF15 to ob/ob mice promotes weight loss and enhances insulin sensitivity.

Conclusion: This provides robust evidence for the cell non-autonomous factor GDF15 as a regulator of systemic energy homeostasis, and as a potentially useful therapeutic agent in the treatment of obesity-associated insulin resistance.

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Disclosure: **H. Chung:** None.

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Regulation and functional impact of miRNAs in alpha cells during type 2 diabetes

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Background and aims: miRNAs are small non-coding RNAs of 21 to 23 nucleotides regulating the expression of target genes by inhibiting translation or by inducing mRNA degradation. The expression of miRNAs themselves, like mRNAs, is submitted to both transcription factor and epigenetic regulations. miRNAs are found in various genomic locations and are often observed to be clustered. Several studies give evidence of the implication of miRNAs on glucose homeostasis. Although most of the studies on miRNAs have been performed in beta-cells, it was suggested that miRNAs also play a role in alpha-cell. The aim of this study is to identify miRNAs regulated in alpha-cell during type 2 diabetes as well as their function.

Materials and methods: We used mice, expressing the Venus fluorescent protein under the control of the rat glucagon promoter as well as the Cherry fluorescent protein under the control of the rat insulin promoter, fed with a control low (10%) fat diet or a high (60%) fat diet during 16 weeks to generate obesity and hyperglycemia. miRNA profiling was performed through a microarray analysis on FACS-purified alpha and beta-cells of obese hyperglycemic and control mice. Selected dysregulated miRNAs were mimicked or inhibited in primary culture of rat alpha-cells to evaluate their molecular and functional impact in alpha-cells.

Results: We identified 16 miRNAs significantly regulated in alpha-cells and 28 in beta-cells from hyperglycemic obese mice. Among the 16 miRNAs regulated in alpha-cells, 14 are down-regulated and 2 are up-regulated. Interestingly, 8 of the down-regulated miRNAs in alpha cells are localized in a common genomic cluster. Moreover 125 miRNAs were differentially expressed between alpha and beta-cells. The analysis showed a significant interaction between cell type and diet for 14 miRNAs. We focused then our analysis on miR-132-3p, the most regulated miRNA in diabetic alpha-cells with a 2.24 fold decrease (adj P.Val=6.9 10^{-4}), which was also regulated in an opposite manner in corresponding beta-cells (1.67 Fold increase, adj P.Val=1.3 10^{-2}). *In vitro* inhibition of miR-132-3p in primary alpha-cells led to an increase of mRNA levels of specific genes previously identified in diabetic alpha-cells, among which *Gcg*, *Foxa1*, *Cacna1C* and *Pcsk1*. Interestingly, mimicking of miR-132-3p in the same conditions was correlated to decreased expression of these genes, suggesting that part of the diabetic molecular footprint of alpha-cells may be due to the regulation of miR-132-3p expression. We did not observe significant modification of glucagon content and release 48h after transfection with miR-132-3p mimics and inhibitors.

Conclusion: Several miRNAs in pure populations of alpha and beta-cells are regulated by high fat diet and hyperglycemia. As described for beta-cells, miR-132-3p seems to have a functional impact on alpha-cells, at least on their molecular identity. The mechanism implicated in this effect remains to be identified.

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Pericentrin regulates insulin secretion and relates closely with the development of impaired glucose tolerance

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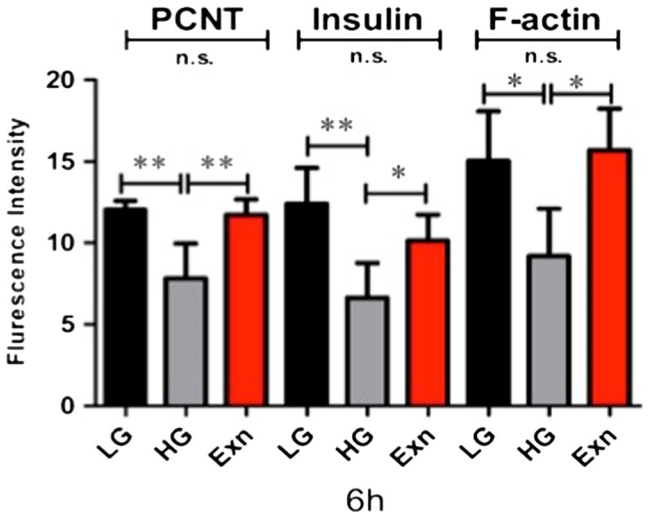
Background and aims: Diabetes has become a serious threat to human health, how to make the β cells secreting insulin on-demand becomes a hot spot. Pericentrin (PCNT), a component of the pericentriolar material, was found positively relating with intracellular insulin expression in our former research. This study further investigated the effect and mechanism of PCNT on insulin secretion and its role in the development of impaired glucose tolerance.

Materials and methods: Two kinds of mouse models and MIN-6 cells were used. Mouse model of PCNT reduction specifically in β cells (ΔPCNTβ) was built by Tet-on induction system; impaired glucose tolerance model (IR) was built by high-glucose high-fat feeding (HG-HF). Glucose tolerance, insulin secretion, and the distribution of intracellular insulin granules, pancreas PCNT, insulin and F-actin expressions were compared among control, ΔPCNTβ and IR mice at baseline, 4 weeks, 12 weeks by intraperitoneal glucose injection (IPGTT), WB, IF, IHC, ELISA and transmission electron microscope. MIN-6 cells were divided into control, exenatide group (Exn, 100 nm) and repaglinide group (Reg, 50 nm); control, Si-PCNT group and Si+Exn group. PCNT, Insulin, F-actin expressions were observed at baseline, 15 min, 30 min, 2 h and 6 h.

Results: (1) Compared with control, ΔPCNTβ had similar glucose level, but higher fasting insulin level at baseline. While after IPGTT, ΔPCNTβ showed

higher blood glucose area under the curve ($AUC_{Glu0-15}$) ($P < 0.01$) and lower insulin area under the curve ($AUC_{INS0-15}$) ($P < 0.05$) at 0-15 min. There was no difference at 30-120 min in IGPTT. Lower F-actin level, and reduced insulin granules of rapid release pool and anchoring pool (0-300 nm under the cyto-membrane) were detected in ΔPCNTβ mice ($P < 0.01$). (2) The PCNT expression decreased in IR group with the extension of HG-HF feeding. At 4 weeks, compared with control, PCNT expression of IR mice reduced ($P < 0.05$) and AUCs increased ($P < 0.05$ and $P < 0.01$) significantly; while ΔPCNTβ had further lower PCNT and higher AUCs than IR. At 12 weeks, the PCNT expression of IR mice dropped to that of ΔPCNTβ mice level, and there were no difference in AUCs between these 2 groups. (3) Compared with control, the PCNT, insulin and F-actin expressions of MIN-6 cells in Exn group decreased obviously at 2 h, while dramatically increased after 6 h (Figure). No difference could be found between Rep mice and control. Exenatide could not change the insulin and F-actin expressions of Si-PCNT group.

Conclusion: PCNT could adjust the first phase insulin release by regulating granules of the rapid release and anchor pools through F-actin. Decreased PCNT plays an important role in the process of impaired glucose tolerance induced by HG-HF feeding. GLP-1 has two-way regulating effect on beta cells at early stage (< 2 h) and late stage (> 6 h) through PCNT.



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Effects of nocturnal rise in cortisol on gluconeogenesis and glycogenolysis in type 2 diabetes

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Background and aims: We have previously shown that nocturnal glucose concentrations and rates of endogenous glucose production (EGP) correlate with higher overnight cortisol concentrations in individuals with type 2 diabetes (T2D). However the contribution of gluconeogenesis (GNG) and glycogenolysis (GGL) to EGP in response to physiological rise in cortisol and the extent to which this is altered in T2D in the overnight fasted state has not been investigated.

Materials and methods: To do so, we utilized the deuterated water method in combination with isotope dilution technique using [3-^{3}H] glucose to compare EGP in T2D and controls (ND) in the presence vs. absence of a nocturnal rise in cortisol. 17 ND (9M, age 59 ± 8 yr, HbA1c 35 ± 2 mmol/mol) and 16 T2D (9M, age 62 ± 8 yr, HbA1c 59 ± 12 mmol/mol) matched for BMI were studied on two occasions in random order. Subjects were admitted to the Clinical Research Trials Unit at our clinic for overnight stays. Subjects with sleep apnea were excluded from participation. Endogenous cortisol was blocked with metyrapone 500 mg given orally every 4 hours from 1800 to 0600 starting on the evening of each visit. At 1800, 2000 and 2200 subjects ingested 1.7 g/total body weight deuterated water for estimation of fractional GNG. At 2200, hydrocortisone infusion was started at 0.15 μg/kg/min and continued until 0700 on one occasion; on the other occasion infusion rates were sequentially changed (0.3 μg/kg/min from 2200 to 2400,0.6 μg/kg/min from 2400 to 0400,1.0 μg/kg/min from 0400 to 0700) to mimic physiological nocturnal rise in cortisol. [3-^{3}H] glucose was infused starting at 2200 until 0700. Blood was sampled from an arterial catheter.

Results: Data are presented in the table. Fasting glucose, glucagon and insulin concentrations were not different between visits in either group but glucose concentrations were higher in T2D than in ND. Cortisol concentrations by design and rates of EGP at 0700 were higher in the presence than absence of a nocturnal rise in cortisol in both ND and T2D. GNG was significantly higher in ND and T2D at 0700 and higher in the latter irrespective of cortisol concentrations achieved. On the other hand, whereas GGL also was higher in the presence than in the absence of a nocturnal rise in cortisol in T2D, it did not differ in ND.

Conclusion: Higher GNG and GGL in response to high cortisol contribute to the physiological nocturnal rise in EGP observed in T2D; the relative contribution of GNG is higher than that observed for GGL. Further studies are required to test whether medications that block cortisol and/or GNG are clinically relevant in reducing EGP in T2D.

	Diabetes Status	Low Cortisol 7 AM	High Cortisol 7 AM	7 AM p-value Low vs High
Glucose	ND	5.1 ± 0.4	5.7 ± 0.4	p < 0.001
(mmol/L)	T2D	11.7 ± 3.6	12.9 ± 3.6	p < 0.002
		(p < 0.001)	(p < 0.001)	
Cortisol	ND	7.2 ± 1.6	19.0 ± 4.2	p < 0.001
(μg/dl)	T2D	6.9 ± 1.2	17.4 ± 2.9	p < 0.001
		(p = 0.56)	(p = 0.20)	
Insulin	ND	43.7 ± 18.2	52.4 ± 26.9	0.09
(pM)	T2D	41.1 ± 15.0	43.9 ± 14.9	0.19
		(p = 0.66)	(p = 0.26)	
Glucagon	ND	85.9 ± 21.6	84.2 ± 26.2	0.52
(pg/ml)	T2D	86.8 ± 17.7	92.1 ± 18.7	0.09
		(p = 0.90)	(p = 0.33)	
EGP	ND	12.2 ± 2.6	13.5 ± 1.8	p < 0.01
(μmol/kgFFM/min)	T2D	17.0 ± 3.8	20.6 ± 4.8	p < 0.001
		(p < 0.001)	(p < 0.001)	
Gluconeogenesis	ND	7.7 ± 1.7	9.2 ± 1.3	p < 0.001
(μmol/kgFFM/min)	T2D	11.5 ± 2.7	13.3 ± 3.1	p < 0.001
		(p < 0.001)	(p < 0.001)	
Glycogenolysis	ND	4.5 ± 1.5	4.2 ± 1.4	0.52
(μmol/kgFFM/min)	T2D	5.5 ± 1.6	7.2 ± 2.0	p < 0.001
		(p = 0.07)	(p < 0.001)	

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Glucagon secretion in type 2 diabetes: results of the pilot clinical trial "Low dosE GlibENclamide in Diabetes - part A (LEGEND-A)"

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Background and aims: Fasting hyperglucagonaemia is a feature of type 2 diabetes (T2DM), though the mechanism by which it occurs and its metabolic consequences are not clear. Previous *in vitro* studies using islets from T2DM donors have recapitulated this phenomenon, and demonstrated that low concentrations of the sulphonylurea tolbutamide could normalise glucagon secretion. We designed a clinical trial to assess the impact low-dose sulphonylureas would have on patients with T2DM.

Materials and methods: In this open-label, dose-titration trial, patients with T2DM (either diet-controlled or on metformin) received increasing doses of an oral suspension of glibenclamide. The dosing schedule ranged from 0.3mg to 6mg per day (split between morning and evening), and the dose was increased every 3-4 days. Two early morning baseline fasting blood samples were taken prior to starting glibenclamide, and further fasting samples were taken prior to each dose change. A subset of participants also agreed to wear a continuous glucose monitor (CGM) throughout the study. The primary objective was to identify the dose of glibenclamide which resulted in a significant decrease in fasting glucagon concentration. Samples were also assayed for c-peptide and glucose. The results were analysed using two-way repeated measures ANOVA, grouped into either normal (N) or high (H) depending on baseline glucagon levels. Statistical significance was set at $p<0.05$.

Results: A total of 16 patients (7 men, 9 women) were recruited, whose characteristics were as follows (median ±interquartile range): age 66 (±17), HbA1c at screening 51mmol/mol (±8), BMI 30 (±7.3), and 10 participants were on metformin (average dose 1g/day). CGM data was collected from 11 patients during the study. Four patients had high fasting (group H) glucagon levels at baseline (mean 25.6 pmol/L, ±SEM 2.1), while 12 had baseline levels (group N) within the normal fasting range (mean 6.1 pmol/L, ±SEM 1.2). There was a significant reduction in fasting glucagon secretion in group H at a glibenclamide dose of 0.3mg/day (17.4 pmol/L ± 2.1), which did not occur at higher doses (0.6mg - 6mg), nor was there a change in glucagon levels in group N. There was no significant difference or change in fasting c-peptide levels between groups at any dose, however there was significant reduction in fasting glucose in group H at 6mg/day (change from baseline = 1.5mmol/L) and in group N at 1.8mg/day (0.9mmol/L), 3mg/day (1.2mmol/L), and 6mg/day (1.5mmol/L). Hypoglycaemia episodes were also reported as adverse events over this dose range. CGM data showed a significant reduction in mean amplitude of glycaemic excursion (MAGE) at 3mg and 6mg glibenclamide (difference of means 2.5 and 1.6 respectively), however this was associated with a 16% increase ($p<0.05$) in hypoglycaemic-range recordings at the highest dose.

Conclusion: This pilot clinical trial is the first to use such low doses of sulphonylurea in patients with T2DM, and indicates that $1/20^{th}$ the normal starting dose of glibenclamide can significantly reduce fasting hyperglucagonaemia by 30%, without having an effect on insulin secretion nor causing hypoglycaemia. Whether this reduction in glucagon has a beneficial impact on glucose control over a longer period of time remains to be investigated.

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Disclosure: **I.I. Spiliotis:** None.

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Membrane estrogen receptor alpha signalling contributes to the prevention of diet-induced obesity and insulin resistance through enhanced energy expenditure

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Background and aims: Clinical and experimental observations demonstrated that the beneficial influence of estrogens on energy balance and glucose metabolism is mediated by estrogen receptor α (ERα). These metabolic effects absolutely require the transcriptional actions of ERα, mainly mediated by its activation function (AF)-2. However, beside these classical nuclear actions, a palmitoylated ERα sub-population located at the plasma membrane has been shown to elicit Membrane Initiated Steroid Signaling (MISS) which could also contributes to the metabolic influence of estrogens. This study thus aimed to characterize the involvement of ERα MISS in the prevention of obesity and metabolic disorders by estrogens thanks to a recently generated transgenic mouse model harbouring a point mutation at the palmitoylation site of ERα (C451A-ERα) that confers a membrane-specific loss of function of the receptor.

Materials and methods: We analyzed several metabolic parameters (body weight, body composition, glucose tolerance, insulin sensitivity using hyperinsulinemic-euglycemic clamps, energy expenditure, thermoregulation in response to cold exposure) in C451A-ERα and their wild-type littermates (WT-ERα) female mice fed with a normal chow diet (NCD) or a high-fat diet (HFD). In some experiments, mice were ovariectomized and supplemented or not with 17β-estradiol (E2).

Results: Although almost no differences were observed in mice maintained on a NCD, HFD-fed C451A-ERα female mice developed accelerated obesity, adiposity and liver steatosis, and were characterized by a significant insulin resistance (hyperinsulinemic euglycemic clamp) and glucose intolerance, as compared to their wild type littermates (WT-ERα). Physiological E2 supplementation prevented WT-ERα controls from obesity and hyperglycemia but failed to protect C451A-ERα ovariectomized mice. Finally, abrogation of MISS-ERα signaling did not alter food intake and physical activity level but indirect calorimetry revealed a significant decrease in energy expenditure in HFD-fed C451A-ERα females. Accordingly, brown adipose tissue dysfunctions (significant decrease in UCP1 and PGC1a expression) and altered thermogenesis adaptation were observed in HFD-fed C451A-ERα mice.

Conclusion: The present data demonstrate that, in addition to the previously demonstrated role of ERα nuclear actions, MISS ERα is required for the protective effects of estrogens against HFD-induced obesity, insulin resistance and impaired glucose tolerance. This work thus provides important insights into the metabolic actions of estrogens and will open new perspectives to design optimized strategies for ERα selective modulation.

Disclosure: **P. Gourdy:** None.

OP 11 What can we learn from animal and in vivo models of cardiorenal complications?

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SNogo-B overexpression ameliorates diabetic glomerulopathy

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Background and aims: Endothelial dysfunction is a mechanism for chronic vascular complications in diabetes. Diabetic glomerulopathy (DG) is characterized by defects in glomerular capillaries, resulting in altered vascular remodelling, increased vascular permeability and albuminuria. Neurite Outgrowth Inhibitor (Nogo)-B and its soluble form (sNogo-B) have been implicated in the regulation of cell survival, cell migration and vascular remodelling. Both Nogo-B (at the cellular level) and sNogo-B (systemically) bind to NgBR receptor (expressed mainly in endothelial cells), and promote vascular remodelling. Full-length Nogo-B is expressed in glomerular endothelial cells and podocytes and is downregulated in in vitro and in vivo experimental model of diabetic nephropathy. Our aims were to investigate whether overexpression of sNogo-B could ameliorate DG in an experimental animal model of diabetes.

Materials and methods: Eight to ten weeks-old male DBA/2J mice were made diabetic (streptozotocin/low-dose), and administered (12-14 week-old) with an adeno-associated viral vector (AAV) expressing sNogoB or GFP (control vector). Animals were divided into four groups: non-diabetic (ND) and diabetic (D) mice treated with either control vector (GFP-AAV), or vector over expressing sNogo-B (sNogo-B-AAV). Mice were followed for 12-14 weeks of diabetes. Blood pressure was assessed with tail-cuff methodology. Plasma sNogo-B levels were analyzed by ELISA, full-length Nogo-B levels, AKT levels and phosphorylation in kidney cortex lysate with immunoblotting, creatinine by mass spectrometry and albuminuria by fluorescence. Glomerular ultrastructural morphology was assessed with electron microscopy.

Results: Treatment with sNogo-B-AAV allowed a sustained expression of the transgene (sNogoB) in the circulation (GFP vs sNogo-B in both ND and D mice, $p<0.05$). Albuminuria was increased by diabetes in both GFP and sNogo-B AAV mice ($p<0.01$). sNogo-B upregulation ameliorated diabetes-mediated albuminuria (D GFP-AAV vs D sNogo-B-AAV, $p=0.04$). Similarly, diabetes-mediated increase in creatinine clearance was corrected by sNogo-B overexpression to control ND mice levels ($p<0.006$). Preliminary blood pressure determinations showed a similar systolic and diastolic pressure in ND GFP and sNogo-B AAV mice, conversely blood pressure in D animals was lower in sNogo-B mice when compared to GFP ones ($p<0.04$); diabetes was paralleled by a fall in blood pressure observed only in sNogo-B AAV mice ($p<0.04$). Diabetes-mediated increase in mesangial expansion was also ameliorated by sNogo-B overexpression ($p<0.05$). sNogo-B overexpression prevented the diabetes-mediated full-length Nogo-B downregulation ($p=0.02$), and blunted diabetes-mediated Akt-Serine phosphorylation ($p=0.01$). Total Akt protein levels were downregulated in diabetes ($p<0.01$), but not affected by sNogo-B overexpression.

Conclusion: Overexpression of sNogo-B ameliorates DG possibly via amelioration of the diabetes-mediated glomerular haemodynamic perturbations. sNogo-B could represent a potential novel future treatment for DG.

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Disclosure: **I.P. Hernandez-Diaz:** None.

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Glyoxalase-1-deficient mice show only mildly enhanced deficits in methylglyoxal detoxification under control or streptozotocin-treated conditions

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Background and aims: The pathogenesis of diabetic long-term complications involves the accumulation of reactive metabolites, such as the dicarbonyl methylglyoxal (MG). The rate-limiting enzyme for MG detoxification is glyoxalase1 (Glo1) and it has been shown that Glo1 knockdown mice, generated by injecting shRNA-expressing lentiviral particles in mouse zygotes, exhibit signs of diabetic nephropathy comparable to streptozotocin (STZ)-diabetic wildtype (WT) mice, supporting the hypothesis of MG accumulation as a leading cause of diabetic late complications, in particular nephropathy. In this study, MG metabolism in the kidneys of mice homozygous for a Glo1 null allele were analyzed and the consequences of a complete Glo1 inactivation under STZ-evoked hyperglycemia was studied.

Materials and methods: Glo1-deficient mice were generated using CRISPR/Cas9-mediated genome editing by injecting mRNA encoding Cas9 and a sgRNA targeting exon 1 of the mouse Glo1 gene in C57Bl6/N mouse zygotes. Adult male Glo1-deficient and WT controls (n=12) were injected i.p. with either citrate or streptozotocin (60mg/kg) for five consecutive days. Following the establishment of robust hyperglycemia, the mice were maintained in the diabetic condition for four months. Glomerular filtration rate (GFR) was assessed using FITC-labeled sinistrin as a surrogate parameter for diabetic nephropathy, and MG and MG-H1 was determined by LC-MS/MS.

Results: 17 out of 19 (90%) founder mice showed successful genome editing. Mice with an allele exhibiting a 8 bp deletion ($\Delta 8$) at the 3' end of exon 1 were mated with C57Bl6/N and intercrossed to obtain $Glo1^{\Delta 8/\Delta 8}$ homozygous mice. Western Blot and qPCR analysis together with measurements of Glo1 enzyme activity in several organs verified the $Glo1^{\Delta 8/\Delta 8}$ allele as a *null* allele. $Glo1^{\Delta 8/\Delta 8}$ ($Glo1^{-/-}$) mice appeared normal and were born according to mendelian ratios. STZ-diabetic $Glo1^{-/-}$ mice showed little or no change in either insulin or glucose tolerance and glucose tolerance tests compared to STZ-diabetic WT mice. No differences in hyperfiltration were observed in GFR measurements between the WT and $Glo1^{-/-}$ STZ-diabetic mice. Plasma MG levels were found to be elevated in the STZ-diabetic mice, however no differences were observed between the different genotypes. No differences in the levels of MG-derived hydroimidazolone (MG-H1) were found in total kidney lysates from the mice. The activity of AKR was found to be increased ca.2-fold in the kidney of $Glo1^{-/-}$ mice as compared to WT (p0.001), although this cannot be explained by a corresponding increase in the expression of AKR (AKR1b3, AKR1a1).

Conclusion: In contrast to shRNA-mediated Glo1 knockdown mice, $Glo1^{\Delta 8/\Delta 8}$ KO mice show no elevation of MG-H1 content in kidneys neither in the citrate nor the STZ group. Also hyperglycemia-evoked hyperfiltration was unaltered in Glo1-deficient mice. MG detoxification in the kidney might occur by an increase in AKR activity upon increased MG load, as have been reported in Glo1-deficient Schwann cells.

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Disclosure: **D. Schumacher:** None.

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Adipocyte Fatty Acid Binding Protein (A-FABP) is a pathological mediator of obesity-related renal dysfunction

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Background and aims: Chronic kidney disease (CKD) is an emerging global public health problem which is characterized by irreversible deterioration of kidney function and can gradually progress to end-stage renal disease (ESRD). Obesity is the major risk factor of type 2 diabetes, hypertension and cardiovascular disease which are shown to promote CKD. Obesity is also an independent risk factor for the development and progression of this disease. Adipocyte fatty acid binding protein (A-FABP) is an adipokine expressed in

adipocytes, macrophages and endothelial cells. Its circulating level is increased in obese patients and is closely associated with various obesity-related cardiometabolic diseases. Emerging clinical evidences suggest that A-FABP is implicated in renal dysfunction. Our clinical findings also showed that circulating levels of A-FABP are independently associated with nephropathy staging and macrovascular complications of type 2 diabetic patients. However, the underlying mechanism whereby A-FABP mediates renal dysfunction is so far not explored. Here we investigated the pathological role of A-FABP in the development of renal dysfunction associating with obesity.

Materials and methods: A-FABP knockout (KO) mice and their wildtype (WT) littermates were fed with either standard chow (STC) or high fat high cholesterol (HFHC) diet for 20 weeks. Another batch of WT mice was fed with HFHC diet in the presence of pharmacological inhibitor of A-FABP, BMS309403 (BMS). Various serum and urine biochemical parameters, morphological change and lipid accumulation in kidney were determined.

Results: HFHC-diet feeding increased the circulating and renal expression of A-FABP in WT mice. Pharmacological inhibition of A-FABP by treatment with BMS not only significantly attenuated HFHC diet-induced hyperglycemia and hyperinsulinemia but also alleviated renomegaly in WT mice which was accompanied by reduced glomerulus volume, glycogen deposition and fibrotic area. Treatment of BMS markedly reduced diet-induced urinary albumin level comparing to respective controls. HFHC diet-induced renal lipid accumulation was remarkably attenuated in A-FABP KO mice as indicated by reduced oil droplets and diminished renal triglyceride levels when compared to respective WT controls. A-FABP deficiency also protects against the development of diet-induced hypertension in mice. The expression of A-FABP was significantly increased in the renal sections of HFHC diet-induced WT mice comparing with that of the STC-fed mice and was co-localized with endothelial cell marker CD31. In addition, treatment of palmitate significantly induced the expression of A-FABP in the human umbilical vein endothelial cells (HUVECs) compared to the vehicle-treated control.

Conclusion: Genetically ablation and pharmacological inhibition of A-FABP protect mice against HFHC-diet induced nephropathy. A-FABP as a lipid chaperone, may be a pathological mediator of obesity-related renal dysfunction at least partially through enhancing lipid transportation into the renal tissues.

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Disclosure: **R.L.C. Hoo:** None.

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Adult stem/progenitor cells as a personalised treatment for peripheral vascular disease

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Background and aims: Peripheral vascular disease in people with diabetes affects principally medium and small caliber vessels which are less amenable to surgical treatment Stem/progenitor cell (SPCs) treatments with bone-marrow-derived cells show promising outcomes in treating vascular diseases, albeit not without adverse events related to cell mobilization and collection. BGC101 is a preparation of enriched endothelial progenitor cells (EnEPCs) generated from peripheral blood using a novel one day technology employing dendritic cells (DCs) to specifically direct potentially therapeutic stem/progenitor cell (SPC) activity in-vitro. Previous animal studies have shown promising results in reversing induced limb ischemia. We describe a "first in man" pilot study of BGC101 for treating patients with severe peripheral vascular disease with no surgical option available.

Materials and methods: We performed a pilot open-label study of treatment with BGC101 in 5 patients, 3 with diabetes, with critical limb ischemia (CLI) characterized by rest pain and/or ulceration, and with no surgical option for revascularization. The primary end-point was assessment of safety, with efficacy as a secondary end-point. BGC101 was prepared from 250ml of peripheral blood. Co-culture of activated DCs for 12-18 hours with SPCs from the same patient sample generated 83.7±7.4×10^6 BGC101 cells with 97% viability, comprising 52.4±2.5% EPCs. Treatment consisted of a single treatment session with 30 intramuscular injections of BGC101 into the gastrocnemius muscle of the diseased leg, with acute safety follow-up during the initial 24±6 hours at the hospital and long-term follow-up at 1 week and at 1, 3, and 6 months.

Results: Preliminary safety data of several months follow-up showed that the therapy was well tolerated. A total of 29 adverse effects (AEs) reported by the 5 patients were typical to CLI disease and happened or could have happen regardless of the therapy. Indeed, 18 of them were judged as being unrelated or unlikely related to therapy, including one episode of hospitalization due to leg infection. Of the remaining 11 AEs, 8 were defined as possibly related, 2 probably related and 1 related. One of these, moderate necrosis of toes, recovered spontaneously without any further treatment. Preliminary efficacy results showed leg and toe salvage and substantial improvements for the patients comprising increased blood flow, enhanced wound healing, reduced pain and recovery of walking ability.

Conclusion: These observations indicate that the cell product BGC101 of EPC-enriched SPCs, generated by a novel culture process taking less than one day, shows promising preliminary results in the treatment of CLI and may also be therapeutic in other vascular conditions.

Clinical Trial Registration Number: NCT02805023

Disclosure: **M.J. Niven:** None.

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Liraglutide inhibits vascular smooth muscle cell proliferation by enhancing p-AMPK and cell cycle regulation, and delays atherosclerosis in APO-E deficient mice

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Background and aims: Several studies have demonstrated that both native glucagon-like peptide-1 (GLP-1) and GLP-1 receptor agonists suppress the progression of atherosclerosis in animal models. Recently, the liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results (LEADER) trial showed a striking reduction in the relative risk of cardiovascular (CV) mortality and all cause death among patients with type 2 diabetes and high CV risk on liraglutide therapy. Then, we investigated whether liraglutide, a GLP-1 analogue, could prevent the development of atherosclerosis in apolipoprotein E knockout mice ($ApoE^{-/-}$) on a high-fat diet. We also examined the influence of liraglutide on angiotensin II-induced proliferation of rat vascular smooth muscle cells (VSMCs) via enhancement of AMP-activated protein kinase (AMPK) signaling and regulation of cell cycle progression.

Materials and methods: $ApoE^{-/-}$ KO mice aged 5weeks and control C57/BL6 mice of the same age were used in this study. We divided total 23 mice into 4 groups ; control, vehicle, liraglutie150ug/day, and liraglutide400ug/day. Control mice were fed with normal chow. $ApoE^{-/-}$ KO mice were fed with high-fat diet for 4 weeks. These isolated aorta and aorta-ring section of each groups were measured for plaque lesion of oil red staining, hematoxylin and eosin staining and immunohistochemistry for AMPK phosphorylation. In an ex-vivo study, we performed the measurement of endothelium-dependent vascular reactivity on each groups. In a vivo study, we used rat vascular smooth muscle cells(VSMCs). Western blot and flow cytometry were performed by standard procedure methods. Measurement of cell proliferation was evaluated by xcelligence systems.

Results: Treatment of Apo-$E^{-/-}$ mice with liraglutide (400 μg/day for 4 weeks) suppressed atherosclerotic lesions and increased AMPK phosphorylation in the aortic wall. liraglutide also improved the endothelial function of thoracic aortas harvested from Apo- $E^{-/-}$ mice in an ex vivo study. Furthermore, liraglutide increased AMPK phosphorylation in rat VSMCs, while liraglutide-induced activation of AMPK was abolished by exendin 9-39, a

GLP-1 antagonist. Moreover, angiotensin (Ang) II-induced proliferation of VSMCs was suppressed by liraglutide in a dose-dependent manner, and flow cytometry of Ang II-stimulated VSMCs showed that liraglutide reduced the percentage of cells in G2/M phase (by arrest in G0/G1 phase).

Conclusion: These findings suggest that liraglutide may inhibit Ang II-induced VSMC proliferation by activating AMPK signaling and inducing cell cycle arrest, thus delaying the progression of atherosclerosis independently of its glucose-lowering effect.

Disclosure: **T. Iijima:** None.

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Empagliflozin, an SGLT2 inhibitor, improves survival after myocardial infarction in diabetic rats by up-regulating anti-oxidative stress proteins

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Background and aims: EMPA-REG OUTCOME demonstrated that empagliflozin, an SGLT2 inhibitor, improves cardiovascular outcomes in patients with type 2 diabetes mellitus (T2DM), but its mechanism remains unclear. It has been reported that treatment of T2DM patients with an SGLT2 inhibitor increased serum β-hydroxybutyrate (βOHB) concentration by ~2 fold and that the expression of anti-oxidative stress proteins was increased through inhibition of class I histone deacetylases in βOHB-treated mice. In the present study, we examined whether empagliflozin improves survival rate after myocardial infarction (MI) by suppressing oxidative stress in T2DM rats.

Materials and methods: Coronary artery ligation was performed to induce MI in a rat model of T2DM (25-30-week-old; OLETF) and its control (LETO).. Rats were fasted for 12 hrs before induction of MI, and survival rate at 48 hrs after MI was monitored. Empagliflozin (10 mg/kg/day) or a vehicle was administered for 14 days before MI. In separate groups of rats, myocardial tissue in the non-infarcted region was sampled 12 hrs after MI for biochemical analyses.

Results: Body weight (641±11 vs. 531±7 g), fasting plasma glucose (FPG: 174±10 vs. 122±4 mg/dl) and mean blood pressure (122±2 vs. 111±4 mmHg) were significantly higher in OLETF than in LETO. Treatment with empagliflozin reduced FPG (118±8 mg/dl, $p<0.05$) and increased serum βOHB (1.15±0.09 vs. 0.60±0.04 mmol/L, $p<0.05$) in OLETF, but the effects on body weight and blood pressure were modest. Empagliflozin had no effects on left ventricular function before MI. As in our previous studies, survival rate at 48 hrs after MI was lower in OLETF than in LETO (40% vs. 84%, $p<0.05$) due to exacerbating heart failure. Empagliflozin treatment significantly improved survival rate to 70% in OLETF, although infarct size was unchanged. ATP level in the non-infarcted myocardium was lower in OLETF than in LETO after MI (3571±726 vs. 5692±804 nmol/g, $p<0.05$) and empagliflozin increased ATP to a level similar to that in LETO (5790±1238 nmol/g). Although myocardial βOHB level was significantly higher in empagliflozin-treated OLETF than in LETO and OLETF (1719±670 vs. 189±33 and 184±31 nmol/g), acetyl-CoA level was not changed by empagliflozin in OLETF. Protein levels of βOHB dehydrogenase 1 and succinyl-CoA:3-oxoacid-CoA transferase, key enzymes in ketone oxidation, were also not changed by empagliflozin in OLETF. On the other hand, levels of anti-oxidative proteins, including manganese superoxide dismutase, catalase, and Sirt3, were increased by empagliflozin in OLETF. Empagliflozin also increased the acetylation level of histone H3, suggesting histone deacetylase inhibition. Myocardial levels of Akt and S6 phosphorylation, well-known protective signals, after MI were lower in OLETF than in LETO, regardless of empagliflozin treatment.

Conclusion: Treatment with empagliflozin improved survival after MI in a rat model of T2DM. Empagliflozin up-regulated anti-oxidative proteins presumably via βOHB-induced inhibition of histone deacetylases and preserved ATP in the non-infarcted myocardium. These results suggest that restoration of myocardial energy metabolism by up-regulating anti-oxidant proteins contributes to the beneficial effect of empagliflozin on post-MI outcomes in T2DM.

Supported by: Grants from Boehringer Ingelheim.

Disclosure: **H. Oshima:** None.

OP 12 Lipid handling in metabolically active tissues

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Fatty acid spillover: A signature of a metabolic health?

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Background and aims: Spillover of fatty acids (FAs) into the plasma non-esterified fatty acids (NEFA) pool, due to an inability of adipose tissue to accommodate sufficient fat uptake, has been suggested to be a contributing factor to insulin resistance in obesity. Using specific labelling techniques, we compared the proportion of spillover-derived NEFA in men and women across a range of adiposity.

Materials and methods: Seventy-one healthy adults (55 men, 16 women) aged 22-64 years with a BMI 20-35 kg/m^2 were studied. Participants were fed a mixed meal containing [U^{13}C]palmitate to assess the contribution of dietary-derived spillover FAs to the systemic NEFA pool. To investigate adipose tissue specific spillover, arterio-venous difference and stable-isotope methodologies were used in a sub-study (6 men, 6 women). Dietary FA oxidation was assessed by appearance of ^{13}C into breath CO2 as markers of whole-body FA oxidation.

Results: Dietary FA spillover was higher in individuals with a low BMI (<25kg/m^2, n=18) than in individuals with a high BMI (≥25kg/m^2, n=53, AUC 22.2±1.6% vs. 18.6±0.7%, p=0.02). Women had significantly higher dietary FA spillover than men when matching for BMI (AUC 21.9±1.1% vs. 15.0±1.6%, p=0.001). Assessing spillover across subcutaneous abdominal adipose tissue showed significantly higher proportions in women than in men (28.5±6.1% vs. 9.9±1.3%, p=0.01). The appearance of $^{13}CO_2$ in breath was also greater in women compared with men (p=0.0004).

Conclusion: Our data show that there is a considerable degree of dietary FA spillover into the systemic NEFA pool. This process is greater and more dynamic in lean individuals and in women. Contrary to general perception, dietary spillover of FA into the systemic circulation is a physiologically normal feature most easily observed in people with a high plasma triglyceride clearance capacity and not a pathway providing excess NEFA in people with obesity or insulin resistance.

Supported by: Bristish Heart Foundation

Disclosure: **M. Piche:** None.

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Effect of gender and metformin on hepatic fat handling in early type 2 diabetes

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Background and aims: Type 2 diabetes and fatty liver disease are more common among men than women, and are associated with lower BMI at diagnosis in men. Liver fat content is now known to be determinant for the onset of type 2 diabetes. We have evaluated fat content and VLDL1-triglyceride (VLDL1-TG) production of the liver in early type 2 diabetes at baseline in a sub-group of the prospective, randomised Diabetes Remission Clinical Trial (DiRECT).

Materials and methods: 95 participants were initially recruited and complete data are available on 89 people (51M/38F; 53.1±7.8 years; weight 99.5±16.2 kg; BMI 34.4± 4.2 kg/m^2; diabetes duration 2.9±1.7 years). Magnetic resonance using 3 point Dixon, and an enzyme competitive blocking methods were used respectively to quantify liver fat content and VLDL1 production rate in vivo. Data are presented as mean ±SD.

Results: Mean liver fat content was elevated at 14.7±9.8%, and VLDL1-TG production rate and TG pool size were 545±179mg/kg/day, and 2291 ±1645mg, respectively. Both VLDL1-TG production rate and pool size were positively correlated with liver fat content (r=0.43, p<0.0001; r=0.31, p=0.003). There was a positive relationship between both liver fat and fasting plasma glucose (r=0.35, p=0.001) and fasting plasma insulin (r=0.52, p<0.0001). Although fasting liver fat and VLDL1-TG production rate were similar in males and females (14.2±9.8 vs. 15.2±9.8%, p=0.63; 531±181 vs. 563±177mg/kg/day, p=0.40), men had a TG pool size 59% larger than that of women (2722±1726 vs. 1712±1346 mg, p=0.003). All physical characteristics of males and females were similar (BMI 33.9±4.1/ 35.1±4.4 kg/m^2, age 54.0 ±6.7/ 51.8±9.0 years, diabetes duration 2.8±1.5/ 2.9±1.8 years) except for body weight (men: 105.6±16.7kg, women: 91.2±11.2kg, p<0.0001). However, the difference in VLDL1-TG pool size remained after normalization for body weight (men: 25.6±15.6mg/kg, women: 18.7±13.9mg/kg, p=0.03).Women had lower fasting TG (0.54±04 vs. 0.73±04 mmol/l) and higher fasting NEFA (0.69±02 vs. 0.56±02 mmol/l) but similar fasting plasma glucose and insulin levels. At the time of the baseline tests, all remained on their usual therapy (38.2% diet alone, 32.6% metformin alone, 29.2% combined oral agents). HOMA-S tended to be higher in Metformin group compared with the combined treatment (89.6±52.4% vs. 62.7±33.1%, p=0.03).There were no differences in liver fat content, metabolic or body characteristics between the groups other than higher fasting plasma glucose in the combined treatment group compared with metformin or diet alone (9.8 vs. 8.1 or 7.6 mmol/l, p<0.05). In the metformin group only, liver fat content positively correlated with VLDL1-TG production rate and TG pool (r=0.61, p<0.0001 and r=0.56, p=0.002, respectively).

Conclusion: These data demonstrate clear gender differences in the dynamics of fat handling in type 2 diabetes. The lower TG pool size together with the raised NEFA and lower plasma TG suggest that adipose tissue clearance of VLDL1-TG is more rapid in women. Regardless of gender, metformin therapy aligned VLDL1-TG production with liver fat content.

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Disclosure: **A. Al-Mrabeh:** Grants; Diabetes UK.

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Bariatric surgery improves brown adipose tissue lipid metabolism in morbidly obese subjects

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Background and aims: Brown adipose tissue (BAT) plays a role in human energy balance and insulin sensitivity. BAT functional activity is reduced in obese and insulin resistant subjects. Whether BAT metabolism is stimulated with weight loss in humans is unclear. We aimed to investigate BAT free fatty acid uptake and triglyceride content in morbidly obese subjects before and after bariatric surgery.

Materials and methods: We studied 23 morbidly obese women (BMI 41.1 ±4.2 kg/m^2) using [^{18}F]-FTHA-PET/CT imaging after overnight fast before and 6 months after bariatric surgery. 15 age- and sex-matched controls (BMI 22.6±2.8 kg/m^2) were studied once. Fractional uptake rate (FUR) and free fatty acid uptake (FAU) were measured from PET/CT images in the supraclavicular fat depots which is typical location for human BAT. Adipose tissue radiodensity [a marker for intracellular triglyceride content] of supraclavicular fat depots was obtained by voxel-based thresholding using CT images (values from -250 HU to -50 HU). Whole body fat oxidation rate (FOX) was measured using indirect calorimetry and insulin sensitivity was assessed as the insulin sensitivity index (ISI).

Results: Before surgery, FUR (0.0055±0.0034 vs. 0.0016±0.0018 1/min, p=0.001) and FAU (0.39±0.27 vs. 0.57±0.50 μmol/100g/min, p=0.01) were lower in obese compared to lean controls. Adipose tissue radiodensity was lower (-101.24±10.10 vs. -82.48±5.84 HU, p<0.001) and FOX was higher (p<0.001) in obese compared to lean controls. In the whole study group, radiodensity was associated with FUR (r=0.45, p=0.004), and inversely with FOX (r=-0.60, p<0.001) and with FFA levels (r=-0.45, p=0.01). After surgery, BMI decreased by 23% and ISI improved by 79%. FUR and FAU increased (from 0.0055±0.0034 to 0.0074±0.0035, p=0.004, and 0.39±0.27 to 0.56 ±0.50, p<0.01, respectively), and radiodensity increased (from -101.2 ±10.1 to -86.5± 9.6, p<0.001) to levels similar to controls (p=0.135). Lipid oxidation tended to decrease after surgery (p=0.068). The increment in adipose tissue radiodensity correlated with the improvement in insulin sensitivity (r=0.66, p<0.001) and the decrease in BMI (r=-0.72, p<0.001).

Conclusion: BAT lipid metabolism is impaired in obesity and associates with increased adipose triglyceride content. Marked weight loss after bariatric surgery stimulates BAT lipid metabolism and decreases triglyceride content. The improvement in BAT lipid metabolism interrelates with improvements in BMI and whole-body insulin sensitivity.

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Supported by: Academy of Finland

Disclosure: **P. Dadson:** None.

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High protein levels of PLIN2 and low levels of ATGL are associated with insulin resistance and low number of intramyocellular lipid droplets

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Background and aims: Although intramyocellular lipid (IMCL), stored in lipid droplets (LDs) negatively correlates with insulin sensitivity, highly insulin sensitive athletes also present high levels of IMCL. Upon matching for IMCL, we observed that in patients with type 2 diabetes (T2DM), lipid was stored in fewer, but larger LDs than in athletes, suggestive of reduced lipid droplet dynamics (the process of lipid hydrolysis (re-)esterification). Cell and animal studies suggest that ATGL and PLIN2 are players in LD dynamics. Therefore, we examined the hypothesis that LD dynamics in the trained and the T2DM state involves differential regulation of PLIN2 and ATGL.

Materials and methods: Over a wide range of insulin sensitivity (endurance trained athletes, lean sedentary, obese subjects, and T2DM patients), we analyzed muscle biopsies (n=8 per group) for PLIN2 and ATGL protein content (Western blotting). Correlations were performed for these parameters with insulin sensitivity (2-step hyperinsulinemic euglycemic clamp) and LD size and number.

Results: PLIN2 protein content was significantly lower in athletes than T2DM patients (0.54±0.08 vs. 1.36±0.16 AU, p<0.001) who were matched for total IMCL content, with LDs being smaller and more numerous in the athletes. For ATGL it was observed that athletes had higher protein levels than T2DM patients (1.76±0.25 vs. 0.37±0.13 AU, p<0.001). PLIN2 protein content was negatively associated with insulin sensitivity and LD number (insulin sensitivity: r=-0.583, p=0.001; LD number: r=-0.498, p=0.016). In contrast, ATGL protein content was positively associated with insulin sensitivity and LD number (insulin sensitivity: r=0.610, p<0.001; LD number: r=0.706, p<0.001). No associations were observed for PLIN2 or ATGL protein content with LD size (PLIN2: r=0.221, p=0.310; ATGL: r=-0.178, p=0.418).

Conclusion: The high protein levels of PLIN2 and low levels of ATGL may reflect compromised LD dynamics in T2DM patients with larger and fewer LDs as a consequence. Along with the negative association of PLIN2 and positive association of ATGL with insulin sensitivity and LD number, these data indicate that the process of PLIN2- and ATGL mediated LD dynamics (rather than IMCL content per se) plays a role in modulating muscle insulin sensitivity.

Disclosure: **A. Gemmink:** None.

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Specific hepatic sphingolipids relate to insulin resistance and predict both oxidative stress and inflammation in non-alcoholic fatty liver disease

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is associated with obesity and type 2 diabetes. Several lipid metabolites in liver and plasma are related to hepatic steatosis and hepatic insulin resistance in animal models, but their role for human insulin resistance and NAFLD remains unclear.

Materials and methods: To examine the relationship between total and specific sphingolipids with insulin sensitivity and NAFLD progression, we examined 14 obese patients with or without NAFLD and 7 healthy lean individuals (CON), who underwent liver biopsies during bariatric surgery or elective abdominal surgery. Before surgery, hyperinsulinemic-euglycemic clamps with D-[6,6-$^2H_2$] glucose were performed to measure hepatic and peripheral insulin sensitivity. Hepatic oxidative capacity, H_2O_2 and lipid peroxidation were measured to assess mitochondrial function and oxidative stress.

Results: Total liver ceramides were 8% and 19% higher in NAFLD+ compared to NAFLD- and CON. Hepatic dihydroceramides, sphingosine and sphinganine were exclusively increased by 12%, 25% and 40% respectively in NAFLD+. Serum ceramide species 14:0, 16:0 and 20:0, total dihydroceramides, dihydroceramide 16:0, 20:0 and 22:0 correlated negatively with peripheral insulin sensitivity (all $r>0.55$, $p<0.05$). Hepatic maximal respiration correlated positively to total and certain serum dihydroceramides, liver lactosylceramide 16:0 and sphingomyeline 18:0 (all $r>0.50$, $p<0.05$). Liver H_2O_2 (ceramide 16:0, certain hexosyl- and lactosylceramides), lipid peroxides (dihydroceramide 24:1, total ceramides, ceramide 24:1) and pJNK (total dihydroceramides, ceramide 22:0, lactosyl- and hexosylceramides) correlated all positively with the respective hepatic sphingolipids ($r>0.47$, $p<0.05$). Ceramide subsets (total dihydroceramides and specific species) as well as certain sphingomyelins in serum reflected the respective species in the liver, which could be helpful for the future use of certain serum sphingolipids as biomarkers for the detection and progression of NAFLD.

Conclusion: In conclusion, specific sphingolipids are increased in obese people with NAFLD and correlate with higher oxidative capacity and oxidative stress in liver, thus indicating a possible role of sphingolipids in the NAFLD progression.

Clinical Trial Registration Number: NCT01477957

Disclosure: **M. Apostolopoulou:** None.

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Tankyrase inhibition ameliorates lipid disorder in diabetic db/db mice

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Background and aims: Tankyrases (TNKSs) catalyze a post-translational modification of proteins by transferring ADP-ribose moiety of NAD^+ to target proteins. TNKSs are associated with metabolic disorders, but the underlying mechanism remains largely unclear. We aimed to dissect the molecular mechanism by which TNKSs regulate energy metabolism in obese and diabetic db/db mice.

Materials and methods: Male C57BLKS/J db/db and db/+ mice served as a diabetes model and its control, respectively. TNKS inhibition was conducted in both db/db and db/+ mice in two parallel groups: regular chow vs chow spiked with G007-LK, a specific TNKS inhibitor, for 15 weeks. The effects on lipid metabolism and molecular mechanisms involved were assessed in white adipose tissue (WAT), muscle and liver.

Results: In diabetic db/db mice, TNKS inhibition with G007-LK reduced body weight gain by 10-12% and abdominal fat mass by 25%, lowered serum LDL cholesterol, and decreased hepatic steatosis. In addition, TNKS inhibition led to a reduction of the fasting insulin level after 8 weeks of treatment and a tendency toward improved insulin response ($p=0.068$). Consistent with a trend of improved insulin sensitivity, G007-LK treatment induced upregulation of GLUT4 in muscle. Furthermore, TNKS inhibition restrained lipolysis in WAT, as demonstrated by downregulation of triglyceride lipase and active (Ser660-phosphorylated) hormone sensitive lipase. In line with restrained lipolysis, low serum glycerol and a tendency for NEFA to decrease appeared in G007-LK-treated mice compared to non-treated controls. Notably, TNKS inhibition augmented NAD^+ content and SIRT1 activity manifested as a decline in PGC-1α acetylation level in WAT. Moreover, the reduction of adiposity was associated with increased fatty acid oxidation and reduction of cholesterol efflux in muscle, as illustrated by the upregulation of transcriptional coactivator PGC-1α and genes involved in fatty acid oxidation, and by the downregulation of ATP-binding cassette transporter A1, a major transporter mediating the efflux of cholesterol and phospholipids. Notably, TNKS inhibition attenuated PARylation of PGC-1α in muscle of db/db mice, likely contributing to PGC-1α stability.

Conclusion: Our findings demonstrate that pharmacological inhibition of TNKSs ameliorates lipid disorder observed in obesity and diabetes via distinct mechanisms in a tissue-specific manner. In addition to supporting SIRT1-dependent effects of TNKS inhibition on metabolism in WAT, our data also provide a novel mechanism whereby TNKS-mediated PARylation directly regulates PGC-1α in muscle. The effects of G007-LK in db/db mice do not occur in nondiabetic db/+ mice. These findings highlight inhibition of TNKSs as a potential pharmacotherapy for obesity and type 2 diabetes mellitus.

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Disclosure: **H. Wang:** None.

OP 13 Insulin therapy in type 2 diabetes: novel concepts

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What determines treatment satisfaction of type 2 diabetes patients on insulin therapy? An observational study in eight European countries

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Background and aims: Type 2 diabetes (T2DM) patients on insulin therapy are less satisfied with their diabetes treatment than those on oral hypoglycaemic therapies or lifestyle advice only. Determinants of treatment satisfaction in T2DM patients on insulin therapy are not clearly known. The aim of this study was to determine the association of treatment satisfaction with demographic and clinical characteristics of insulin treated T2DM patients.

Materials and methods: For this study we used data from the GUIDANCE study, a cross-sectional study among 7597 T2DM patients from Belgium, France, Germany, Ireland, Italy, Sweden, the Netherlands and the United Kingdom. The majority of patients were recruited from primary care. Treatment satisfaction was assessed with the Diabetes Treatment Satisfaction Questionnaire (DTSQ, score 0-36; higher scores reflecting higher satisfaction). Missing data were handled with multiple imputation. To account for the hierarchical three level structure (i.e. countries-physicians-patients), we used a linear mixed model with random intercepts for country and physician to determine which patient characteristics and laboratory values were independently associated with treatment satisfaction.

Results: In total 1984 T2DM patients on insulin therapy were analysed; the number of included patients per country ranged from 166 (the Netherlands) to 384 (Italy). The mean DTSQ score was 28.50 ±7.52 and ranged from 25.93 ±6.57 (France) to 30.11 ±5.09 (the Netherlands). Higher DTSQ scores were associated with having received diabetes education (β 1.64 for having received diabetes education, versus not having received diabetes education, 95% CI 0.95-2.32), presence of macrovascular complications (β 0.76 for presence versus absence, 95% CI 0.21-1.31) and better health status (β 0.08 for every one unit increase on a 0-100 scale, 95% CI 0.07-0.10). Lower DTSQ scores were associated with more frequently perceived hyperglycaemia (β -0.32 for every one unit increase on a seven-point Likert scale, 95% CI -0.50 - -0.13), and higher HbA1c (β -0.52 for every percentage increase, 95% CI -0.75 - -0.29). No association was found between treatment satisfaction and insulin regimen, nor insulin injection frequency.

Conclusion: These findings underline the importance of diabetes education in insulin-treated T2DM patients. Moreover, health care providers should be attentive to patients with a lower health status, frequently perceived hyperglycaemia and higher HbA1c levels, to discuss and improve their diabetes treatment satisfaction. Patients with macrovascular complications were more satisfied with their treatment; this may be because patients with incident diabetes-related comorbidity are more intensively treated. The lack of an association between treatment satisfaction, and insulin regimen or insulin injection frequency is favourable.

Disclosure: **A. Boels:** None.

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Efficacy and safety of oral basal insulin: eight-week feasibility study in people with type 2 diabetes

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Background and aims: Oral insulin 338 formulated into a GIPET® I tablet (OI338GT) is a basal, acylated insulin analog with a half-life of ~70 hours. We investigated efficacy and safety of OI338GT in comparison to subcutaneous insulin glargine U100 (IGlar) in 50 insulin naïve T2DM subjects (mean±SD age 61±7 years, BMI 30.5±3.7 kg/m²) insufficiently controlled (HbA_{1c} 7-10%) on metformin alone or in combination with other oral agents.

Materials and methods: Patients were randomized 1:1 to OI338GT or IGlar once daily for 8 weeks in a double-blind, double-dummy fashion as add-on to existing metformin with or without DPP-4 inhibitor. Insulin doses were uptitrated weekly on an individualized basis aided by a pre-specified algorithm aiming at achieving fasting plasma glucose (FPG) in the target range of 80-126 mg/dL.

Results: Both treatments substantially improved glycemic control as indicated by FPG (primary endpoint), HbA1c and fructosamine with no significant differences between treatments at 8 weeks (table). C-peptide was comparable at end of treatment. The incidence of treatment-emergent hypoglycemia was low (OI338GT: 7 events in 6 subjects, IGlar: 11 events in 6 subjects). No severe hypoglycemia occurred. The rate of adverse events was similar.

Conclusion: In conclusion, this study demonstrated for the first time that an oral basal insulin safely improves glycemic control to a similar extent as does IGlar.

Parameter	OI338GT		IGlar		Treatment difference*/ratio⁺ [95% CI]	p-value
	Baseline	EOT	Baseline	EOT		
FPG (mg/dl)	175±50	129±33	164±31	121±17	5.2* [-8.8;19.1]	0.4567
HbA_{1c} (%)[&]	8.1±0.6	7.3±0.8	8.2±0.8	7.1±0.6	0.30* [-0.03;0.63]	0.0774
Fructosamine (µmol/l)[&]	275±44	235±45	273±50	223±34	9.6* [-11.7;30.9]	0.3700
Fasting C-peptide (nmol/l)[&]	1.02±0.37	0.64±0.29	0.97±0.29	0.65±0.27	- 0.02* [-0.13;0.08]	0.6799
Daily insulin dose (nmol/kg OI338GT, U/kg IGlar)[&]	30±11[#]	114±52	0.11±0.03[#]	0.33±0.15	347⁺ [262;459]	-

Table shows mean±SD values at baseline and end of treatment (EOT). Treatment difference/ratio, 95% CI and p-value are based on a linear mixed model for repeated measurements.
*Treatment difference OI338GT - IGlar; ⁺Treatment ratio OI338GT/IGlar; [#]Initial dose; [&]Post-hoc statistical analysis

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Disclosure: **L. Plum-Mörschel:** None.

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Association of fasting C-peptide levels with glycaemic efficacy and risk of hypoglycaemia in people with type 2 diabetes commencing insulin glargine 100 U/ml

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Background and aims: To investigate the prognostic value of fasting C-peptide levels for efficacy and safety outcomes when starting insulin glargine 100 U/ml (Gla-100) at bedtime in combination with oral agents.

Materials and methods: Standardized patient-level data were pooled from 16 randomized controlled trials (≥ 24 weeks duration). Outcomes were assessed descriptively up to week 24 stratified according to fasting C-peptide (≤ 0.40, > 0.40-1.20, > 1.20-2.00, >2.00 nmol/l) at start of Gla-100 (baseline).

Results: Overall, 2,165 participants (54% male) were included. Mean ± SD age by baseline C-peptide level (≤ 0.40, > 0.40-1.20, > 1.20-2.00, >2.00 nmol/l) were 58 ± 11, 58 ± 10, 59 ± 9, and 59 ± 10 years. Increasing baseline fasting C-peptide levels were associated with a shorter duration of diabetes (10.4 ± 7.0 vs 10.0 ± 6.6 vs 8.5 ± 5.6 vs 7.6 ± 5.5 years), higher BMI (25.7 ± 3.6 vs 29.5 ± 4.8 vs 31.9 ± 5.2 vs 32.2 ± 5.3 kg/m²), lower baseline fasting plasma glucose (11.5 ± 3.3 vs 10.9 ± 3.0 vs 10.8 ± 2.8 vs 10.3 ± 2.7 mmol/l), and slightly lower baseline HbA_{1c} (9.0 ± 1.1 vs 8.8 ± 1.0 vs 8.7 ± 1.0 vs 8.8 ± 1.0 %). Week 24 outcomes for HbA_{1c}, Gla-100 dose and hypoglycaemia are shown in the Table. From a slightly higher HbA_{1c} level, with smallest titrated and final Gla-100 doses, those in the lowest C-peptide group had a lesser HbA_{1c} reduction (-1.34 vs -1.40 to 1.54%), thus being less likely to achieve the target HbA_{1c} of

<7.0% (26%, 43%, 42%, and 44% in the lowest to highest C-peptide groups, respectively). Lower FPG levels were achieved in the lower C-peptide groups (6.3 ± 2.2 vs 6.5 ± 2.1 vs 6.7 ± 2.1 vs 7.0 ± 2.2 mmol/l). Final Gla-100 insulin dose was least in the lowest C-peptide group (0.34 U/kg), 0.42 U/kg in the >0.40−1.20 C-peptide group, and highest in the upper two C-peptide groups at 0.51, 0.50 U/kg, respectively. For all definitions and time periods of hypoglycaemia, incidence and event rates were higher with lower C-peptide levels (**Table**). The percentage of participants achieving HbA_{1c} <7.0% without hypoglycaemia (confirmed plasma glucose < 3.9 mmol/l) was 9%, 18%, 20%, and 25% in the lowest to highest C-peptide groups, respectively. Body weight change was greater with lower C-peptide levels (3.3 ± 3.7 vs 2.3 ± 3.5 vs 2.0 ± 4.0 vs 1.7 ± 3.2 kg).

Conclusion: This pooled analysis in people with T2D suggests that fasting C-peptide levels may help predict hypoglycaemia, insulin dose, and blood glucose control achieved when commencing Gla-100 therapy.

Table: Clinical outcomes in people with T2D starting Gla-100 stratified by baseline fasting C-peptide levels

		Baseline fasting C-peptide groups (nmol/l)			
		≤0.40 n=100	>0.40–1.20 n=1267	>1.20–2.00 n=621	>2.00 n=177
C-peptide	baseline (nmol/l)	0.30 (0.08)	0.84 (0.21)	1.51 (0.22)	2.62 (0.73)
Gla-100 dose	at start (U/kg)	0.21 (0.10)	0.17 (0.08)	0.16 (0.08)	0.15 (0.10)
	week 24 (U/kg)	0.34 (0.20)	0.42 (0.22)	0.51 (0.29)	0.50 (0.27)
Hypoglycaemia[a]	overall (% people)	66	51	43	34
	overall (events/person-yr)	12.8 (2.2)	6.6 (0.4)	4.0 (0.3)	2.5 (0.4)
	nocturnal (% people)	35	22	16	11
	nocturnal (events/person-yr)	3.3 (0.8)	1.4 (0.1)	0.8 (0.1)	0.6 (0.2)
Severe hypoglycaemia[b]	(% people)	5.0	2.4	2.1	0
	(events/person-yr)	0.18 (0.09)	0.11 (0.03)	0.10 (0.05)	0

Mean (SD) or percent.; Group numbers may vary if missing values; [a], confirmed plasma glucose < 3.9 mmol/l; [b], ADA definition; yr, year

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Contributions of fasting and postprandial hyperglycaemia in type 2 diabetes

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Background and aims: The Relative contribution of basal and postprandial hyperglycemia (HG) was previously documented in type 2 diabetes subjects treated by oral anti-hyperglycemic agents or basal insulin but not in subjects with an intensified insulin regimen with multiple daily injections (MDI). Our aim was to measure basal and postprandial HG in type 2 diabetes subjects on MDI before randomization in the OPT2MISE study. We also analyzed the predictive value of these variables of the metabolic response to continuous subcutaneous insulin infusion (CSII).

Materials and methods: We performed an analysis of CGM recordings after 8-week run-in period in 259 MDI patients. HG Area under curve (AUC) was calculated in the basal (AUC-B), nocturnal (AUC-N) and postprandial (AUC-PP) periods according baseline HbA1c level (Gr1 :<8, Gr2 :8-8.5, Gr3 :8.5-9, Gr4 :9-9.5, Gr5 :>9.5%). AUC changes were analyzed in 131 subjects switched from MDI to CSII. Non parametric tests were used for comparisons between groups.

Results: AUC-B was 29% to 79% higher in Gr5 vs Gr4 to Gr1 (p=0.002). AUC-N was 18% to 96% higher in Gr5 vs Gr4 to Gr1 (p=0.0001). Conversely, AUC-PP did not differ between groups HbA1c≥9.5% vs <9.5% (p=0.75). Accordingly, HbA1c correlated with AUC-N (R=0.32, p=0.0001) and AUC-B (R=0.183, p=0.003) but not with AUC-PP. After switch from MDI to CSII, both AUC-B and AUC-N decreased significantly (-15% and -19%) while AUC-PP tended to increase by 8% (p=0.087). When comparing responders to non responders to CSII, the latter had higher AUC-B, AUC-N and AUC-PP, but differences did not reach significance.

Conclusion: Fasting and nocturnal HG are the major determinants of HG in type 2 diabetes with MDI failure. Both significantly correlated with baseline HbA1c level. In contrast, postprandial HG accounts for 20-30% overall hyperglycemia only, whatever the HbA1c level. Pump therapy reduces mainly the fasting component of overall hyperglycemic exposure across a large range of A1c levels above 8%.

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Real-world titration of insulin glargine in type 2 diabetes patients poorly controlled on oral antidiabetic drugs: an analysis in 2308 patients in primary care in Germany

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Background and aims: Addition of insulin glargine 100 U/mL (Gla-100) improves glycemic control in patients (pts) not controlled on oral antidiabetic drugs (OADs). While a number of titration schemes for basal insulin exist, actual titration behavior in daily clinical practice remains unclear.

Materials and methods: TOP-1 is a prospective, observational study in T2DM patients attending primary care offices in Germany. Patients with an HbA1c of 7.5-10% despite being treated by ≥1 OAD (with/without a non-Gla-100 basal insulin) and a physician decision to start Gla-100 therapy were included. The primary endpoint (PE) was achievement of FBG ≤110 mg/dL or individual HbA1c target at 12 months.

Results: In the analysis (n = 2308), patients were grouped by the magnitude of insulin titration during the first month (no titration [+0 U: 39.2%], +1-4 U [31.0%], +5-8 U [17.7%], or +>8 U [12.1%]). At baseline, +>8 U patients were younger (64 vs. 66 years), more often female (58% vs. 50%), and had a higher mean BMI (33 vs. 31 kg/m^2), FBG (201 vs. 179 mg/dl) and HbA1c (8.8% vs. 8.4%) as compared to the +0 U group (all p<0.05). At 12 months, the PE was met by a comparable proportion of patients in each group (65.0%, 68.4%, 66.7%, and 62.9% for +0 U, +1-4 U, +5-8 U and +>8 U, respectively [p = NS]). As expected mean reductions in HbA1c and FBG were greater for +>8 U than +0 U pts (-1.6% vs. -1.2% and -75.1 vs. - 51.2 mg/dl, respectively, all p<0.05). Furthermore, compared to the +0 U group, the proportion of pts who achieved FBG ≤110 mg/dL and their HbA1c target at 12 months was higher in the +5-8 U (27.2% vs. 20.1%; p=0.03) and +>8 U groups (26.2% vs. 20.1%; p=0.02). Confirmed, symptomatic hypoglycemia was documented in 1.5%, 2.4%, 1.2%, and 2.2% of pts in ascending order of titration with no difference between groups.

Conclusion: The majority of patients were not titrated or were titrated slowly in a real word primary care setting. More aggressive titration had only minor effects on the improvement of glycemic control at 12 months.

Supported by: Sanofi-Aventis Deutschland GmbH, Germany.

Disclosure: **J. Seufert:** Honorarium; Sanofi.

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Glycaemic variability and risk for hypoglycaemia on insulin glargine 300 U/ml versus insulin glargine 100 U/ml in people with type 2 diabetes

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Background and aims: It has been observed that the new insulin glargine 300 U/ml (Gla-300) has a smoother 24-hour pharmacokinetic (PK) and pharmacodynamics (PD) profile when compared to insulin glargine 100 U/ml (Gla-100). Here we assess whether these PK/PD differences translate into differences between the daily profiles of glycaemic variability (GV) and risk for hypoglycaemia on Gla-300 vs. Gla-100.

Materials and methods: Edition 2 and Edition 3 are 12-month multicentre trials comparing Gla-300 with Gla-100, both administered in the evening in type 2 diabetes. These trials collected self-monitoring (SMBG) daily profiles and records of documented symptomatic hypoglycaemia (DSH) confirmed by SMBG below 3mmol/l from N=796 insulin users (Edition 2) and N=839 insulin naive patients (Edition 3). The data included 39,388 SMBG readings and 693 DSH episodes (Edition 2), and 41,548 SMBG readings and 235 DSH episodes (Edition 3). GV metrics were computed from SMBG data, including the Low Blood Glucose Index (LBGI) - a GV measure of the risk for hypoglycaemia.

Results: The LBGI and the Night-time LBGI were lower on Gla-300 compared to Gla-100 (P-values <0.001 for both indexes in Edition 2 and P-values =0.09 and =0.02 in Edition 3). These differences were evident throughout the study, and more apparent during the titration phase (mean LBGI Gla-300 vs Gla-100 = 0.333 vs 0.507 in titration and 0.410 vs 0.498 in maintenance in Edition 2; 0.241 vs 0.300 in titration and 0.376 vs 0.410 in maintenance in Edition 3). Figure 1 presents the LBGI daily profiles for Edition 2 (Panel A) and Edition 3 (Panel B), indicating that the largest differences in the risk for hypoglycaemia between Gla-300 and Gla-100 occurred in the second half of the night. Further, among the measures of glucose variability (e.g. SD, CV), the LBGI was the only metric significantly correlated with DSH: r=0.35, p<0.001 (Edition 2) and r=0.26, p<0.001 (Edition 3). While Edition 2 and Edition 3 had different frequencies of hypoglycaemia, the LBGI identified patients at risk uniformly well across the two studies. Compared to those who had LBGI≤1.1, subjects who had LBGI>1.1 experienced several-fold more DSH: 0.63 vs. 3.49 DSH episodes per person, p<0.001 (Edition 2), and 0.25 vs. 1.06 DSH episodes per person, p<0.01 (Edition 3).

Conclusion: Smoother PK/PD profile on Gla-300 compared to Gla-100 is associated with reduced daily glycaemic variability and lower risk for hypoglycaemia, particularly in the second half of the night. Thus, while Gla-300 and Gla-100 maintained similar average glycaemia, Gla-300 may be better suited for treatment intensification than Gla-100. This observation was recently supported by continuous monitoring data in type 1 diabetes. Moreover, type 2 diabetes patients experiencing symptomatic hypoglycaemia when treated with basal insulin are identifiable from SMBG data using risk analysis of glycaemic variation.

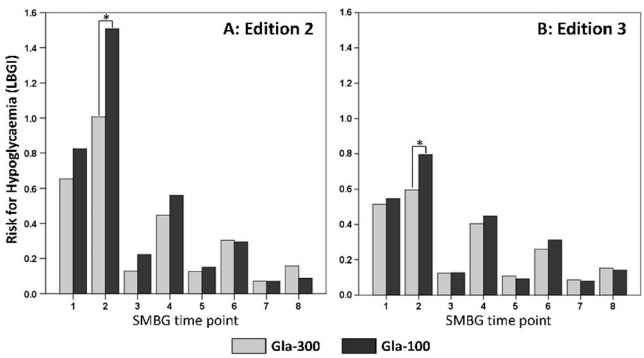

Figure 1: Daily profiles of risk for hypoglycemia on Gla-300 and Gla-100 as measured by the LBGI computed from 8-point SMBG profiles taken at time points: **1**-3AM; **2**-before breakfast; **3**-after breakfast; **4**-before lunch; **5**-after lunch; **6**-before dinner; **7**-after dinner; **8**-at bedtime.

Clinical Trial Registration Number: NCT01499095 and NCT01676220
Supported by: Sanofi

Disclosure: **B. Kovatchev:** Employment/Consultancy; Sanofi. Grants; Sanofi. Honorarium; Dexcom. Non-financial support; Dexcom, Roche Diagnostics, Tandem Diabetes Care. Stock/Shareholding; TypeZero Technologies.

OP 14 Epidemiology

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Novel metabolic indices and incident type 2 diabetes among women and men: the Rotterdam study

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Background and aims: Body fat distribution, rather than its magnitude, is increasingly linked to the risk of type 2 diabetes (T2D). Both visceral adipose tissue (VAT); a hormonally active component of total body fat, and truncal fat depot; partitioned into android and gynoid area, have been associated with T2D. While computed tomography (CT) and magnetic resonance imaging (MRI) are the gold standard measures for quantification of VAT, dual energy X-ray absorptiometry (DXA) can measure compartment body compositions; android and gynoid fat. Several easily measured indices, product of combined anthropometric and blood lipid measures, have recently been suggested as indicators of insulin resistance or adiposity. In the large population-based Rotterdam Study, we aimed to investigate the associations of these novel combined metabolic indices with incident type 2 diabetes among women and men and compare them with DXA measurements on body fat.

Materials and methods: Novel combined metabolic indices included visceral adiposity index (VAI), lipid accumulation product (LAP), and the product of triglycerides and glucose (TyG). We used Cox proportional hazard models to investigate associations between VAI, LAP, TyG, the anthropometric [body mass index (BMI) and waist circumference (WC)] and laboratory components [inverse high-density lipoprotein cholesterol (HDL) and triglycerides (TG)] included in their formulas, as well as DXA measurements on body fat (android, gynoid, android to gynoid ratio, total fat mass) with incident type 2 diabetes among women and men in the Rotterdam Study. Associations were adjusted for systolic blood pressure, treatment for hypertension, smoking, prevalent cardiovascular diseases, and serum lipid reducing agents.

Results: We included 9564 subjects (5576 women and 3988 men) free of diabetes at baseline. During a median follow-up time of 6.5 years, 899 incident T2D cases (511 women and 388 men) were identified. In the multivariable adjusted models, VAI (Hazard ratio - HR; 95% confidence interval - CI: 1.49; 1.36, 1.65 in women and 1.37; 1.22, 1.53 in men), LAP (HR; 95% CI: 1.35; 1.16, 1.56 in women and 1.19; 1.01, 1.42 in men), TyG (HR; 95% CI: 1.73; 1.52, 1.98 in women and 1.43; 1.26, 1.62 in men), BMI (HR; 95% CI: 1.37; 1.26, 1.49 in women and 1.45; 1.28, 1.65 in men), and inverse HDL (HR; 95% CI: 1.29; 1.14, 1.46 in women and 1.32; 1.14, 1.52 in men) remained associated with incident T2D in both women and men. WC (HR; 95% CI: 1.24; 1.07, 1.45), TG (HR; 95% CI: 1.24; 1.10, 1.39), gynoid fat mass percentage (HR; 95% CI: 0.64; 0.45, 0.89), and the ratio of android to gynoid fat mass percentage (HR; 95% CI: 1.48; 1.14, 1.94) were associated with incident T2D only in women.

Conclusion: Novel combined metabolic indices including VAI, LAP and TyG were stronger risk markers for incident type 2 diabetes than the traditional anthropometric and laboratory measures included in their formulas. These novel metabolic indices were also comparable to DXA measured body fat compositions. While the association of the combined indices with incident T2D was stronger in women, TyG (the product of triglycerides and glucose) was the best risk marker associated with T2D in both genders.

Disclosure: **A. Brahimaj:** None.

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Spousal diabetes and obesity as risk factors of incident type 2 diabetes: analysis from the English Longitudinal Study of Ageing

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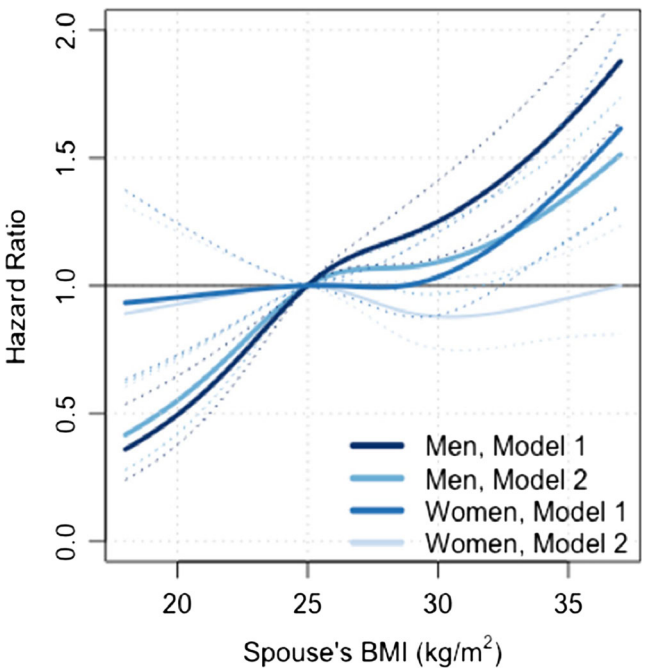
Background and aims: Obesity and family history of diabetes are among the strongest risk factors for type 2 diabetes. Conversely, there is little evidence on the effect of spousal diabetes and obesity as risk factors for type 2 diabetes. Therefore, we investigated the association of spousal diabetes and obesity with incident type 2 diabetes in an elderly population.

Materials and methods: We analysed data from the English Longitudinal Study of Ageing (ELSA), a representative cohort of the English population aged >50 years. We included 3151 men and 3050 women in the study. All participants attended wave 0 of ELSA, serving as baseline. We excluded participants with known diabetes, no follow-up information and missing information on variables of interest. Incidence of diabetes was based on the clinical examinations at waves 2, 4 and 6 (screen-detected: HbA_{1c} >= 6.5% or fasting plasma glucose >= 7 mmol/L) or on the questionnaires at each wave (self-reported diagnosis or medication use). We applied Cox proportional hazards model to obtain hazard ratios (HR) having spousal diabetes status or obesity (BMI and waist circumference) as exposures and diabetes status of the index individual as outcome. The effects of continuous exposures were modelled using splines to reveal potential non-linearity. Model 1 was adjusted for age, ethnicity and socio-economic status, while Model 2 was further adjusted for the index individual's BMI or waist circumference, depending on the exposure.

Results: Women were more likely than men to have a spouse with diabetes at baseline (6.4% vs. 3.3%). Incidence of diabetes was 12.6 and 8.6/1000 person-years among men and women, respectively. There was a tendency that women with a husband with diabetes had higher diabetes risk (HR: 1.45 (95% CI: 0.97,2.19), p=0.07), but this was not seen for men with a wife with diabetes. Non-linear associations between BMI and diabetes risk are shown in the *Figure*. The association between waist circumference and diabetes risk exhibited a similar pattern. After adjustment for a woman's own obesity level, the initial association between the husband's obesity level and the woman's diabetes risk was attenuated and no longer statistically significant. For men, the respective associations were somewhat attenuated but remained statistically significant.

Conclusion: This is the first study investigating the sex-specific effect of spousal diabetes and obesity on diabetes risk. Having an obese wife increases a man's risk of diabetes over and above the effect of his own obesity level, while among women, having an obese husband gives no additional diabetes risk beyond that of her own obesity level. Our results indicate that on finding obesity in a person, screening of their spouse for diabetes may be justified.



Supported by: AH and DRW are supported by the Danish Diabetes Academy.

Disclosure: **A. Hulman:** Grants; Danish Diabetes Academy funded by the Novo Nordisk Foundation.

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Associations of serum fatty acids with insulin resistance and risk of prediabetes and type 2 diabetes

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Background and aims: Few studies have examined the associations between serum fatty acids with insulin resistance and risk of type 2 diabetes (T2D), with conflicting results. Whether and how serum fatty acids are related to risk of prediabetes is still unknown. Therefore, we aimed to investigate the associations between serum fatty acids with insulin resistance and risk of prediabetes and T2D in in a large population-based cohort study.

Materials and methods: Our current study was embedded within the Rotterdam Study (RS), a population-based cohort study in the Ommoord District of Rotterdam, the Netherlands. The study has been approved by the Medical Ethics Committee of Erasmus University Medical Center and all participants gave written informed consent. For the current analyses, we followed 2142 participants aged 65 years and over since 2002-2004 without diabetes, of whom 1653 were without prediabetes, and 1313 had assessments of glucose and insulin, from which we calculated the homeostatic model assessment for insulin resistance (HOMA-IR). Serum phospholipid fatty acids were measured in serum collected from study participants in 2002-2004. Serum fatty acids were categorized into saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) and were expressed individual serum fatty acid in percentages of total serum fatty acids. We used multivariable cox proportional hazard regression models to analyze the associations between serum fatty acids and risk of prediabetes and T2D. We used multivariable linear mixed models to analyze the associations between serum fatty acids and insulin resistance.

Results: Serum SFA, MUFA and PUFA comprised 38% (SD 1.6%), 23% (SD 3.2%) and 38% (SD 3.3%) of the total phospholipid fatty acids at baseline. During a median follow-up of 8.1 (SD 2.4) years, we documented 193 cases of T2D; during a median follow-up of 8.0 (SD 2.3) years, we documented 232 cases with prediabetes. In multivariable models including age, sex, BMI, physical activity, diet quality score, smoking, education level, total energy intake and family history of diabetes; serum SFA was positively associated with insulin resistance (β=0.07 in HOMA-IR; 95%CI: 0.04-0.10; per 1 SD difference) and risk of prediabetes (HR=1.17; 95%CI: 1.02-1.35; per 1 SD difference) and T2D (HR=1.15; 95%CI: 1.00-1.32; per 1 SD difference). Serum PUFA was inversely associated with insulin resistance (β=-0.11 in HOMA-IR; 95%CI: -0.14- -0.07; per 1 SD difference) and risk of prediabetes (HR=0.34; 95%CI 0.16-0.71; per 1 SD difference) and T2D (HR=0.79; 95%CI: 0.64-0.97; per 1SD difference), which was mainly driven by serum n-6 PUFA. Serum MUFA or n-3 PUFA was not associated with insulin resistance or risk of prediabetes or T2D.

Conclusion: Our findings suggest that serum SFA is associated with a higher risk of developing type 2 diabetes; and that PUFAs, particularly n-6 PUFAs, are associated with less insulin resistance and a lower risk of prediabetes and T2D.

Disclosure: **T. Voortman:** None.

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Clinical profile of post-load glucose curves and their association with cardiometabolic risk factors over time

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Background and aims: Subclasses of different glycaemic disturbances could explain the variation in characteristics of people with prediabetes and type II diabetes (T2DM). We aimed to identify subgroups with distinct glucose curves following an oral glucose tolerance test (OGTT) or a mixed meal test (MMT), describe their baseline clinical characteristics and explore associations with cardiometabolic risk factors at follow-up.

Materials and methods: The study included 2179 people with prediabetes and 819 newly diagnosed T2DM patients within the Diabetes Research on Patient Stratification (DIRECT) Study. Latent class trajectory analysis was used to identify subgroups with distinct glucose curves from OGTT for those with prediabetes and MMT for T2DM patients at baseline. Using general linear models, these subgroups were associated with clinical characteristics at baseline and with insulin resistance (HOMA-IR) or HbA1c at 18 months, adjusted for adjusted for potential confounders.

Results: We identified five subgroups from OGTT and three from MMT, labelled in order of increasing peak values as C1 to C5 (OGTT) and T1 to T3 (MMT). At baseline, C4 and C5 had similar fasting glucose (6.0 and 6.2 mmol/l) but different 2-hour glucose (5.5 and 8.4 mmol/l). C5 had higher body mass index (BMI), blood pressure and Hba1c values than C4. C3 compared to C2, had similar fasting glucose (5.7 mmol/l), different 2-hour glucose (5.4 and 7.7 mmol/l), higher BMI and Hba1c. In both analyses, medium (C3 and T2) and highest (C5 and T3) peak curves had the highest BMI, blood pressure and Hba1c. At 18 months, change in HOMA-IR were higher in C5 [beta=1.72; 95%CI=1.28-2.16] , C4 [beta=0.70; 95%CI=0.35-1.06], C3 [beta=1.04; 95%CI=0.73-1.35], C2 [beta=0.38; 95%CI=0.13-0.64] compared to C1. At 18 months, Hba1c (mmol/l) increased across groups with beta=1.88; 95%CI=-0.44-4.20 for T2 and beta=2.93; 95%CI=0.10-5.75 for T3, relative to T1.

Conclusion: Using OGTT and MMT, different glycemic profiles can be identified with different clinical characteristics and cardiometabolic risk. Subgroups with the highest 2-hour glucose had greater cardiometabolic risk than those with high fasting levels only.

Supported by: n° 115317 IMI-FP7/2007-2013 and EFPIA

Disclosure: **M. Obura:** None.

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Sex-gender differences in diabetes associated risk of cardiovascular events in a young population

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Background and aims: Excess risk of cardiovascular diseases linked to diabetes is higher in women compared to men, mostly in peri-post-menopausal age. What happens in a younger population (aged lesser than 40yr) is however more uncertain. To answer this question we carried out an observational study concerning the whole population living in Tuscany, a region of centre of Italy, retrospectively followed up along a period of five years (from January1st, 2007 to December 31st, 2012) comparing, by gender, the effect of diabetes on the risk of first hospitalizations for cardiovascular diseases (CVD).

Materials and methods: Using the regional hospital database, first hospitalizations due to any CVD: (acute myocardial infarction, ischemic stroke, lower limbs amputations or chronic heart failure) were recorded during period January 1st, 2007 to December 31st,2012 in the population with age ranging between 16 and 40yr. Presence of baseline diabetes was identified by a regional diabetes registry in year 2006, and current insulin therapy in year 2006 was considered as a proxy for type 1 diabetes (T1D), compared with those with no insulin therapy considered as affected by type 2 diabetes (T2D). Analysis of proportional incident risk of incident hospitalizations, adjusting for confounders, was carried out by Cox-regression analysis model.

Results: Population without diabetes was composed by 514,732 males and 515,799 females while those with diabetes were 2,052 males and 2,598 females of mean age:32±7yr, [934 T1D/1,664 T2D, among females and 1,256 T1D/796 T2D among males]. Hospitalization rates for CVD (p-yr per 100,000) were much higher in those with diabetes in both sexes [32p-yr in people without diabetes vs. 209p-yr for T1D and vs. 510p-yr in T2D among males, and 14p-yr vs. 194p-yr in T1D and vs. 48p-yr for T2D among females]. In T1D the hazard ratio (HR) of hospitalizations for CVD, adjusted for age and co-morbidities, as compared with those without diabetes, was significantly higher in women [HR:6.04 (95%CI: 1.74-16.17); p=0.0012], not in men [HR: 1.47 (95%CI: 0.53-4.05); p=NS], even if CVD incidence rate in diabetic people was similar in both sexes. On the contrary in T2D the excess risk was significantly higher in men [HR:6.32 (95%CI: 4.01-9.95); p=0.0001]but not in women [HR:1.99 (0.74-5.37);p=NS].

Conclusion: In this young population (with age between 16 and 40yr) followed up for 6 years, diabetes associated excess risk of first hospitalizations due to CVD shows an opposite sex-gender dimorphism, according to diabetes type. The incident risk of hospitalization for CVD in patients with T1D, compared to those without diabetes, was six fold significantly higher among females, while on the contrary the excess risk of CVD in patients with T2D was to the same extent higher only among males.

Disclosure: **G. Seghieri:** None.

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Long-term risk of aortic aneurysm and aortic dissection among individuals with type 2 diabetes: a nationwide observational study

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Background and aims: Decreased short-term risk for aortic aneurysm (AA) and aortic dissection (AD) has been suggested among individuals with type 2 diabetes (T2DM). No studies have examined long-term risks, or mortality after an AA and AD event in T2DM patients.

Materials and methods: In this nationwide, longitudinal, observational cohort study we linked data for individuals with T2DM in the Swedish National Diabetes Register, and 5 individually matched population-based control subjects (CS) without diabetes (based on sex, age and county), to other national databases to capture hospitalisations and death. We examined the risk of AA and AD in individuals with T2DM and mortality risk after an event with AA or AD using Kaplan-Meier curves and Cox-regression hazards models.

Results: Data on 448,319 individuals with T2DM and 2,251,015 CS was obtained between 1998 and 2015. Mean follow-up time was 7.0 years for T2DM and 7.2 years for CS. In total, there were 2,878 cases of AA in T2DM patients and 16,740 in CS, and 200 cases of AD among T2DM and 2,020 cases among CS.Patients with T2DM had a relative risk reduction (RRR) of 28% (hazard ratio [HR]: 0.72, 95% CI 0.68-0.77, p<0.001) for AA and a 47% RRR (HR 0.53, 0.42-0.66) for AD compared to CS.Adjusted survival rates after an event with AA after 3 months, 1 year and 2 years among T2DM patients were: 84.2, (82.9-85.4), 74.7 (73.2-76.2), and 66.7, (65.1-68.3), respectively. Corresponding rates for CS were: 80. 9 (80.3-81.4), 71. 7 (71.0-72.3) and 64.2 (63.5-64.9). For AD, survival rates were non-significantly higher among T2DM patients.

Conclusion: Among T2DM patients there were significantly decreased risks of AA and AD as well as decreased risk of mortality after an event of AA. Data may suggest that glycated cross-links in aortic tissue may play a protective role in progression of aortic diseases in diabetic patients.

Supported by: Swedish Association of Local Authorities and Regions

Disclosure: **T. Avdic:** None.

OP 15 The failing heart in type 2 diabetes

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Risk of heart failure in intensively treated patients with type 2 diabetes and microalbuminuria: 21 years follow-up in the multifactorial Steno-2 intervention

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Background and aims: In type 2 diabetes mellitus, congestive heart failure is a frequent, fatal and often forgotten complication and both antidiabetic agents as well as adjuvant therapies modify the risk of developing heart failure. The aetiology of heart failure in patients with diabetes is multifactorial, with both metabolic dysregulation and atherosclerotic disease as risk factors and heart failure might be classified as primary (metabolic/diabetic) or secondary (ischaemic). We have recently reported that 7.8 years of multifactorial intensified intervention in patients with type 2 diabetes and microalbuminuria in the Steno-2 study reduced the risk of atherosclerotic cardiovascular disease. Here, in a post hoc analysis, we examine the impact of intensified multifactorial intervention in patients with type 2 diabetes and microalbuminuria on the risk for hospitalization for heart failure with up to 21.9 years of follow up.

Materials and methods: 160 patients with type 2 diabetes and microalbuminuria were randomized to conventional or intensified, multifactorial, target-driven intervention involving both behavioral and pharmacologic approaches including RAS-inhibition. After 7.8 years, all patients were recommended treatment similar to the intensive-therapy group and the study continued as an observational follow-up study. Heart failure hospitalizations were identified from nationwide registries and supported by mining of patient records. Event-rates were compared using Log-rank test and time to event was analysed using a Cox-regression adjusted for age and sex.

Results: Ten intensive-therapy group patients were hospitalized for heart failure during follow up vs 19 patients in the conventional-therapy group. Cumulative incidence curves are shown in figure 1.The unadjusted hazard ratio was 0.39 [0.18; 0.84], p = 0.017 in the intensive group compared to the conventional group. Adjusted for age and sex, the HR was 0.37 [0.17; 0.81], p = 0.013. Including death in the end-point kept signal stable with HR 0.50 [0.33; 0.75], p = 0.001 after adjustment. In the conventional group, co-existing atherosclerotic coronary disease was more frequent (n = 1 vs. n = 10; p = 0.009).

Conclusion: Intensified, multifactorial intervention in type 2 diabetes with microalbuminuria reduced the risk of hospitalization for heart failure. Heart failure was more frequently associated with ischaemic heart disease in the conventional group, suggesting that intensified multifactorial intervention most importantly reduces secondary (ischaemic) heart failure.

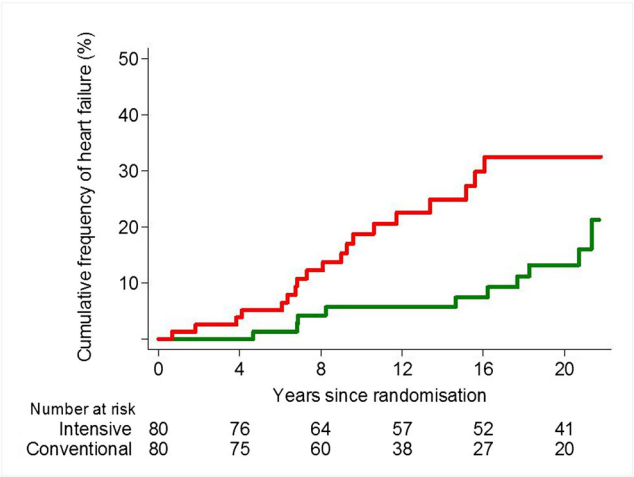

Figure 1: Cumulative incidence of hospitalization for heart failure. Red: conventional therapy. Green: Intensive therapy. HR 0.39 [0.18; 0.84], p = 0.017.

Clinical Trial Registration Number: NCT00320008

Supported by: an unrestricted grant from Novo Nordisk A/S

Disclosure: **J. Øllgaard:** Grants; unrestricted grand from Novo Nordisk A/S.

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Novel biomarkers predicting heart failure hospitalisation in people with dysglycaemia in the ORIGIN trial

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Background and aims: Heart failure (HF) develops in 1 to 2% of middle aged or older people with diabetes per year and increases with age and HbA1c levels. Adding novel serum biomarkers covering multiple pathways to routine clinical risk factors may identify people at high risk for HF.

Materials and methods: One ml of stored serum from 8018 ORIGIN participants was assayed for multiple novel biomarkers using Myriad RBM's customized Human Discovery Multi-Analyte Profile 250+ panel (N=237) as well as for high sensitivity Troponin I. During a median follow-up of 6.2 years, 449 had at least 1 hospitalization for HF. A Cox regression model using a forward selection approach was used to identify the subset of assayed biomarkers that independently predicted this outcome after accounting for 8 commonly measured clinical CV risk factors (sex, age group, prior CV event, albuminuria, smoking, established diabetes, LDL/HDL ratio, and hypertension).

Results: A Bonferroni-corrected P value for inclusion in the model of less than 0.00021 (i.e. 0.05/238) identified 6 significant biomarkers that included: a) hsTroponin I (HR 1.37); b) NTproBNP (HR 1.69); c) growth differentiation factor 15 (HR1.28); d) angiopoietin- 2 (HR 1.17); e) chromogranin A (HR 0.72); and f) cystatin C (HR 1.26), where angiopoietin-2 was calculated at 5 years to account a significant time-by-covariate interaction. When the model was rerun after also accounting for baseline serum creatinine as a 9th clinical risk factor for heart failure hospitalization, cystatin C was no longer significant and a new biomarker, YKL-40 (HR1.20) became significant. Inclusion of HbA1c or baseline use of statins, or ACE inhibitor/ARB use did not change the list of identified biomarkers. Troponin I, NTproBNP, growth differentiation factor 15, angiopoietin-2 and chromogranin A were previously shown to be independently associated with CV outcomes.

Conclusion: Novel biomarkers can help identify dysglycemic people who are at risk for future heart failure and are potential therapeutic targets.

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Endoplasmic reticulum stress and autophagy are impaired in epicardial adipose tissue from heart failure subjects

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Background and aims: Cardiovascular disease (CVD) is a primary cause of morbidity and mortality among diabetics. Epicardial adipose tissue (EAT) thickness appears to be correlated with the risk of CVD and the metabolic syndrome. Proper protein turnover is essential for cardiac homeostasis.

Endoplasmic reticulum (ER) stress and dysregulated autophagy are associated with CVD and heart failure. ER stress may induce autophagy in the diabetic heart, in addition to playing a crucial role in adipose tissue dysfunction. Similarly, autophagy is impaired in obesity. However, little is know regarding either of these pathways in EAT. We sought therefore to evaluate ER stress and autophagy in EAT from heart failure patients with and without diabetes (DM).

Materials and methods: Paired subcutaneous adipose tissue (SAT) and EAT biopsies were obtained from 64 (48 male) subjects, (39 non-diabetic (NDM), 65±2 years and 25 DM, 69±2 years), with and without coronary artery disease (CAD) during elective heart surgery. Gene and protein expression was evaluated for markers of ER stress and autophagy by conventional methods.

Results: *GRP94* gene levels were increased in EAT (median=0.3285) compared to SAT (median=02679), with an F ratio of F(1,70) = 10.378, p=0.002, while *GRP78* gene expression was not altered. On the other hand, the protein expression of both of these key chaperones in the ER unfolded protein response, was increased ($p \leq 0.01$) in EAT compared to SAT, but no differences were observed when comparing diabetic versus non-diabetic subjects. Moreover, *BECN1* gene levels were significantly increased in EAT (median=0.0415) compared to SAT (median=0.0312), with and F ratio of F(1,70) = 11.226, p=001. In addition, Beclin1 protein levels were also significantly increased in EAT (median=0.8917) compared to SAT (median=0.3984), F ratio of F(1,16) = 6.180, p=0024. Furthermore, autophagy markers LC3-I (p=0.011) and LC3-II (p=0.002) were significantly increased in EAT compared to SAT. No diferences were observed when comparing diabetic versus non-diabetic subjects.

Conclusion: This study unravels significant metabolic differences between the EAT and SAT depots from heart failure subjects with and without diabetes, in regard to the ER stress and autophagy pathways. However, EAT remains to be investigated in healthy subjects in order to understand whether these differences are characteristic of EAT as a special fat depot or whether these differences are due to heart failure, suggesting that EAT might be a possible therapeutic target.

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Disclosure: **D. Espinoza:** None.

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Hospitalisation for heart failure and death in new users of SGLT-2 inhibitors in patients with and without cardiovascular disease: the CVD-REAL study

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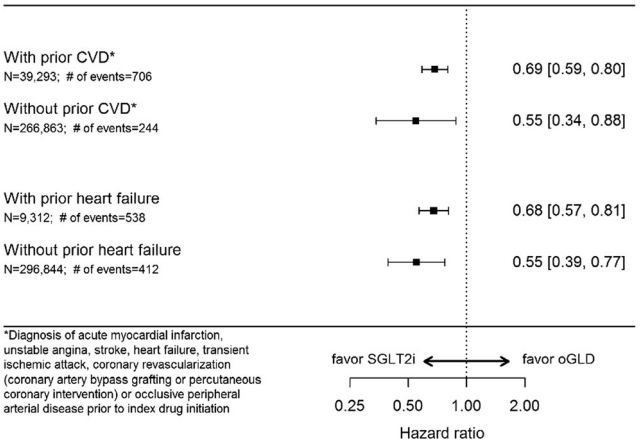
Background and aims: A reduction in cardiovascular death and hospitalization for heart failure (HHF) has been reported with a sodium-glucose co-transporter-2 inhibitor (SGLT-2i) in patients with type 2 diabetes and established cardiovascular disease (CVD). Using observational data from clinical practice (2012-2016), we compared HHF and death in patients with/without prior CVD or heart failure in new users of SGLT-2i vs other glucose lowering drugs (oGLD) in the US, UK, Sweden, Norway and Denmark.

Materials and methods: Both cohorts were matched 1:1 by propensity score. HHF and death were collected via medical records (UK), medical claims, electronic health and death records (US), and national registers (Sweden, Norway, Denmark). Hazard ratios (HR) for HHF, death and the composite endpoint (HHF or death) were estimated by country and pooled as a weighted average.

Results: After matching, baseline characteristics were balanced between groups. In total, 306,156 patients with >150,000 person years (PY) (100,947 PY for SGLT-2i; 89,208 PY for oGLD) and 950 new HHF events were analyzed. SGLT-2i, when compared with oGLD, was associated with significantly lower rates of HHF in patients with and without prior CVD (HR 0.69; 95% CI 0.59-0.80; HR 0.55 95% CI 0.34-0.88, respectively) and with and without prior heart failure (Figure). Similar results were seen for death and the composite endpoint (HHF or death), irrespective of prior history of CVD or heart failure. Findings were consistent across countries with varying proportions of SGLT-2i use by individual agents.

Conclusion: In this large cohort of patients, both with and without CVD or heart failure, SGLT-2i were associated with a significant reduction in HHF and death vs oGLD. This suggests that the benefit of SGLT-2i applies to a broad population of patients with Type 2 diabetes.

Figure: Pooled hazard ratios from meta-analyses for 'hospitalization for heart failure in patients with and without established CVD* and heart failure respectively at initiation of the index drug in five countries: US, UK, Sweden, Norway and Denmark. Analyses of first events on treatment.



Clinical Trial Registration Number: NCT02993614
Supported by: AZ

Disclosure: **M.A. Cavender:** None.

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Total events of hospitalisation for heart failure in new users of SGLT-2 inhibitors: real world data from 5 countries and more than 298,000 patients: the CVD-REAL study

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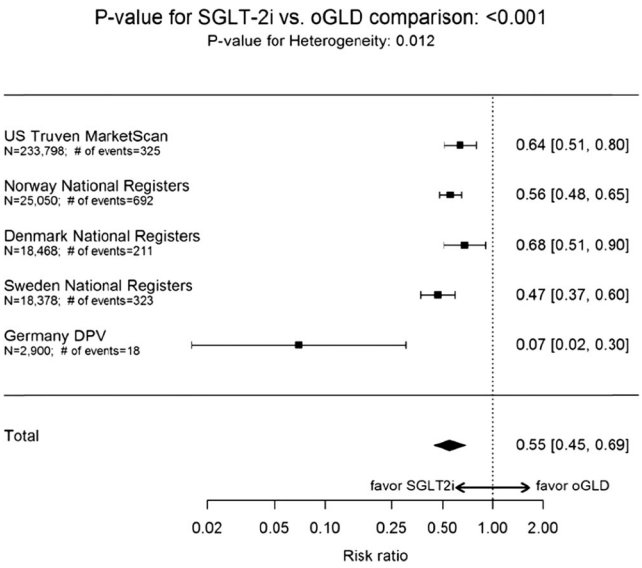
Background and aims: A reduction in hospitalization for heart failure (HHF) was recently reported with a sodium-glucose co-transporter-2 inhibitor (SGLT-2i) in patients with type 2 diabetes and established cardiovascular disease. Whether similar results are seen in clinical practice, and across the SGLT-2i class, is unknown. We compared occurrence of all HHF events in new users of SGLT-2i vs other glucose lowering drugs (oGLD) in the US, Norway, Denmark, Sweden and Germany, while still on drug.

Materials and methods: We used medical claims, electronic health and death records (US), prescription data from national registers (Norway, Denmark, Sweden) and electronic health data (Germany) to identify new users of SGLT-2i and oGLD. Non parsimonious propensity scores for SGLT-2i initiation were developed and used to match patients in the two treatment groups 1:1. Incident and recurrent HHF were collected. The relative risk for HHF was estimated by country/database and pooled to provide an overall weighted average.

Results: After matching, baseline characteristics were balanced between the two groups. The percentage of exposure time to individual agents in the SGLT-2i group was 54.2% canagliflozin, 40.1% dapagliflozin and 5.6% empagliflozin. There were 1569 HHF cases among 298,594 patients with 183,732 person-years follow up (incidence rate: 0.85 per 100 person-years). The average follow-up time was 0.54 years in the US, 1.01 years in Norway, 0.93 years in Denmark, 0.75 years in Sweden and 0.49 years in Germany. Initiation of SGLT-2i was associated with significant reduction in the risk of all HHF events (relative risk for SGLT-2i vs oGLD was 0.55 [95% CI: 0.45-0.69]; p-value <0.001).

Conclusion: Treatment with SGLT-2i vs oGLD in this large multi-country cohort was associated with marked reduction in total events of HHF suggesting a class effect applicable to real world practice.



Clinical Trial Registration Number: NCT02993614
Supported by: AZ
Disclosure: **M. Kosiborod:** None.

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Dapagliflozin is associated with lower risk of major adverse cardiovascular events compared to DPP-4i in type 2 diabetes patients. Results from CVD-REAL Nordic

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Background and aims: Despite advances in management, patients with type 2 diabetes (T2D) continue to experience a 2-fold increased risk of cardiovascular (CV) complications, highlighting the need for treatments that improve CV outcomes in addition to lowering HbA1c We aimed to investigate the effects of a SGLT2i dapagliflozin compared to DPP-4i on the risks for MACE (time to first non-fatal stroke, non-fatal myocardial infarction [MI] or CV death), MACE+ (MACE plus unstable angina) and MACE++ (MACE plus unstable angina and hospitalization for heart failure) in using national registers from Sweden and Norway.

Materials and methods: All T2D patients dispensed GLD during 2013—2015 (2014 in Norway) were identified in nationwide mandatory registries in Norway and Sweden. Patients were divided in two groups; new users of dapagliflozin and new users DPP-4i, matched 1:3 by propensity score, calculated using 90 variables covering patient characteristics (age, sex, time since first GLD, frailty), co-morbidities (CV-, kidney-, microvascular complications, cancer, major bleedings, amputations, and respiratory diseases) and drug treatments (antihypertensives, statins, GLD, anticoagulants/platelets). Hazard ratios estimated by Cox survival models were calculated separately for each country, and meta-analyzed using weighted averages.

Results: A total of 60,108 T2D patients were identified as new users of DPP-4i or dapaglifozin. Following matching 25,448 remained and were diveded into the dapagliflozin (n=6362) or DPP-4i group (n=19,086). The groups were well balanced at baseline; mean-age was 61 years, 40% were women, 21% had established CVD, and 17% microvascular complications. Mean follow-up time was 0.75 years, with a total of 19,006 patient-years. Dapagliflozin was associated with 30%, 28% and 31% lower risk of MACE, MACE+ and MACE++ compared to DPP-4i, respectively (Table; p <0.01 for all comparisons).

Conclusion: Treatment with dapagliflozin vs. DPP-4i was associated with significantly lower risk of all three defined MACE outcomes in a broad population of T2D patients from a real-world clinical practice. As CVD is the leading cause of death in T2D, treatments that reduce morbid CV complications should be prioritized.

	Dapagliflozin N=6362		DPP-4i N=19,086		Weighted average estimates N=25,448		
	No. events	Rate/100 PYR	No. events	Rate/100 PYR	Hazard ratio	95% CI	p-value
MACE	**83**	**1.83**	**373**	**2.58**	**0.70**	**(0.55-0.89)**	**0.004**
- Non fatal myocardial infarction	*40*	*0.88*	*170*	*1.17*	*0.75*	*(0.53-1.06)*	*0.102*
- Non fatal stroke	*37*	*0.81*	*147*	*1.01*	*0.80*	*(0.56-1.15)*	*0.222*
- CV death	*18*	*0.39*	*77*	*0.53*	*0.74*	*(0.44-1.23)*	*0.241*
MACE+	**95**	**2.09**	**416**	**2.88**	**0.72**	**(0.58-0.90)**	**0.004**
- Unstable angina	*17*	*0.37*	*53*	*0.36*	*1.05*	*(0.61-1.82)*	*0.863*
MACE++	**140**	**3.10**	**632**	**4.41**	**0.69**	**(0.58-0.83)**	**<0.001**
- Hospitalization for heart failure	*50*	*1.10*	*261*	*1.80*	*0.60*	*(0.44-0.81)*	*0.001*

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Disclosure: **A. Norhammar:** Honorarium; advisory panel, AstraZeneca, Boehringer Ingelheim Pharmaceuticals Inc, MSD Sweden; consultant, AstraZeneca, Boehringer Ingelheim Pharmaceuticals Inc, MSD Sweden; research support, Swedish Heart Lung.

OP 16 Pregnancy: from model to clinic

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Socs2$^{-/-}$ mouse as a potential model of macrosomia and gestational diabetes

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Background and aims: Foetal macrosomia, the predominant complication of gestational diabetes mellitus (GDM), is associated with negative maternal and neonatal outcomes. Macrosomic newborns have increased risk of respiratory distress and neonatal hypoglycemia, and high risk for obesity and DM. The etiopathogenic factors associated with macrosomia are maternal obesity/overweight, high lipid and glucose levels, and older maternal age. Its prevalence is increasing worldwide simultaneously to obesity and DM, but its lifelong impact is not fully understood. Animal models of GDM and macrosomia are necessary to study both conditions, but most are induced, only very few transgenic strains mimic GDM and only *db*$^{-/+}$ shows the poor foetal outcome of macrosomia. We have observed apparent macrosomia in the offspring of the cytokine signalling 2 knockout mice. SOCS2, through the Janus kinases and signal transducers and activators of transcription (JAK/STAT) pathway, acts mediating cytokine responses to control growth, development, metabolism/DM and immunity. Due to its valuable importance as a possible model, we aimed to analyze observational data of the *Socs2*$^{-/-}$ mice as a potential model for foetal macrosomia.

Materials and methods: During routine colony management all pregnant females were followed up. If inability to give birth was detected, they were humanely euthanized. Mothers' age and body weight (BW) were obtained. BW; head length, lateral height and dorsolateral diameter; abdomen width (ventral view) and body length were determined in undelivered and delivered (healthy control) neonates. A retrospective analysis was also assessed in the mouse colony archive (Jan-Dec 2016) to identify related data. To compare groups, Mann-Whitney's U & Student's test were used. A two-tailed $p<0.05$ was considered significant.

Results: Eight pregnant females were euthanized and their 39 non-delivered neonates and six new-born littermates were analyzed. Undelivered neonates were 40% heavier (1.59 ± 0.15 vs 1.14 ± 0.08 g, $p<0.001$), had a 16% increase in dorsolateral head diameter (1.33 [0.7-1.2] vs 0.7 [0.6-0.75] cm, $p<0.045$) and 22% in abdomen width (1.14 [0.75-1.4] vs 0.93 [0.75-1.1] cm, $p<0.025$) than their new-born littermates, but had similar body length. Teratomes were identified in 13% of the necropsied mothers. Retrospective analysis showed high rates of infertility or neonatal mortalities in 60% of all mated females (77 females; 83 mating). Mean age of *Socs2*$^{-/-}$ females with infertility or delivery problems was 175 days (±14.52). No incidences were seen in younger females.

Conclusion: Undelivered neonates were much larger and heavier than the delivered littermates. This macrosomia seemed to cause the inability to deliver in all cases. This condition was seen in mature-old females, along with high rates of infertility. Thus, we hypothesised that macrosomia, mothers' maturity and infertility could be all associated with subsequent GDM or pre-gestational DM. Since same-mother-offspring differences are seen, a placental role or other regulatory mechanisms can be present. Besides, previous insulin resistance in *Socs2*$^{-/-}$ males has been described. Further studies will be performed to evaluate the presence of DM and the role of *Socs2*$^{-/-}$ influence on foetal development. The potential role of pregnant *Socs2*$^{-/-}$ as a spontaneous model of GDM and macrosomia will be established.

Disclosure: **Y. Brito-Casillas:** None.

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Role of peroxisome proliferator-activated receptor gamma in the energy homeostasis and metabolic adaptations of pancreas during late pregnancy

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Background and aims: Reversible β-cell adaptations occur during pregnancy due to the increased maternal insulin resistance, with compensation of β-cell through hyperplasia and hyper-functionality for optimal glucose homeostasis. A defective maternal β-cell adaptation can lead to gestational diabetes mellitus (GDM) and could lead to derived metabolic complications in the offspring. Peroxisome proliferator-activated receptor γ (PPARγ) plays an important role in β-cell proliferation in other pathologic situations such as obesity. Our aim was to study the role of PPARγ in β-cell adaptation during gestation and its consequences in the offspring at later stages.

Materials and methods: We have generated a specific PPARγ knockout mouse in pancreatic β-cell (βγKO). Groups of mice were fed with high fat diet (HFD) or control diet for 3 weeks before pregnancy. At day 15 (D15) of gestation GTT (glucose 1 g/kg BW) and/or ITT (insulin 0.75 U/kg BW) were performed and at D18 animals were sacrificed. Serum concentration values of glucose, insulin and different cytokines were quantified by Bio-plex ProTM Diabetes Assays. Metabolomic analysis in placenta was performed. Immunohistochemistry of insulin and immunofluorescence of insulin/glucagon were performed in pancreas. Indirect calorimetry, locomotor activity, food and drink intake were measure in metabolic cages in adult offspring.

Results: Although our data did not showed differences in body weight in the different experimental groups, βγKO mice fed with HFD showed certain insulin resistance (IR) at late stages of pregnancy and showed higher glucose intolerance than the other experimental groups. Of note, under conditions of HFD, insulin serum concentrations in pregnant βγKO mice were higer (4.8 ± 0.3 *vs* 3.8 ± 0.2 ng/ml) than in WT mice. Furthermore, the adaptation in the expansion of β-cell mass during pregnancy and specific diet was defective (lower β-cell mass in βγKO mice than WT). On the other hand, there were also differences in placental morphology and metabolites between pregnant βγKO and WT mice. Metabolic differences were present in adult offspring depending on the sex, genotype and diet of the mother such as substrate utilization, energy expenditure, activity and drink/food intake.

Conclusion: These data indicated that an appropriate expression of PPARγ in β-cell is necessary to ensure normal pancreas metabolism during the late phase of pregnancy, when a state of insulin resistance is established, contributing to lead normal gestation to term and correct metabolic homeostasis in adult offspring.

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Study of the maternal-foetal interface in women with obesity and gestational diabetes as a potential mirror of foetal programming

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Background and aims: Pre-gestational obesity and GDM impair fetal development and enhance adverse outcomes for both, mothers and newborns, by affecting the uterine milieu and fetal programming. hUCMS, located at the maternal-fetal interface, hold immunomodulatory properties by production of humoral factors (TGFβ, IDO, NO, IL6, PGE2, HGF, VEGF) and expression of immune molecules (HLA E-F-G), and may mirror fetal effects induced by maternal metabolic alterations. We assessed how pre-gestational obesity and GDM, in accurately and intensively managed patients, may influence hUCMS at the maternal-fetal interface.

Materials and methods: We studied 628 pregnant women, that were classified by pre-gestational BMI (normal b.w, overweight, obesity), and presence

of GDM or overt diabetes (OGTT 75 g. at 16-18th or 24-28th week of gestation), and monitored throughout the third trimester of pregnancy. Glycometabolic control (by HbA1c, fasting and 1 hr post-prandial BG, plasma total/HDL cholesterol, triglycerides) and anthropometric parameters (gestational weight gain) including nutritional or drug (ie, insulin) prescriptions were assessed at third trimester. Fetal parameters (metabolic, birth weight, birth complications or hypoglycemia) were assayed and, in a representative sample, maternal systemic inflammatory parameters (plasma cytokines) were tested. We also compared hUCMS stemness and immunoregulatory properties of diabetic and obese vs. normal weight and euglycemic mothers, well monitored during pregnancy. In particular, mesenchymal (CD90, SCF, CD117, vimentin E/N-cadherins, nestin), and stemness (Oct4A, Oct4B, Sox2, Nanog, ABCG2) markers, as well as hUCMS capability to differentiate into osteogenic (osteopontin), adipogenic (FABP4, PPARγ), neural (Map2ab, TUBβ3, Nestin), endocrine (MAFB/A, NEUROD, PDX1, NKX6.1), and definitive endoderm (SOX17, CXCR4, FOXA2) cell phenotypes were evaluated. Immunomodulatory molecules (IDO, iNOS, HLAG1, IL6, IL10, PGE2, TGFβ1 - in basal and after exposure to peripheral blood mononuclear cells in alginate beads) were also examined. Cytokines from isolated hUCMS were evaluated.

Results: The mothers ranked by pre-gestational b.w. and of GDM or T2D under intensive monitoring, terminated pregnancy under optimal metabolic conditions, with final incremental body weight on target (compared to pre-gestational b.w.) and glucose control similar between BMI classes. An optimized metabolic control during pregnancy translates into tangible benefits of neonatal outcomes. However, hUCMS plasticity was different in obese/diabetic vs. normal glucose tolerant mothers, in terms of differentiation potential, expression of pro-inflammatory cytokines and immunoregulatory markers, all these reflecting an inflammatory "milieu", in obese/diabetic vs. normal mothers.

Conclusion: We preliminarily showed that the obesity/DM-induced inflammatory environment, at the maternal/fetal interface, is mirrored by hUCMS, as a possible marker for fetal programming.

Disclosure: **S. Parrettini:** None.

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Apelinergic system in placenta of women with gestational diabetes

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Background and aims: With respect to the finding that maternal insulin resistance and GDM are associated with fetal brain insulin resistance, placental Apelin (APL) could be one key player in the establishment of fetal brain insulin resistance and transgenerational diabetes. APL is a protein discussed to improve insulin sensitivity at physiological concentrations. By contrast, high cerebral APL concentrations associate with increased hypothalamic inflammation and development of insulin resistance. Usually, APL plasma levels are markedly elevated in pregnancy due to its strong expression and release by the placenta. Gestational diabetes mellitus (GDM) increases APL plasma levels even more. Additionally, maternal overnutrition was shown to increase maternal and fetal APL plasma levels and placental APL and APL receptor (APJ) expression, in animal models. However, GDM-specific effects on placental and trophoblastic APL and APJ expression awaits further investigation. As high levels of saturated fatty acids (SFA) are found in placental tissue of GDM women, we also focused on trophoblastic APL release and APJ expression after stimulation with SFA.

Materials and methods: Placental tissue from normal glucose-tolerant (NGT) and GDM women was collected in the Tübingen PREG study. RNA was isolated from homogenized placental tissue (N=30). Quantitative PCR (qPCR) was conducted for relative quantification of APL and APJ. Additionally, immunohistochemical staining of paraffin-embedded, serial sections of placental tissue was conducted with antibodies against APL and APJ. Furthermore, primary human trophoblasts (N=6) were stimulated with palmitic and oleic acid (100μM each) and with insulin (10nM and 100nM), lysed, and subjected to APL and APJ gene expression analysis by qPCR

Results: As expected, placental APL (P=0.01) and APJ (P=0.06) mRNA expression was elevated in GDM compared to NGT women after adjustment for pregnancy week at birth, maternal BMI, fetal sex, and maternal smoking behavior. Immunohistochemical studies revealed that trophoblasts express both APL and APJ. Stimulation of primary trophoblasts with palmitic acid (100μM) triggered increased APL release into the cell culture supernatant (P=0.003). No changes in concentrations of APL in cell culture supernatants were seen upon oleic acid (100μM) and insulin (10nM and 100nM) treatment. Furthermore, stimulation of primary trophoblasts with palmitic acid, but not oleic acid or insulin, was associated with increased APJ mRNA expression (P=0.04).

Conclusion: We identified the saturated fatty acid palmitate as a novel and potent inducer of trophoblastic APL and APJ expression. Thus, saturated fatty acids, e.g., derived from insulin-resistant maternal adipose tissue and/or from the lipid-laden placenta itself, may contribute to the GDM-induced increase in APL release from the placenta and may trigger, via the apelinergic system, fetal brain insulin resistance.

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Disclosure: **L. Stirm:** None.

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High incidence of foetal macrosomia in cohort of pregnant women with type 1 diabetes on continuous subcutaneous insulin infusion despite excellent glycaemic control

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Background and aims: Type 1 diabetes mellitus (T1DM) in pregnancy may lead to adverse outcomes. Poor glycaemic control at around the time of conception is associated with congenital anomalies, while persistent hyperglycaemia during pregnancy may result in increased rates of stillbirths, neonatal death, preterm delivery and fetal macrosomia. Achieving good glycaemic control is therefore essential. Our objective was to characterize the relationship between the use of continuous subcutaneous insulin infusion (CSII) use in pregnant women with T1DM and their obstetric outcomes.

Materials and methods: We performed a retrospective analysis of fifteen patients from three hospitals in South Glasgow who were on CSII during pregnancy over a three-year period from 2013 to 2016. Data was obtained from maternity records and the electronic Scottish Care Information Diabetes Collaboration (SCI-DC) database.

Results: This cohort had a mean age of 32.3 years (range 27-40 years) and a mean BMI of 26.7 pre-pregnancy. All were on CSII pre-pregnancy. Twelve of the women were of white European descent, and three were white American. Mean duration of diabetes was 15.8 years (range 3.1-24.0 years). Ten women had retinopathy, one had microalbuminuria and one had peripheral neuropathy. Mean HbA1c was 59 mmol/mol pre-pregnancy and 49 mmol/mol at the end of pregnancy. Twelve of the fifteen women underwent structured education. Twelve were planned pregnancies on pre-conceptual folic acid, and twelve were commenced on low dose aspirin from 12 weeks gestation. Five women underwent induction of labour as per National Institute for Health and Care Excellence (NICE) guidance for women with diabetes on insulin therapy. Ten women required pre-term delivery, and two suffered from pre-eclampsia. Ten were delivered by caesarian section. Thirteen women had a fetus with an abdominal circumference (AC) of over the 95th centile. One had polyhydramnios. No patient sustained a third degree tear at delivery, and two women suffered from postpartum haemorrhage.

Conclusion: This cohort demonstrated a marked improvement in HbA1c during pregnancy. Thirteen of fifteen patients had a fetus with an AC over the 95th centile. The reasons for this high rate of fetal macrosomia are unclear and require further exploration. The possibilities include better placental function as a result of CSII use in early pregnancy leading to improved fetal blood

supply and consequential greater exposure to glucose and insulin, over insulinisation, or marked glucose variability not identified on random capillary blood glucose monitoring.

Disclosure: **M.R. Talla:** None.

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Higher odds for initiation of insulin therapy in MTRN1B rs10830963 G allele carriers with gestational diabetes and pre-pregnancy BMI above 29 kg/m^2

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Background and aims: Gestational diabetes mellitus (GDM) has a high prevalence, which highlights the importance of factors predicting the need for pharmacological treatment, including antenatal insulin therapy (AIT). The rs10830963/G allele, a common variant of the Melatonin receptor 1 B (MTNR1B) gene is associated with GDM development and glycemic traits. We hypothesized that carrying the rs10830963/G risk allele may have an effect on AIT initiation in GDM in a BMI dependent manner.

Materials and methods: Clinical and MTNR1B genotype data of 211 Hungarian and 87 Austrian GDM cases were assessed in a post-hoc analysis of a recently completed Austro-Hungarian case-control genetic association study. The genetic effect on the initiation of AIT was studied in different body mass index (BMI) subgroups and a BMI threshold was determined with a stepwise increase where the genetic effect became the most significant using logistic regression. The odds were calculated using a chi-square test for both countries.

Results: Although the glycemic targets were the same, we recorded different AIT rates between the two countries (74% vs. 14.7%, p=1x10^{-4} in Austria vs. Hungary, respectively). The pre-pregnancy BMI threshold of 29 kg/m^2 was identified where the genetic effect on the initiation of AIT was the most significant. The mean weight gain of Hungarian patients with pre-pregnancy BMI≥29 kg/m^2 was lower (5.2 kg) compared to all other study subgroups (9.36-10.56 kg, p<0.007). We detected a significant genetic effect on AIT initiation in Hungary (real-life AIT OR=5.2, p=0.02) in patients with GDM and pre-pregnancy BMI≥29 kg/m^2, which even remained significant after adjusting the data to macrosomia (OR=3.29, p=0.05). In addition, there was a NS trend for higher odds of AIT initiation in Austrian MTNR1B rs10830963 G allele carrier GDM patients with pre-pregnancy BMI≥29 kg/m^2 (real-life OR=2.7, NS), however this could not reach the level of statistical significance due to the higher AIT use rate (81%) and the lower number of cases in Austria.

Conclusion: To our knowledge this is the first report that carrying a common gene variant (MTNR1B rs10830963/G allele) increases the odds of AIT initiation in patients with GDM and pre-pregnancy BMI≥29 kg/m^2. This observation might serve as a basis for future studies about the effect - of reporting MTNR1B rs10830963 genotype (or more complex prediction scores) - on GDM management strategies and outcomes, however the results should be first confirmed in replication studies with larger sample sizes.

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OP 17 Beta cell vulnerability

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MANF is required for the postnatal expansion of the pancreatic beta cell mass and for adult beta cell maintenance in mice

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Background and aims: Mesencephalic astrocyte neurotrophic factor (MANF) is an endoplasmic reticulum (ER) located, but also secreted protein that is essential for the β cell proliferation and survival in vivo since conventional MANF knockout mice (Manf$^{-/-}$) show progressive postnatal reduction of β cell mass resulting in severe insulin-deficient diabetes. MANF absence in mouse pancreatic islets in vivo caused ER stress and chronic activation of UPR signalling pathways. In this study we aimed 1) to verify that diabetic phenotype of the conventional Manf$^{-/-}$ mice is caused by the lack of MANF from β cells and not from other organs, and 2) to investigate whether MANF-removal in postnatal/adult mouse β cells affects the survival and proliferation of β cells using conditional Pdx1Cre and inducible β cell specific Manf$^{-/-}$ mice.

Materials and methods: We generated pancreas-specific conditional MANF-deficient mice by crossing Manf$^{fl/fl}$ mice with transgenic Pdx1Cre^{Tuv} mice. Inducible β cell specific Manf$^{-/-}$ mice were created by crossing of Manf$^{fl/fl}$ mice to MIP1-CreERT mice. At 8 weeks of age MIP1-CreERT:: Manf$^{fl/fl}$ were injected with 33 mg/kg of Tamoxifen for 5 consecutive days and control mice with corn oil. Mice were analysed four weeks post injection. β cell mass, β cell proliferation and death analysis were quantified from pancreatic sections. RNA was extracted from isolated islets and cDNA was assessed by quantitative real-time PCR for β cell specific- and ER stress markers.

Results: We found that pancreas-specific Pdx-1$^{Cre/+}$::Manf$^{fl/fl}$ mice similarly to Manf$^{-/-}$ mice, developed insulin deficient diabetes due progressively reduced β cell mass after birth. However, the severe growth defect found in Manf$^{-/-}$ mice was absent. We found mosaic MANF expression in the postnatal pancreases of conditional Pdx-1$^{Cre/+}$::Manf$^{fl/fl}$ mice, which probably was due to incomplete and individually variable recombination efficiency, reported previously using Pdx1Cre mice. Importantly, we found that the number of MANF-positive β cells significantly correlates with the β cell mass in individual mice, demonstrating the importance of MANF expression in restoring the β cell mass. Histological analysis of MIP1-CreERT::Manf$^{fl/fl}$ pancreases revealed decreased insulin staining and loss of β cells in the Tmx-injected mice compared to controls. β cell mass was quantified and a significant reduction was seen in the Tmx injected MIP1-CreERT::Manf$^{fl/fl}$ mice compared to oil injected mice. The reduction of the β cell mass was accompanied by enhanced β cell apoptosis and diminished β cell proliferation. Additionally, we observed reduced intensity of immunofluorescent GLUT2 and PDX1 staining in β-cells lacking MANF. Accordingly, decreased mRNA expression of β cell markers and increased expression of ER stress markers was detected in islets isolated from adult Tmx-injected MIP1-CreERT::Manf$^{fl/fl}$ compared to control islets.

Conclusion: Our results show that 1) Embryonic MANF removal specifically from pancreas leads to progressive β cell loss and diabetes in mice 2) MANF is needed for the expansion of β cell mass postnatally 3) MANF is needed for maintaining the adult β cell phenotype and for the survival of adult β cells in mice. Our results makes MANF a promising candidate for regenerative β cell therapy in diabetes.

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MicroRNA miR-184 islet expression is regulated by NKX6.1 and is involved in beta cell dedifferentiation and protection from cytokine- and palmitate-induced apoptosis

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Background and aims: Beta-cell dysfunction in Type 2 diabetes (T2D) involves several molecular mechanisms, including microRNAs deregulation. A new proposed mechanism of beta-cell dysfunction in T2D is the loss of the mature phenotype or dedifferentiation. We recently demonstrated that miR-184 is reduced both in T2D donors and in in vitro dedifferentiated human islets, and that its downregulation leads to overexpression of CRTC1, protecting from palmitate- and cytokine-induced apoptosis. However, the mechanism leading to miR-184 downregulation is not known. Therefore, the aim of this study is to shed light onto the regulatory pathway of miR-184 expression to better understand whether the dedifferentiation process is a protective mechanism against β cell stress and apoptosis, occurring in T2D.

Materials and methods: The algorithm MatInspector was used to identify the predicted TFBS (transcriptional factor binding sites) for miR-184, and allowed to putatively identify NKX6.1 as regulator gene on miR-184 promoter. Consequently, NKX6.1 expression was evaluated by Taqman qRT Real-time PCR in purified human islets from 7 T2D and 10 non-diabetic multiorgan donors, as well as in 5 in vitro dedifferentiated and 5 native human islet preparations. NKX6.1 nucleus-cytoplasm translocation was induced through H_2O_2 in MIN6 cell line, and miR-184 expression was evaluated in treated vs non-treated samples by Taqman qRT Real-time PCR. Moreover, the binding of NKX6.1 to miR-184 promoter was analyzed by Chromatin Immunoprecipitation (ChIP). Statistical analyses were performed using Mann-Whitney U test and Spearman test; p values <0,05 were considered statistically significant.

Results: We identified 3 binding sites for NKX6.1 on human promoter and 1 binding site on mouse promoter of the miR-184 gene. Results obtained by qRT Real-time PCR demonstrated that NKX6.1 is downregulated in human islets of T2D vs non-diabetic donors (p=0,0004) and in in vitro dedifferentiated vs native human islets (p=0,0079), in line with reduced expression of miR-184. Moreover, NKX6.1 expression positively correlated with miR-184 expression in human pancreatic islets (r=0,68; p=0,0023). Immunofluorescence analysis on MIN6 cells, in which oxidative stress was induced, showed the translocation of NKX6.1 from the nucleus to the cytoplasm and, as hypothesized, a decrease in miR-184 expression (p=0,01). Finally, ChIP data revealed the actual binding of NKX6.1 on miR-184 promoter, suggesting that this gene is indeed one of miR-184 regulators and acts on the expression and function of this miRNA, pivotal for the protection against β-cell death.

Conclusion: In summary, we found that NKX6.1 is a regulator of miR-184 expression in β-cells. Given the pivotal function of NKX6.1 in the maintenance of β-cell identity, we speculate that its downregulation, and the consequent reduction of miR-184 expression, is involved in β-cell dedifferentiation process. We hypothesize that the reduced expression of NKX6.1 and of miR-184, and the consequent increased expression of CRTC1, is a dedifferentiation-induced protection (DiP) pathway, suggesting a new concept of β-cell dedifferentiation as a protection mechanism against apoptosis in T2D.

Disclosure: **G.E. Grieco:** None.

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Overexpression of eukaryotic translation initiation factor 2A (eIF2A) in pancreatic beta cells attenuates diabetes in Akita mouse model

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Background and aims: Endoplasmic reticulum (ER) stress-induced unfolded protein response (UPR) is a major mechanism mediating beta-cell apoptosis in diabetes. Inhibition of general protein translation via phosphorylation of eIF2α is a key event during the UPR. Previously, we have demonstrated that overexpression of an alternative translation initiation factor eIF2A, that can deliver initiator tRNA to the ribosome during inhibition of general translation, protects MIN6 cells, primary mouse and human islets from ER stress-induced apoptosis. Therefore, we investigated the potential protective effect of eIF2A in beta cells *in vivo* using Akita mice, which carry a mutated *Ins2* gene leading to spontaneous diabetes due to ER stress-induced apoptosis in beta cells.

Materials and methods: For beta-cell specific overexpression we designed adeno-associated virus 6 (AAV6), encoding either eIF2A-GFP or control GFP and driven by an insulin promoter. 1.5 x 10^11 particles of either virus were injected into the pancreatic duct of 6-week old $Ins2^{Akita/WT}$ female mice randomized into two groups (n=5). Body weight and 4h fasting blood glucose levels were monitored weekly. A glucose tolerance test (GTT) (1 g/kg, i. p.) and a glucose stimulated insulin secretion test (1 g/kg, i. p.) were performed 3 weeks after AAV injection. Plasma insulin and proinsulin levels were measured with commercially available ELISA kit. Pancreatic islets were collected for RNA and protein extraction 4 weeks post AAV injection.

Results: $Ins2^{Akita/WT}$ mice with beta-cell specific eIF2A overexpression had lower fasting blood glucose levels compared to GFP control mice at 2 weeks (11.3±0.8 vs 17.1±1.0 mmol/l, p=0.0022) and 3 weeks (12.8±1.0 vs 19.2±0.4 mmol/l, p=0.0003) after AAV6 ductal injections. As expected GFP control $Ins2^{Akita/WT}$ mice showed increased fasting blood glucose levels with age, but this increase was attenuated by eIF2A overexpression in beta cells. No significant difference in body weight gain was observed between groups at any time point. Three weeks after viral injection, $Ins2^{Akita/WT}$ mice overexpressing beta-cell specific eIF2A showed significantly improved glucose tolerance in comparison to GFP overexpressing $Ins2^{Akita/WT}$ mice (AUC 2646±97 vs 3208 ±85 respectively, n=5, p=0.0024). Overexpression of eIF2A in $Ins2^{Akita/WT}$ was associated with increased insulin secretion at 15 min post glucose challenge (0.73±0.09 vs 0.47±0.07 ng/ml, n=4, p=0.04) when compared with controls infected with GFP alone. In fed state, no significant difference in plasma insulin (0.20±0.06 vs 0.23±0.04 ng/ml, n=5, p=0.7) or proinsulin levels (13.7±5.2 vs 10.9±4.7 pmol/l, n=5, p=0.7) were detected between eIF2A-GFP and control GFP expressing $Ins2^{Akita/WT}$ mice. The evidence of the specific molecular mechanisms involved in this remarkable beta-cell protection was further provided using qPCR and Western Blotting analysis of pancreatic islets.

Conclusion: We conclude that overexpression of eIF2A in pancreatic beta cells prevents progression of diabetes and potentiates glucose stimulated insulin secretion in $Ins2^{Akita/WT}$ mice under the conditions of our study.

Supported by: CDA Operating Grant, JDRF Postdoctoral Fellowship

Disclosure: **E. Panzhinskiy:** None.

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Human pancreatic beta cell dedifferentiation

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Background and aims: Clinical and experimental evidences indicate a reduced functional β-cell mass in type 2 diabetes. A recent hypothesis implicates β-cell dedifferentiation in this reduction of functional β-cell mass. Majority of

data related to β-cell dedifferentiation derive from rodent models, and only indirect evidences are available in human. Our goal was to study human β-cell dedifferentiation using the functional human pancreatic β-cell line, EndoC-βH1, and primary human pancreatic islets.

Materials and methods: We first screened for molecules that decrease insulin and MAFA gene expression in EndoC-βH1 cells. We next searched for additional markers of dedifferentiation both in EndoC-βH1 cells and in primary human islets, and studied whether dedifferentiated cells could be protected from extra-cellular signals.

Results: By screening a number of molecules, we first found that FGF2 treatment dramatically reduces insulin production and MAFA expression, a beta cell specific transcriptional activator in EndoC-βH1 cells. RNASeq of EndoC-βH1 cells treated with FGF2 revealed the down-regulation of additional β-cell specific markers, including MAFB, SLC2A2, SLC30A8, GCK. In parallel, FGF2 treatment activated the expression of β-cell disallowed genes. This is the case for transcription factors such as MYC, HES1, SOX9 and NEUROG3. This is also the case for hormones such as GASTRIN and PYY. Such data were further confirmed by qPCR and immunostaining on primary human islets, attesting that dedifferentiation process occurs in human beta cells. We also showed that FGF2-induced dedifferentiation is time- and dose-dependent, and is reversible upon wash-out. Furthermore, transcriptomic analysis revealed an increase of TNFRSF11B (osteoprotegerin) expression upon FGF2 treatment. TNFRSF11B is a decoy receptor for the receptor activator of nuclear factor kappa B ligand (RANKL). Our experimental data on EndoC-βH1 demonstrated that FGF2-induced TNFRSF11B and protected beta cells against TNFSF11 (RANKL) signaling by preventing P38 phosphorylation.

Conclusion: We developed a robust model to study β-cell dedifferentiation in a human context. We discovered SOX9, HES1 and MYC as positive markers of human β-cell dedifferentiation, demonstrating for the first time direct evidence for dedifferentiation process in human beta cell. Finally, we observed that beta-cell dedifferentiation prevents against activation of the RANKL pathway.

Disclosure: **F. Chimienti:** None.

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Metformin restores insulin secretion from palmitate-treated human islets by normalising mitochondrial metabolism and reducing ER stress and apoptosis

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Background and aims: Insulin levels in the blood of obese individuals are elevated, which has been related to high concentrations of free fatty acids (FFAs). In long-term, hyperinsulinemia causes exhaustion of beta cells, insufficient insulin secretion and development of type 2 diabetes mellitus (T2DM). In line with this, experiments on isolated human islets showed that palmitate treatment accentuated glucose-stimulated insulin secretion (GSIS) at earlier time points and caused beta cell dysfunction after prolonged palmitate exposure. Metformin is the first-line drug for the treatment of T2DM, in particular, in overweight and obese individuals. However, it remains controversial whether metformin has a beneficial effect on FFA-treated beta cells. Here, we investigated if metformin prevents beta cell dysfunction induced by chronically elevated palmitate levels.

Materials and methods: Study was performed on isolated human pancreatic islets. Human islets were cultured in CMRL medium containing 5.5 mM glucose. For treatments, human islets were exposed to 0.5 mM palmitate with 0.5% BSA in the absence or presence of 25 μM metformin for 0, 2, and 7 days. After treatments, GSIS, intracellular insulin content, mitochondrial function (Extracellular Flux Analyzer XF96e), ER stress (pEIF2α and CHOP), and apoptosis (active caspase 3) were determined.

Results: GSIS from 2-day palmitate treatment was almost doubled compared with the control islets and this was accompanied by a 65% rise in oxygen consumption rate (OCR). After exposing the islets to palmitate for 7 days, GSIS was significantly lowered to 50% of the control islets but there was no difference in OCR compared with control islets. The introduction of metformin restored insulin secretion and OCR in islets exposed to palmitate for 2 days. After 7-day treatment with metformin palmitate-exposed islets showed restored GSIS but exerted no effect on OCR. Insulin content was decreasing during palmitate culture, from 70% of the control level after 2 days to 30% after culture for 7 days. The inclusion of metformin during palmitate culture had no effect on insulin content after 2 days, but almost doubled it after 7 days. Markers of ER stress (pEIF2α and CHOP) and apoptosis (active caspase 3) were not affected after 2 days but significantly increased after 7-day palmitate treatment. They were reduced to the control level in palmitate-exposed islets treated with metformin.

Conclusion: Metformin prevents palmitate-induced increase of GSIS from human islets after 2 days and decrease of GSIS after 7 days. These effects are associated with decreased mitochondrial metabolism at earlier time points and reduced ER stress and apoptosis at later time points.

Supported by: Beta-JUDO (EU FP7)

Disclosure: **J. Cen:** None.

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ER stress induces diabetes by impairing early postnatal beta cell proliferation

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Background and aims: ER stress plays an important role in the pathophysiology of type 1 and type 2 diabetes (T1D and T2D); it is widely believed that chronic ER stress contributes to reduced beta cell mass in diabetes, but the underlying mechanisms remain largely unknown. Akita diabetes is a genetic form of ER stress-induced diabetes resulting from a mutation in the proinsulin gene, leading to its irreparable misfolding. We studied the life-long changes in β-cell mass in Akita mice and the role of apoptosis, altered proliferation, or dedifferentiation in mediating β-cell dysfunction.

Materials and methods: Pancreatic sections were immunostained for islet hormones, transcription factors (PDX-1 and NKx6.1), proliferation markers (Ki67, PCNA and phospho-histone H3) and apoptosis (TUNEL). β-cell lineage tracing was performed using Cre-mediated labeling of differentiated beta cells. Mice metabolic tests included IPGTT and measurement of glucose-stimulated insulin secretion.

Results: In 2-3 months old diabetic Akita mice, β-cell mass was decreased by ~70% compared to age-matched controls. TUNEL labeling was rare and proliferation was observed in <1% of beta cells in both Akita and controls, indicating that altered beta cell mass at this stage reflects earlier events. Genetic lineage tracing showed that <2% of lineage-labeled β-cells were completely degranulated or mis-expressed somatostatin or glucagon without insulin, indicating minimal contribution of reprogramming to loss of beta cell mass. We therefore studied early postnatal β-cell dynamics. In control mice, during the first 3 weeks of life β-cell proliferation was ~10-fold higher than in adult animals, leading to a 3-fold increase in β-cell mass. In Akita mice, β-cell proliferation and the expression of Nkx6.1 and PDX-1 were decreased by ~50% and β-cell mass was reduced by 60% compared with controls at day 21. At this stage, mice were normoglycemic despite marked depletion of pancreatic insulin content, attenuation of insulin secretion, and glucose intolerance. Treatment of wildtype mouse islets with a low concentration of the SERCA inhibitor thapsigargin decreased the expression of cyclin D2, Pdx-1, Nkx6/1 and proinsulin.

Conclusion: ER stress during early postnatal life inhibits β-cell proliferation, possibly by reducing the expression of transcription factors required for β-cell proliferation and maturation, resulting in reduced beta cell mass and silent impairment of beta cell function. Later in life, these early postnatal defects give rise to overt diabetes. These findings may have important implications for the pathophysiology and treatment of T1D and T2D.

Disclosure: **G. Leibowitz:** None.

OP 18 Impact of exercise on metabolism

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Mini-dose glucagon as a novel approach to prevent exercise-induced hypoglycaemia in type 1 diabetes

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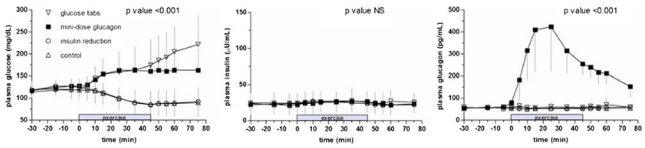
Background and aims: Patients with type 1 diabetes (T1D) who do aerobic exercise have a drop in blood glucose concentration that can result in hypoglycemia. Current approaches to prevent exercise-induced hypoglycemia include reduction in insulin delivery or ingestion of carbohydrates, but these strategies may still result in hypo- or hyperglycemia. We sought to determine whether mini-dose glucagon (MDG) given s.c. before exercise could prevent subsequent glucose lowering, and to compare the glycemic response to current approaches.

Materials and methods: We conducted a randomized, 4-period crossover trial involving 15 adults with T1D who exercised at ~55% VO_{2max} for 45 min with no intervention (control), 50% basal insulin reduction, 40 g oral glucose tabs, or 150 μg glucagon, all administered 5 min before exercise.

Results: During exercise, mean plasma glucose increased slightly with MDG compared to a decrease with control and insulin reduction, and with a greater increase with glucose tabs. Insulin levels were not different, while glucagon increased with MDG. Six subjects experienced hypoglycemia (< 70 mg/dl) during control and 5 during insulin reduction and none with glucose tabs or MDG; 5 subjects experienced hyperglycemia (≥250 mg/dl) with glucose tabs and 1 with MDG.

Conclusion: MDG may be more effective than insulin reduction for preventing exercise-induced hypoglycemia, and may result in less post-intervention hyperglycemia than ingestion of carbohydrate.



Clinical Trial Registration Number: NCT02660242

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Disclosure: **M. Rickels:** None.

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Evaluation of changes in cerebral oxyhaemoglobin during and after a 20-minute moderate-intensity cycling exercise: a near-infrared spectroscopy study

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Background and aims: Aerobic exercise is highly recommended for patients with type 2 diabetes mellitus to reduce or control their blood glucose level and body weight. During aerobic exercise, cortical activities change, and these changes can be measured noninvasively using near-infrared spectroscopy. Increased oxygenation (O_2Hb) of the cerebral prefrontal cortex during exercise and improved cognitive function following exercise have been observed. However, aftereffects of exercise on cerebral O_2Hb have not been well established. This study aimed to investigate changes in oxygenation during and after a 20-minute moderate-intensity cycling exercise.

Materials and methods: Twelve healthy volunteers (9 women, 3 men) participated in this study. After an incremental exercise test was performed on a cycle ergometer to determine the maximal oxygen consumption (VO_2peak), subjects performed a cycle ergometer exercise on a separate day. After a 3-minute pre-exercise rest, exercise was initiated at workloads corresponding to 50% VO_2peak for 20 minutes followed by a 15-minute post-exercise rest. O_2Hb levels in the right prefrontal cortex (R-PFC) and left prefrontal cortex (L-PFC) were measured using a near-infrared spectrometry system. O_2Hb levels of each area were expressed as changes from the mean pre-exercise rest phase values, and averaged values of the last 5 minutes during the exercise and post-exercise rest phases were calculated. One-way ANOVA was performed to compare these variables according to the factor of time.

Results: The O_2Hb level increased during the exercise and remained following exercise. However, the O_2Hb level changed throughout the experiment significantly in both areas (ANOVA, $p<0.01$). The O_2Hb level significantly increased from the pre-exercise rest phase to 6.60 ± 4.56 (a.u.) in the R-PFC (post-hoc test, $p < 0.01$) and to 8.50 ± 5.23 (a.u.) in the L-PFC (post-hoc test, $p < 0.01$) during the last 5 minutes of exercise. After the exercise, the O_2Hb level did not return to the pre-exercise value during the 15-minute post-exercise rest phase in both areas. The O_2Hb level in the L-PFC during the last 5 minutes of the post-exercise rest phase was 5.94 ± 4.65 (a.u.), which was significantly higher than that during the pre-exercise rest phase (post-hoc test, $p < 0.01$), and there was no difference in O_2Hb levels during exercise (post-hoc test, $p = 0.25$).

Conclusion: Oxygenation of the L-PFC persists at least 15 minutes after a 20-minute moderate-intensity aerobic exercise. The L-PFC plays an important role in improving cognitive task after moderate-intensity exercise. Our results suggest that improvement of cognitive function following exercise is based on increased oxygenation of the prefrontal cortex.

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Disclosure: **A. Tsubaki:** Grants; Grant-in-Aid for Scientific Research(C).

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Effects of short-term exercise training on intestinal metabolism and gut microbiota in subjects with insulin resistance

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Background and aims: Insulin-stimulated intestinal glucose uptake is impaired in obese and insulin resistant subjects but ameliorates after weight loss. We have previously shown in healthy sedentary men that already short-term exercise training improves insulin-stimulated intestinal glucose uptake. Whether training improves intestinal metabolism also in insulin resistance is unclear. Here we studied the effects of two different exercise training methods (moderate intensity continuous training (MICT)) and high intensity interval training (HIIT)) on intestinal metabolism in insulin resistant subjects. We also studied the effects of training on gut microbiota.

Materials and methods: Twenty six, sedentary subjects (IFG/IGT n=9, T2D n=17; males/females 16/10, age 49[SD 4] years; BMI 30.5[SD 3]) were randomized into HIIT and MICT for two weeks including six training sessions. Of T2D subjects 13 used glucose lowering medication. Intestinal (duodenum, jejunum, colon) insulin-stimulated glucose uptake (GU) and fasting free fatty acid uptake (FAU) from circulation were measured using PET and [^{18}F]FDG and [^{18}F]FTHA respectively. Fecal samples were successfully collected from eighteen subjects before and after the exercise intervention 16S rRNA gene sequencing (MiSeq). The medication (metformin/no-metformin) and gender were used as additional factors in statistical analyses.

Results: Body adiposity associated positively with intestinal FAU and inversely with aerobic capacity. In the whole study group whole-body insulin

sensitivity associated inversely with *Ruminococcus (r= -0.49, p= 0.054)* and *Blautia (r= -0.53, p= 0.04)* genus. Intestinal GU associated positively with microbiota diversity and Bacteroidetes phyla and inversely with *Dorea* genus. Both HIIT and MICT improved whole-body insulin sensitivity with no group differences (27%,p<0.05) while VO_{2peak} improved only after HIIT (5% p=0.01). FAU decreased in jejunum only after MICT [-34%; p=0.02]. Training had no effect on intestinal insulin stimulated GU. Both training modes increased the Firmicutes/Bacteroidetes ratio at phylum level (p=0.04) and decreased the levels of *Clostridium* and *Blautia* genus (both p=0.04) without group differences. Training also tended to increase the level of Bacteroidetes phyla (decreased in obesity) (p=0.06) and decrease the levels of genus belonging to *Dorea* and *Ruminococcus* (both p≤0.07) previously related to irritable bowel disease.

Conclusion: Two weeks of MICT decreases fasting free fatty acid uptake in jejunum and both HIIT and MICT enhance the gut microbiota profile in insulin resistance.

Clinical Trial Registration Number: NCT01344928

Supported by: EFSD/Novo Nordisk, SKR, Emil aaltosen Foundation, Orion Foundation

Disclosure: **K.K. Motiani:** Grants; Finnish cultural foundation, Orion foundation.

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Effect of aerobic and resistance exercise training on fat derived mesenchymal stromal cells (MSCs) in subjects with prediabetes

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Background and aims: The effect of aerobic exercise has been studied extensively using various inflammatory biomarkers. Previously, we have shown endothelial progenitor cells (EPCs) can act as a strong cellular biomarker of endothelial function following aerobic exercise as an intervention. In this study, we are examining the effect of aerobic exercise on adipocyte derived Msenchymal Stem Cells (MSCs) as a cellular surrogate of fat activity and metabolism.

Materials and methods: In this study overweight and obese subjects (n=10) were enrolled in a 12 week exercise intervention study. The bi-weekly exercise sessions were supervised by a trained exercise physiologist and consisted of a 1 hour sessions that included warm-up and cool-down and 30 min of combined aerobic and resistance training at an exercise intensity of 50-80% of heart rate reserve. The patients were also encouraged to be physically active during the rest of the week. Subcutaneous abdominal fat biopsies were obtained and fat derived stromal cells were cultured in vitro for 2-3 weeks. MSCs were analyzed for mRNA gene expression (qRT-PCR) and cellular oxygen consumption rate (OCR) by SeaHorse, pre and post 12 week exercise.

Results: With the intervention, gene expression analysis showed glucose transporter, Glucose Transporter [GLUT1] (p= 0.04), mitochondrial gene Cytochrome C oxidase [COX4] (p= 0.01), antioxidants superoxide dismutase [SOD3] and Glutathione Peroxidase [GPX3] (p values= 0.04, 0.03, respectively) upregulated significantly with a trend of improvement in other antioxidants such as Catalase [CAT] (p values= 0.07) and reduced expression of inflammatory gene Cyclo-oxygenase [COX2] pre vs post exercise. Unexpectedly, oxygen consumption rate of MSCs between pre and post exercise group, however did not showed any significant difference

Conclusion: Preliminary outcome analysis of this on-going study indicates that aerobic exercise modifies gene expression of MSCs. Exercise appears to augment certain mRNA expressions such as, cellular glucose transporters and cellular anti-oxidants with reduction in inflammatory marker gene cyclo-oxygenase expression in spite of no significant change in IL6 or TNF alpha. Though, no significant difference was observed between oxygen consumption rates in the MSCs, significant upregulation of cytochrome C oxidase gene, COX4, gene indicates better mitochondrial function of fat derived mesenchymal stromal cells, post exercise intervention. Our studies elucidates critical genes and pathways that appears to respond in fat derived stem cells post exercise, that may form a basis for pharmaceutical intervention in a population who may be unable to exercise

Disclosure: **E. Nylen:** None.

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Coordinated regulation of adipose tissue adrenergic- and non-adrenergic-mediated lipolysis during exercise in lean and obese individuals: the effect of exercise training

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Background and aims: Adipose tissue dysfunction, which includes impairments in (adipose tissue) lipolysis, contributes to insulin resistance. Subcutaneous adipose tissue (SCAT) lipolysis in obesity is characterized by catecholamine resistance and an impaired ANP responsiveness. It remains to be established whether exercise training improves non-adrenergically-mediated lipolysis, next to the adrenergic pathway, in metabolically compromised conditions. The aim of the present study was to investigate the effect of local combined α- and β-adrenergic receptor blockade on SCAT lipolysis in obese insulin sensitive (IS), obese insulin resistant (IR) and age-matched lean IS men. Moreover, obese individuals underwent endurance and resistance exercise training to improve metabolic profile and (non-)adrenergically-mediated SCAT lipolysis.

Materials and methods: Abdominal SCAT lipolysis was investigated in 10 obese IS, 10 obese IR and 10 age-matched lean IS men using microdialysis in the presence or absence of local combined α- and β-adrenergic receptor blockade (100 μmol/l phentolamine and 100 μmol/l propranolol) at rest, during 60 min of low-intense (40% VO_{2max}) endurance-type exercise and recovery. Systemic responses were investigated using venous blood sampling. Obese individuals participated in a supervised, endurance and resistance exercise training intervention for 12 weeks (3 sessions/week) after which the microdialysis measurements were repeated in obese IR men.

Results: The exercise-induced increase in abdominal SCAT glycerol concentrations (expressed as total area under the curve) was more pronounced in obese IS (81%) and IR (34%) as compared to lean individuals (P_{group}=0.012). Abdominal SCAT lipolysis was significantly reduced (by ~40%, P=0.020) following local combined α-/β-adrenoceptor blockade in obese IS individuals only. Despite significant improvements in body composition, physical fitness and exercise-induced changes in circulating free fatty acids, lactate and adrenalin, exercise intervention did not significantly affect (non-)adrenergically-mediated lipolysis in abdominal SCAT of obese IR individuals.

Conclusion: Our findings indicate a major contribution of non-adrenergically-mediated lipolysis during exercise in abdominal SCAT of lean and obese individuals. Furthermore, a 12-week exercise training program improved metabolic profile and body composition in obese individuals, but did not affect abdominal SCAT lipolysis.

Clinical Trial Registration Number: NCT02418728

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Disclosure: **R. Stinkens:** Grants; Research Foundation - Flanders (FWO) (KAN 1507217N).

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Two weeks of exercise training improves bone marrow metabolism

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Background and aims: Bone marrow produces blood cells and acts as fat storage. Exercise training improves bone mineral density but little is known about the effects of training on bone marrow metabolism. We studied the effects of short-term high-intensity interval (HIIT) and moderate-intensity continuous training (MICT) on bone marrow metabolism.

Materials and methods: We randomized 54 sedentary subjects of whom 28 were healthy (BMI 26,1 (2,4); age 48 (5)) and 26 had insulin resistance (IR) (8 IGT/IFG and 18 T2D; BMI 30,1 (2,5); age 49(4)) into HIIT and MICT for two weeks. Thoracic vertebral (Th1-Th4), abdominal vertebral (Th12-L3), and femoral bone marrow glucose uptake (GU) were measured during euglycemic hyperinsulinemia and fasting free fatty acid uptake (FFAU) using PET.

Results: At baseline, GU was highest in abdominal vertebral, followed by thoracic vertebral, and lowest in femoral bone marrow (all $p<0.001$). FFAU was higher in abdominal vertebral and thoracic vertebral than in femoral bone marrow (both $p<0.001$). Femoral and abdominal vertebral bone marrow FFAU was higher in healthy compared to IR men ($p=0.02$ and $p=0.002$ respectively) and higher in females than males ($p<0.001$ and $p=0.02$ respectively). Training increased whole-body insulin sensitivity in the whole group and while both training modes increased aerobic capacity in healthy ($p=0.001$) only HIIT improved aerobic capacity in IR men ($p=0.0135$). Training improved femoral bone marrow GU but we found no statistically significant differences between training modes, IR and healthy men, or males and females. MICT decreased FFAU in abdominal bone marrow ($p<0.001$).

Conclusion: Bone marrow substrate uptake differs regarding anatomical region. Short-term training improves bone marrow metabolism in healthy and IR subjects.

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Disclosure: **R.A. Ojala:** None.

OP 19 Novel therapies: future opportunities

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Potent body weight loss, and therapeutic efficacy in a NASH animal model by a novel long-acting GLP-1/Glucagon/GIP tri-agonist (HM15211)

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Background and aims: Obesity and related complications are an increasing public health issue. Existing therapeutic options have limited effectiveness as well as requiring daily injections. Recent clinical and pre-clinical advances indicate that simultaneous targeting more than one GI hormone signaling pathway could lead to improved therapeutic efficacy with fewer adverse events. Thus, we have developed a long-acting GLP-1/glucagon/GIP triple-agonist, HM15211. HM15211 consists of a triple-agonist peptide (TA211) conjugated to the human aglycosylated Fc fragment via a short PEG linker. TA211 is a modified glucagon analog which leads to binding and activation of all three receptors (GLP-1R/glucagonR/GIPR). The human aglycosylated Fc fragment conjugated to TA211 could provide prolonged PK/PD properties. Here, we investigated in vitro properties and therapeutic efficacy of HM15211 for obesity and nonalcoholic steatohepatitis (NASH) in rodent disease models.

Materials and methods: To evaluate triple-agonistic property of HM15211, cAMP accumulation potency was evaluated in CHO cells stably expressing either the human GLP-1 receptor, the glucagon receptor, or the GIP receptor. For the obesity treatment study, diet-induced obesity (DIO) mice were chronically administrated with HM15211 over 4 weeks, and the body weight, food intake, as well as BG were monitored. At the end of treatment, liver TG and blood lipid profiles were determined. For energy expenditure measurement, each mouse was placed in cages for indirect calorimetry, followed by VO_2 and VCO_2 monitoring for 2 days. To evaluate the therapeutic potential treating NASH, methionine choline-deficient (MCD) diet mice, a well-established NASH model was utilized. After 4 weeks treatment, liver TG and the NAFLD activity score (NAS) were determined. Throughout all studies, liraglutide was used as comparative control.

Results: HM15211 induced intracellular cAMP accumulation via all three receptors with a high glucagon activity ratio. The potent glucagon action of HM15211 result in potent weight loss and improved lipid profiles by enhancing energy expenditure and lipid oxidation. In addition, balanced GLP-1/GIP action could neutralize the glucagon-induced hyperglycemic risk. In line with these results, 4 week treatment with HM15211 showed significantly improved BWL (~3.0 fold in DIO mice, ~2.4 fold in DIO rat compared to liraglutide) without hyperglycemia risk with similar food intake. In addition, liver TG and blood CHO level were significantly decreased. Of note, HM15211, but not liraglutide, significantly increased energy expenditure in DIO mice. According to RER (respiratory exchange ratio) value, enhanced energy expenditure by HM15211 primarily resulted from increased fat oxidation, confirming the glucagon action of HM15211 in obesity treatment. As to NASH treatment, HM15211 treatment reduced both liver TG and NAS (1.86 and 0 for liraglutide and HM15211, respectively) in MCD mice.

Conclusion: The unique GLP-1/glucagon/GIP activity of HM15211 could provide a favorable therapeutic profile as well as dosing convenience, compared to existing therapeutics in the treatment of obesity and obesity-related liver disease.

Disclosure: **I. Choi:** None.

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Weight loss unrelated to decrease in calorie intake after a single dose of a bispecific antibody to FGFR1/Klothoβ in obese subjects

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Background and aims: BFKB8488A is a humanized, effector-less, bispecific antibody designed to activate the receptor for fibroblast growth factor 21 (FGF21) in adipose tissue, the FGFR1/Klothoβ complex. Preclinical studies demonstrated that BFKB8488A largely recapitulates the metabolic actions of recombinant FGF21 in rodents and non-human primates. This Phase 1, first-in-human study was performed with obese but otherwise healthy subjects. The primary objectives were safety, tolerability, and pharmacokinetics. Additionally, insulin, glucose, lipids, and other metabolic markers were measured.

Materials and methods: Seventy-one subjects were randomized 3:1 to receive a single subcutaneous injection of escalating doses of BFKB8488A or placebo into the abdomen or thigh. All subjects were confined to a Phase 1 unit and provided a weight-adjusted diet between Day -2 and Day 8; BFKB8488A was administered on Day 1.

Results: The mean age of subjects at baseline was 44.6 ± 11.8 years, and mean baseline weight was 99 ± 12.2 kg. Fifty-eight percent of subjects were male and 45% were of Hispanic ethnicity. Over a wide range of dose levels, BFKB8488A was generally well tolerated. The most common adverse event reported was nausea, which was dose-dependent. BFKB8488A exhibited non-linear pharmacokinetics, with greater than dose-proportional increases in exposures. A significant ($p < 0.01$) dose-dependent decrease in body weight was observed during the confinement period with minimal weight change in the placebo group or the lower dose groups (Table 1). Notably, there was no change in calorie consumption by a visual estimation of percent consumption of standardized meals or in appetite as assessed by a visual analogue scale during the confinement period. To further test the mechanism of BFKB8488A-derived weight loss, subjects completed a food preference survey. In line with previously reported preclinical studies with FGF21 mimetics, a trend towards reduced preference for sweet foods was seen. Substantial dose-dependent improvements in cardiometabolic parameters (decreases in insulin, LDL cholesterol, and triglycerides; increases in HDL cholesterol and adiponectin) were observed, with changes peaking between Days 14 and 21 and persisting for up to 8 weeks post dose.

Conclusion: A single subcutaneous dose of BFKB8488A caused weight loss unrelated to loss of appetite or calorie intake and induced long-lasting metabolic effects similar to those seen with FGF21 mimetics. BFKB8488A may have potential utility in the treatment of obesity-related metabolic disorders.

Table 1. Percent change in mean body weight from Days 1–8 by cohort.

	Weight (kg)			
Cohort	Screening Mean (SD)	Day 1 Mean (SD)	Day 8 Mean (SD)	Change (%), Days 1–8 Mean (SD)
Placebo	102.31 (12.17)	101.06 (12.17)	100.65 (12.49)	-0.28 (0.70)
3 mg	99.79 (8.40)	98.49 (8.49)	96.23 (7.44)	-0.31 (0.78)
10.5 mg	99.05 (8.56)	98.50 (8.76)	97.73 (8.47)	-0.76 (0.70)
39 mg	104.45 (12.42)	103.98 (11.94)	103.15 (12.42)	-0.85 (0.78)
111 mg	99.55 (14.80)	97.88 (15.18)	96.83 (15.93)	-1.17 (1.42)
171 mg	101.40 (18.15)	100.20 (18.53)	99.28 (18.42)	-0.93 (0.98)
171 mg (thigh)	101.60 (10.67)	101.65 (10.23)	99.30 (9.83)	-2.30 (0.25)
250 mg (thigh)	86.62 (10.40)	86.40 (10.57)	85.17 (10.48)	-1.43 (0.97)
342 mg	94.12 (8.24)	92.73 (8.08)	91.20 (7.78)	-1.64 (0.61)
681 mg	103.32 (11.47)	101.88 (11.68)	99.72 (11.70)	-2.15 (0.77)

Note: all injections were given in the abdomen except for the indicated cohorts

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REMD-477, a human glucagon receptor (GCGR) antibody, reduces daily insulin requirements and improves glycaemic control in people with type 1 diabetes

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Background and aims: Studies conducted in rodent models of T1D have shown that glucagon receptor blockade normalizes plasma glucose concentration without the need for exogenous insulin.

Materials and methods: We conducted a randomized, double-blind, placebo (PBO) controlled trial in 21 subjects with T1D (8 men,13 women) to evaluate the effect of a single 70 mg SC injection of REMD-477, a human monoclonal antibody against the GCGR, on daily insulin requirements and glycemic control. After obtaining baseline insulin use and data from Continuous Glucose Monitoring (CGM), subjects were admitted to the Clinical Research Unit for 5 days. Insulin was provided by continuous IV infusion to maintain postabsorptive and postprandial plasma glucose between 90-120 mg/dl and <180 mg/dl, respectively. Standard meals were provided to ensure the same daily energy and macronutrient contents were consumed during the inpatient study. Drug/PBO was given on the second day of admission. The primary endpoint was the comparison between groups in the change in daily insulin requirements on day 4 from day 1.

Results: An interim analysis of the first 17 subjects found REMD-477 treatment reduced daily insulin use by 32% (4.2%, 60%) vs PBO on Day 4 (p=0.027). Average daily glucose assessed by CGM was 19 mg/dL (6.2, 31; p=0.006), and 26 mg/dL (8.2, 45; p=0.008) lower in the REMD-477 group than in the PBO group at Weeks 2 and 3 after treatment, respectively. Glucose time-in-range (70-180 mg/dL) for REMD-477 was 9.5% (2.7%, 16%; p=0.009) and 13% (1.9%, 25%; 0=0.026) greater in the REMD-477 group than in the PBO group during Weeks 2 and 3 after treatment, respectively. REMD-477 therapy was well tolerated and no episodes of severe hypoglycemia were noted.

Conclusion: These data demonstrate that a single SC injection of REMD-477 reduces daily insulin requirements while simultaneously improving glucose control, as measured by average glucose concentration and glucose time-in-range, without increasing hypoglycemia in subjects with T1D.

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Disclosure: **J. Pettus:** Employment/Consultancy; Sanofi-Aventis, Novo Nordisk, Valeritas, Insulet, Mannkind, Sensionics, Dexcom. Lecture/other fees; Sanofi-Aventis, Valeritas.

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Beyond topline results for the oral (non-peptide) GLP-1R agonist TTP273 in type 2 diabetes: How much and when?

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Background and aims: The widespread use of GLP-1 analogues/mimetics that provide glycemic control and weight loss may be hindered by the route of administration and by the high incidence of gastrointestinal side effects. TTP273 is an oral, non-peptide, GLP-1R agonist. The aims of this Phase 2 study were to demonstrate 1) competitive HbA_{1c} reduction, 2) weight loss, and 3) negligible GI side effects following 12 weeks of dosing with TTP273.

Materials and methods: Safety and efficacy were evaluated in a 12-week, multi-center Phase 2, double-blind, placebo-controlled study where 174 patients with Type 2 diabetes (T2DM) on stable doses of metformin were randomized to either placebo or TTP273 (150 mg once (QPM) or twice (BID) daily). Statistical analyses for topline results included Placebo (PBO)-subtracted change from baseline (LSM-CFB) for HbA_{1c} (primary endpoint) and body weight (secondary endpoint) as well as a post hoc concentration/effect analysis to evaluate the effect of exposure on efficacy.

Results: Baseline characteristics were well balanced amongst groups with an overall mean age of 56 years, mean HbA_{1c} of 8.6%, and mean BMI of 32 kg/m^2. TTP273 was well tolerated with no severe hypoglycemia Adverse Events

(AEs). The most common Treatment Emergent AEs was diarrhea (2%, 3% and 12% in PBO, 150mg QPM and 150mg BID, respectively), mostly mild in intensity, with only one subject discontinued due to mild diarrhea (150 mg BID). Less nausea was observed in active groups than placebo and the only incidence of vomiting occurred with placebo dosing. The once and twice daily treatment arms of TTP273 demonstrated placebo-subtracted decreases from baseline in HbA_{1c} and weight at 12 weeks of (i) 0.9 ± 0.2 % ($p<0.001$) and 0.7 ± 0.2 % ($p<0.001$), respectively, and (ii) 0.9 ± 0.5 kg ($p=0.08$) and 0.6 ± 0.5 kg, respectively. Subsequent concentration/effect analysis of completers reveals that lower doses demonstrated more pronounced effects for key efficacy endpoints. This observation is confirmed when correlating TTP273 plasma concentration with efficacy. Patients dosed with lower than 1.35mg/kg of TTP273 had greater efficacy with placebo-subtracted reductions from baseline in HbA_{1c} and body weight of 1.7% ($p<0.01$) and 3.7 kg ($p<0.05$), respectively, at 12 weeks.

Conclusion: All three aims of the study were achieved, suggesting that TTP273 can be efficacious without inducing the side effects of nausea and vomiting. Concentration/effect analysis suggests that lower doses showed more pronounced effects for key efficacy endpoints. Similar findings were observed in the Phase 2 study with our predecessor agonist (TTP054). This surprising result may be explained by two significant differences between TTP273 and GLP-1 that may reduce its effect/increase tolerance at higher exposure levels: 1) TTP273 can signal directly in the intestine (a response known to desensitize over time) and 2) TTP273 has a differential signaling pattern that does not signal through β-arrestin and seems to have sustained activation relative to GLP *in vitro*. These results support the conduct of additional clinical investigation using lower doses to evaluate the efficacy of TTP273.

Clinical Trial Registration Number: NCT02653599

Disclosure: **J.L.R. Freeman:** Employment/Consultancy; vTv Therapeutics LLC. Stock/Shareholding; vTv Therapeutics LLC.

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IDegLira improves cardiovascular risk markers in patients with type 2 diabetes uncontrolled on basal insulin: analyses of DUAL II and DUAL V

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Background and aims: The efficacy and safety of insulin degludec/liraglutide (IDegLira) has been demonstrated in patients with type 2 diabetes (T2D) uncontrolled on basal insulin, with superior HbA_{1c} reductions vs. basal insulin. The cardiovascular (CV) benefit of liraglutide vs. placebo has also been shown in the LEADER CVoutcomes trial. This post-hoc analysis examined the effect of IDegLira vs. insulin degludec (IDeg; DUAL II) and vs. insulin glargine U100 (IGlar U100; DUAL V), both with metformin for 26 weeks, on CV risk markers.

Materials and methods: DUAL II was a 26-week, randomised, double-blind, treat-to-target trial; 413 patients with T2D (HbA_{1c} 7.5-10.0%) on basal insulin (20-40 U) and metformin with or without sulfonylurea/glinides were randomised 1:1 to once-daily IDegLira or IDeg (maximum dose 50 U). DUAL V was a 26-week, randomised, open-label, treat-to-target trial; 557 patients with T2D (HbA_{1c} 7.0-10.0%) treated with IGlar U100 (20-50 U) and metformin were randomised 1:1 to once-daily IDegLira or IGlar U100.

Results: In both trials, there was a greater decrease in systolic blood pressure with IDegLira (Table), and small but statistically significant increases in mean heart rate were observed with IDegLira vs. insulin comparators (both $p<0.001$; Table). IDegLira was associated with weight loss vs. weight gain with insulin comparators (estimated treatment difference [ETD] -2.5kg [-3.2; -1.8]$_{95\%CI}$ $p<0.0001$ and ETD: -3.2kg [-3.8; -2.6]$_{95\%CI}$ $p<0.05$ for DUAL II and DUAL V respectively). Lipid profile improved with IDegLira in both trials; total cholesterol and low-density lipoprotein (LDL) cholesterol were significantly lower vs. insulin comparators (Table). In DUAL II, apolipoprotein B (Apo-B) and brain natriuretic peptide (BNP) were significantly lower with IDegLira vs. IDeg (estimated treatment ratio [ETR] 0.92 [0.88; 0.95]$_{95\%CI}$ $p<0.0001$ and 0.66 [0.55; 0.79]$_{95\%CI}$ $p<0.0001$ respectively), while high-sensitivity C-reactive protein (hsCRP) was similar after 26 weeks of treatment (ETR 0.90 [0.78; 1.04]$_{95\%CI}$ p = non-significant [NS]).

Conclusion: In conclusion, IDegLira is associated with a general improvement in CV risk markers vs. basal insulin therapy after 26 weeks of treatment, which is likely attributable to the liraglutide component.

Table. Cardiovascular markers in DUAL II and DUAL V

	DUAL II (NCT01392573)			DUAL V (NCT01952145)		
	IDegLira (N=199)	IDeg (N=199)	IDegLira vs. IDeg [95% CI] *p*-value	IDegLira (N=278)	Continued IGlar U100 up-titration (N=279)	IDegLira vs. IGlar U100 [95% CI] *p*-value
Δ Heart rate, bpm	2.5	−0.6	ETD 2.9 [1.4; 4.5]*	3.1	−0.2	ETD 3.7 [2.3; 5.1]*
Δ Systolic blood pressure, mmHg	−5.4	−1.7	ETD −3.7 [−6.1; −1.3]*	−3.7	−0.2	ETD −3.6 [−5.5; −1.6]*
Δ Diastolic blood pressure, mmHg	−1.4	−0.7	ETD −0.7 [−2.1; 0.7], NS	−0.8	−1.4	ETD 0.9 [−0.3; 2.1], NS
BL/EOT: Total cholesterol, mmol/L†	4.6/4.3	4.6/4.5	ETR 1.0 [0.9; 1.0]*	4.6/4.4	4.5/4.6	ETR 1.0 [0.9; 1.0]*
BL/EOT: HDL cholesterol, mmol/L†	1.1/1.1	1.2/1.2	ETR 1.0 [1.0; 1.0], NS	1.2/1.2	1.2/1.2	ETR 1.0 [1.0; 1.0], NS
BL/EOT: LDL cholesterol, mmol/L†	2.5/2.2	2.4/2.4	ETR 0.9 [0.9; 1.0]*	2.5/2.3	2.4/2.5	ETR 0.9 [0.9; 1.0]*
BL/EOT: VLDL cholesterol, mmol/L†	0.8/0.7	0.8/0.7	ETR 1.0 [0.9; 1.1], NS	0.8/0.7	0.8/0.8	ETR 0.9 [0.9; 1.0], NS
BL/EOT: Triglycerides, mmol/L†	1.8/1.6	1.8/1.6	ETR 1.0 [0.9; 1.1], NS	1.7/1.6	1.7/1.7	ETR 0.9 [0.9; 1.0], NS
BL/EOT: Free fatty acids, mmol/L†	0.5/0.4	0.5/0.4	ETR 1.0 [0.9; 1.1], NS	0.5/0.4	0.4/0.4	ETR 0.9 [0.8; 0.9]**

*$p<0.05$; **$p<0.0001$; †geometric mean;
Data based on the full analysis set, with the exception of mean heart rate, which is based on safety analysis set. Change in heart rate and lipids were analysed using an ANCOVA model. For lipids, the responses were log-transformed before analysis. Missing data are imputed using last observation carried forward.
ANCOVA, analysis of covariance; BL, baseline; bpm, beats per minute; CI, confidence interval; EOT, end of trial (after 26 weeks); ETD, estimated treatment difference; ETR, estimated treatment ratio; IDeg, insulin degludec; IDegLira, insulin degludec/liraglutide; IGlar U100, insulin glargine 100 units/mL; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NS, not significant; VLDL, very-low-density lipoprotein.

Clinical Trial Registration Number: DUAL II: NCT01392573, DUAL V: NCT01952145

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Disclosure: **T. Vilsbøll:** Employment/Consultancy; Amgen, Boehringer Ingelheim, Eli Lilly, Astra Zeneca, MSD, Sanofi, Novo Nordisk.

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MEDI4166, a novel antibody (PCSK9)-peptide (GLP-1) fusion molecule: single-ascending-dose study in patients with type 2 diabetes

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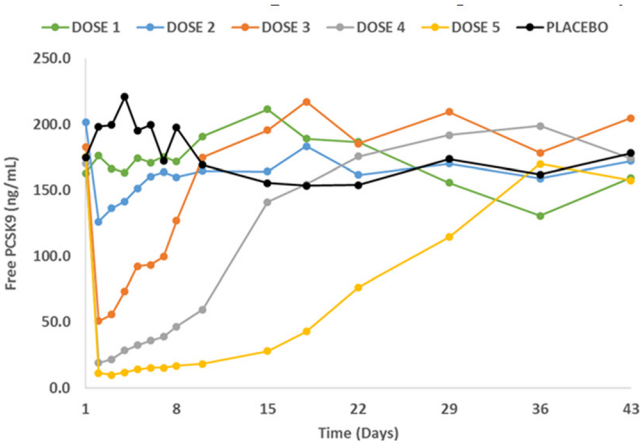
Background and aims: Glucagon-like peptide-1 (GLP-1) agonists are an attractive therapy for type 2 diabetes mellitus (T2DM), having proven effects on glycemic control and, for some, showing positive effects on cardiovascular (CV) outcomes. High-intensity statin therapy is recommended in T2DM patients with established CV disease (CVD) and those at increased CV risk (eg, LDL cholesterol [LDL-c] ≥100 mg/dL, family history of premature CVD). However, some T2DM patients at high CV risk may require additional lowering of LDL-c or may be intolerant to high-intensity statin therapy. Blockade of antiproprotein convertase subtilisin/kexin type 9 (PCSK9) has emerged as an effective method to lower LDL-c and to decrease risk of CV events by decreasing degradation of the LDL receptor. MEDI4166 is a novel antibody-peptide genetic fusion molecule comprising a PCSK9 antibody and a GLP-1 analogue linked to the *N* terminus of the antibody light chain by a peptide linker, and therefore has the potential to effectively lower glucose and LDL-c with a single molecule. This first-in-human study investigated the safety and pharmacokinetics (PK) of single ascending-doses of MEDI4166 in patients with T2DM.

Materials and methods: In this multicenter, double-blind, placebo-controlled, single-ascending-dose study, 40 adult T2DM subjects receiving metformin monotherapy and LDL-c ≥70 mg/dL were randomized to 1 of 5 cohorts receiving SC MEDI4166 or placebo (MEDI4166, $n = 6$; placebo, $n = 2$ in each cohort). MEDI4166 dose was increased by approximately 0.5 log fold increments between cohorts. After dosing on Day 1, subjects were followed for 6 weeks for safety, including immunogenicity (ADA) and PK. Pharmacodynamic (PD) measures (free PCSK9 levels and GLP-1 activity measured by GLP-1 receptor activation assay) were also assessed throughout the study.

Results: Of 40 randomized subjects, 39 completed the study. MEDI4166 was well tolerated; AEs occurred in 20 (66.7%) MEDI4166 subjects and 5 (50%) placebo subjects. GI symptoms were the most common treatment-related AE in both groups (MEDI4166: n = 5 [16.7%]; placebo: n = 3 [30%]); no dose-dependent relationship was observed. No significant laboratory, vital sign, or ECG abnormalities were identified. PK were nonlinear across the dose range and allowed for weekly dosing. Of 30 subjects, 8 (27.6%) receiving MEDI4166 showed treatment-induced or treatment-boosted ADA response. No significant change in PK or PD was observed between ADA-positive and ADA-negative subjects. Free PCSK9 levels declined in a dose-dependent manner (Figure). GLP-1 activity was confirmed at the higher doses by GLP-1 receptor activation bioassay.

Conclusion: In this single-ascending-dose study in patients with T2DM, MEDI4166 had an acceptable safety profile, and dual target engagement was observed. Further investigation of this molecule in a multiple-ascending-dose study is ongoing.



Clinical Trial Registration Number: NCT02524782
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Disclosure: **M. Jain:** Employment/Consultancy; MedImmune.

OP 20 Retinopathy, pathophysiology and new therapeutic targets

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Understanding of the mechanisms involved in vascular leakage in diabetic retinas with glial activation: a proteomic approach

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Background and aims: Glial activation (GA) or reactive gliosis is an early event in the pathogenesis of diabetic retinopathy (DR), which participates in early events of microvascular impairment. One of the most important features in the early stages of DR is the disruption of the blood-retinal barrier (BRB), which results in vascular leakage. BRB is maintained by cell-cell adhesions and cell-matrix adhesion, with the support of glial cells. Most of the cellular and molecular knowledge on the mechanisms of BRB breakdown comes from experimental models and little is known regarding the underlying mechanisms linking GA and vascular leakage by examining the retinas from diabetic patients. In the setting of a general study aimed at identifying new therapeutic targets for early stages of DR by means of a proteomic approach using human retinas, we have herein focused on proteins that are involved in the maintenance of BRB.

Materials and methods: Human retinal samples were obtained from 10 type 2 diabetic donors without (n=5) and with (n=5) GA, and 5 non-diabetic donors (control group). All groups were matched by age. Diabetic donors did not presented microcirculatory abnormalities in the ophthalmoscopic examinations performed during the two years before death. Retinal lysates from each group were pooled and run on an SDS-PAGE gel. Bands were analyzed sequentially by LC/MS using an Orbitrap Mass Spectrometer.

Results: The retinas with GA presented a significant increase of ERM (ezrin, radixin moesin) proteins and fragments of Rho GDP dissociation inhibitor (RhoGDI) compared with those without GA or the control group ($p<0.01$). ERM proteins regulate cytoskeleton conformation directly (by binding actin filaments) and indirectly (binding RhoGDI, which allows Rho GTPases activation). These pathways when activated induce endothelial contractility, favouring intercellular junction opening and paracellular permeability, which is one of the mechanisms implicated in vascular leakage. On the other hand, isoform A2 of the tight junction protein ZO-2 and the proteins talin-1 and talin-2 (which participate in focal adhesions between the endothelial cells and the extracellular matrix) were significantly reduced in retinas with GA compared with those without GA or controls ($p<0.01$). These findings were associated with a significant increase in albumin and immunoglobulins ($p<0.01$), thus indicating the presence vascular leakage.

Conclusion: ERM proteins and RhoGDI are increased in early stages of DR and could be implicated in vascular leakage by inducing changes in the cytoskeleton conformation of endothelial cells. The reduction in ZO-2, talin-1 and talin-2 proteins observed in retinas from patients with GA could contribute to vascular leakage by means of loss of tight junction and focal adhesions. However, further studies are required for elucidating the mechanisms involved in the regulation of these proteins, as well as their role in the crosstalk between GA and vascular leakage.

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Disclosure: **O. Simo-Servat:** None.

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The evaluation of ocular changes in prediabetic individuals

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Background and aims: Prediabetes is the major risk factor for type 2 diabetes, but the prediabetic state itself is also associated with classical macrovascular

and microvascular complications. Studies indicate that other ophthalmic abnormalities can develop during the stage of prediabetes, however data on the occurrence of ocular changes are limited. The aim of our study was to evaluate ocular changes in prediabetic individuals.

Materials and methods: 60 subjects (40 women, 20 men) aged 37-78, with impaired fasting glucose and/or impaired glucose tolerance, were enrolled in the study and compared to 30 volunteers (20 women, 10 men) without prediabetes, aged 39-75. Both groups underwent a complete physical examination, biochemical tests and ophthalmic examination: visual acuity testing, colour vision and letter contrast sensitivity tests, anterior and posterior segment evaluation, intraocular pressure measurement, fundus photographs and optical coherence tomography. Prediabetic patients underwent examinations twice: on the 1st visit and on the 2nd visit after 9 months period.

Results: The prediabetic group was characterised by statistically higher level of HbA1c, HOMA-IR, increased occurrence of BMI≥25 kg/m² and hypertension as compared to healthy controls (table). Patients with IFG and/or IGT had increased prevalence of cataract as compared to control group (31.7% vs 6.7%; p<0.05), significantly higher incidence of corneal surface disorders (21.7% vs 3.3%; p<0.05), posterior vireous detachment (PVD) (76.7% vs 55%; p<0.05), arteries narrowing (81.7% vs 63.3%; p<0.05) and hypertension angiopathy (70% vs 36.7%; p<0.05). There was also difference in prevalence rate of retinopathy between prediabetic and control groups (8.3% vs 3.3%; NS), as well as acquired colour vision impairment (8.3% vs 0%, NS), but results were not statistically significant. We found association between increased rate of arteries narrowing and hypertension. Higher BMI (≥25 kg/m²) was independent factor of PVD in prediabetic patient. There was association between cataract and older age. Comparing visit 1 to visit 2 statistically significant differences were observed in fasting plasma glucose level (106.9 vs 104.1 mg%; p<0.05) and HbA1c (5.80 vs 5.99%; p<0.05). There was no statistically significant difference in ocular changes, however increased prevalence of retinopathy signs were noted on examination after 9 months period (8.3% vs 12.7%; NS).

Conclusion: Prediabetic subjects present increased prevalence of ocular disorders as compared to healthy population. Results of this study indicate that prediabetic state is the independent risk factor of several ophthalmic complications, although many patients with prediabetes have other features of metabolic syndrome. The regular ophthalmic monitoring seems to be essential at the stage of prediabetes in order to detect ocular abnormalities and identify individuals at risk of other diabetic complications.

Parameter	Visit 1 Prediabetic group (n 60)	Visit 1 Control group (n 30)	P value	Visit 2 Prediabetic group (n 55)	P value
BMI [kg/m²]	30.65 ±4.8	27.47 ±4.1	<0.05	30.71 ±4.7	NS
Arterial hypertension	70%	40%	<0.05	72.7%	NS
HbA1c [%]	5.80 ±0.38	5.56 ±0.22	<0.05	5.99 ±0.45	<0.05
HOMA-IR	3.30 ±2.2	1.94 ±0.96	<0.05	3.25 ±2.1	NS
Superficial keratopathy	21.7%	3.3%	<0.05	23.6%	NS
Cataract	31.7%	6.7%	<0.05	30.9%	NS
Retinopathy	8.3%	3.3%	NS	12.7%	NS
Hypertension angiopathy	70%	36.7%	<0.05	72.7%	NS
Impaired colour vision	8.3%	0%	NS	10.9%	NS
Posterior vitreous detachment (PVD)	76.7%	55%	<0.05	76.4%	NS
Epiretinal membrane (ERM)	6.7%	3.3%	NS	7.3%	NS

NS – statistically not significant

Disclosure: **A. Sokolowska-Oracz:** None.

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Topical administration of Dipeptidyl Peptidase 4 (DPP-4) inhibitors prevents retinal neurodegeneration in experimental diabetes

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Background and aims: Glucagon-like peptide 1 (GLP-1) and GLP-1 receptor (GLP-1R) have recently been found in the human retina, and topical administration of GLP-1R agonists prevents retinal neurodegeneration and vascular leakage in experimental diabetes. Since GLP-1 is rapidly degradable by DPP-IV it is possible that DPP-IV inhibitors can enhance the beneficial effects of retinal GLP-1. The main aims of the present study are: a) To determine whether the topical administration (eye drops) of DPP-IV inhibitors is able to reduce GLP-1 degradation, thus preventing retinal neurodegeneration and vascular leakage in db/db mice; b) To investigate whether, as occurs with GLP-1, the reduction of glutamate mediated excitotoxicity is among their mechanisms of action.

Materials and methods: Eye-drops containing sitagliptin, saxagliptin or vehicle were administered for 14 days (twice per day) to db/db mice (n=10 per group). Neurodegeneration was assessed by measuring glial activation (GFAP-IF), the rate of apoptosis (TUNEL) and ERG (Ganzfeld) features. Vascular leakage was examined by assessing the extravasated albumin. The retinal content of GLP-1 and its downstream effector AMPc were assessed before and after the administration of DPP-IV inhibitors. Glutamate and GLAST were determined by HPLC and IF, respectively.

Results: Topical treatment with saxagliptin or sitagliptin prevented glial activation, apoptosis and vascular leakage induced by diabetes. In addition, they also significantly prevented the diabetes induced functional abnormalities in the ERG. A significant increase of GLP-1 was found after treatment with either saxagliptin or sitagliptin. Furthermore, a dramatic prevention of GLAST downregulation induced by diabetes was found, which resulted in a significant reduction of extracellular concentration of glutamate. All these effects were observed without any change in blood glucose levels and, therefore, they cannot be attributed to changes in the diabetic milieu. However, activation of other pathways unrelated to GLP-1R cannot be ruled out.

Conclusion: Topical treatment with DPP-IV inhibitors prevents the neurodegenerative process, as well as vascular leakage, that occur in early stages of diabetic retinopathy (DR). This non-invasive treatment can be contemplated (alone or in combination with GLP-1) as a new strategy for treating DR.

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Disclosure: **C. Hernández:** None.

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Growth factor gene therapy as a therapeutic approach for diabetic retinopathy

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Background and aims: Diabetic retinopathy (DR) is a serious complication of diabetes mellitus (DM) and its prevalence has been increasing worldwide. Early diabetic retinopathy is characterized by a loss of pericytes from retinal capillaries, the appearance of acellular capillaries and microaneurysms, and a breakdown of the blood-retinal barrier. In later stages, this can evolve into the proliferative phase in which there is neovascularization of the retina and vision loss. Aside from pathological damage, treatments such as laser photocoagulation and vitrectomy, applied in advanced disease stages, have severe side effects and further damage the visual field. Additionally, protein-based anti-angiogenic agents used to delay progression of neovascularization require frequent local administration, increasing the risk of retinal detachment, vitreous hemorrhage, and cataract formation. Studies in human diabetic retinas have revealed an imbalance between pro-angiogenic factors such as the vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) and anti-angiogenic factors, like the pigment epithelial-derived factor (PEDF). This imbalance favoring pathological angiogenesis contributes to DR, and can thus constitute a therapeutic target for gene therapy, recently shown to be an adequate intervention for long-term treatment of several retinal pathologies. We have previously described the effectiveness of PEDF gene therapy using the episomal vector pEPito in human RPE cells and in the retina of diabetic mice after a single subretinal injection. PEDF gene therapy reduced DR hallmarks such as microglia reactivity, VEGF expression and the levels of glucose transporter GLUT1, but we have not explored its effect on the retinal vasculature. In this context, our first aim is to evaluate the effect of PEDF gene therapy on DR vasculature, followed by targeting of pathological angiogenesis. For this we have designed a siRNA targeting PlGF due to its role in pathological angiogenesis. In the future, we intend to evaluate the impact of a dual gene therapy approach promoting PEDF overexpression and simultaneously PlGF suppression to treat DR.

Materials and methods: Human retinal pigment epithelial (RPE) cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM). A small interfering RNA (siRNA) corresponding to PlGF mRNA and a scrambled siRNA (scRNA) were used to transfect cells. PlGF, PEDF and VEGF mRNA were quantified by real-time polymerase chain reaction (RT-PCR).

Results: PlGF production is described in the literature as generally occurring in the choroid, but we have analysed the RPE and found these cells to produce and secrete PlGF. To inhibit PlGF, we have tested a range of concentrations of siRNA and our data shows a reduction of PlGF transcripts of up to 50% in RPE cells, after 24 and 48 h of incubation. The maximum inhibition was achieved with a siRNA concentration of 50 pmol/ml. To determine if PlGF inhibition had an effect on the secretion role of RPEs, we analysed the transcript levels of VEGF and PEDF and found that the suppression of PlGF gene *in vitro* had no effect in the production of these factors. Based on these results we have designed an expression system for a shRNA targeting PlGF to assess in vitro and in vivo feasibility of our approach.

Conclusion: Jointly, the previous results from PEDF-pEPito and the efficient knockdown of PlGF in RPE cells, suggest a possible therapeutic potential of gene therapy with a strategy based on simultaneous PEDF overexpression and PlGF inhibition, for the rescue of DR.

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Disclosure: **R. Araújo:** Grants; FCT.

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Diabetic retinopathy after Roux-en-Y gastric bypass

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Background and aims: Roux-en-Y gastric bypass (RYGB) is a effective and durable treatment of obesity. Furthermore, it leads to improvements in or even remission of obesity-related comorbidities such as type 2 diabetes mellitus (T2D). Ophthalmological screening including retinal photo imaging is a cornerstone in preventing eye-threatening complications in individuals with T2D, but there is no consensus on whether or not to end this screening process after surgical remission of T2D. Moreover, rapid deterioration in diabetic retinopathy due to immediate glucose control following RYGB is still a concern. We aimed to evaluate diabetic retinopathy in T2D individuals following RYGB compared to non-operated T2D individuals, and to investigate if there is cause of concern regarding deterioration following RYGB.

Materials and methods: I this clinical trial, 96 RYGB operated obese individuals with T2D were matched on age, gender, and current BMI with 47 non-operated T2D individuals. After induction of cytoplegia and mydriasis fundus photography was performed. We used the Wisconsin Epidemiologic Study of Diabetic Retinopathy (modified by the ETDRS Report 12) to grade retinal lesions from 1-6 (6= most severe). In 49 of the RYGB operated individuals pre-surgical fundus photography were available and assessed as described above. Moreover, HbA_{1c}, BP, anthropometrics, diabetes duration, and smoking were recorded.

Results: Median time since RYGB was 6.1 years (IQR: 5.4; 7.0). Forty-nine of the 96 RYGB operated individuals fulfilled the criteria for T2D remission (HbA_{1c} < 48 mmol/mol and no antidiabetic medication). Group characteristics are presented in table 1. A total of 103 individuals had no detectable retinal changes (grade 1), 25 had grade 2 retinopathy, and 15 had more advanced retinopathy (grade 3-6). The latter were merged due to small numbers in each group. Only 2% of the RYGB individuals with remission displayed severe retinopathy, compared to 11% in the T2D control group and 19% among RYGB individuals still suffering from T2D (p-value <0.001). When adjusting for smoking, gender, and diabetes duration in a multiple regression model the RR of any diabetic retinopathy was 0.67 (0.65; 0.69) and 0.33 (0.14; 0.74) for RYGB individuals with and without remission, respectively. Only one out of 23 RYGB individuals with T2D remission displayed deterioration (from 0 to 1 in grading), while 4 out of 26 RYGB individuals without remission displayed deterioration (p=0.20).

Conclusion: In our cohort RYGB operated both with and without T2D remission have a significant reduced risk of diabetic retinopathy compared to non-RYGB matched controls. RYGB does not seem to provoke deterioration of retinopathy despite rapid improvement in glucose levels.

Clinical characteristics of patient groups (RYGB with diabetes remission, RYGB without diabetes remission and non-RYGB T2D controls)

	n	Age at examination (years)	Women (%)	HbA1c (mmol/mol)	Duration of T2D (years)	Body mass index (kg/m2)	smokers (%)	MAP (mmHg)
REMISSION	49	52.8 ± 9.6	80	40 ± 4	6.3 ± 6.7	33.0 ± 6.6	25	99 ± 12
NON-REMISSION	48	57.5 ± 8.5	75	56 ± 10	16.6 ± 5.4	32.5 ± 6.0	15	102 ± 11
CONTROLS	48	55.3 ± 8.0	75	60 ± 15	7.3 ± 6.5	33.7 ± 6.2	11	109 ± 11
ANOVA	NS	R vs NR, p<0.027	NS	R vs NR, p<0.001	R vs NR, p<0.001	NS	NS	NS
		R vs C, NS		R vs C, p<0.001	R vs C, NS			
		NR vs C, NS		NR vs C, NS	NR vs C, p<0.001			

Data are mean ±SD. When ANOVA indicated significant differences between groups, pairwise comparisons were made and modified using the Bonferroni method.
MAP: Middle arterial pressure, RYGB: Roux-en-Y gastric bypass, T2D: Type 2 diabetes mellitus

Clinical Trial Registration Number: NCT02625649

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Disclosure: **L.R. Madsen:** Grants; Danish Diabetes Academy, Central Denmark Region, AP Møller Foundation, Aarhus University, NOVO nordisk Foundation.

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Topical administration of somatostatin and brimonidine in the early stages of diabetic retinopathy: results of the EUROCONDOR study

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Background and aims: To present the initial results of the first clinical trial using neuroprotective agents (somatostatin 0.1% [SST] and brimonidine tartrate 0.2% [BMN]) administered in eye drops for treating diabetic retinopathy (DR). The primary objective was to assess whether these neuroprotective drugs, administered topically, were able to prevent or arrest neurodegeneration.

Materials and methods: 449 type 2 diabetic patients with either no visible DR (ETDRS level <20; n=193) or only early stages of DR (ETDRS level 20-35; n=256) were enrolled in a prospective (2 years of follow-up), multicenter and randomized clinical trial. Primary outcome: changes in the implicit time assessed by mfERG. Exclusion criteria included retinal diseases associated with neurodegeneration, renal failure, and HbA1c > 10% in the previous 6 months before recruitment. Eye drops were administered twice per day in each eye. Color fundus photography, SD-OCT and mfERG were performed every 6 months and graded by a centralized reading center.

Results: Similar clinical features and degree of metabolic control (HbA1c) were observed among the three arms (placebo, SST and BMN) at baseline and during follow-up. We did not observe differences at the end of follow-up regarding new cases of neurodegeneration among the three arms. However, in those patients in whom neurodegeneration was already detected at baseline (≥6 abnormal hexagons for implicit time in mfERG), SST and BMN arrested the progression of neurodegeneration in comparison with placebo (p<0.01). No effect was observed in the development or progression of microvascular disease assessed by ETDRS scale. The overall drop-out rate was 23%. The

only remarkable secondary effect was the high local side effects reported with BMN, which significantly contributed to the drop-outs.

Conclusion: Topical administration of SST and BMN are useful in arresting the progression of neurodegeneration in early DR. The high rate of local adverse effects observed with BMN seems a limiting factor for using this drug as chronic treatment. Further studies with SST with more longer follow-up in order to determine whether this beneficial neuroprotective effect results in reduction of microvascular disease are needed.

Clinical Trial Registraion Number: NCT01726075

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Disclosure: **R. Simó:** None.

OP 21 From vascular inner layer to cognition

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Irisin improves endothelial dysfunction by upregulating haeme oxygenase-1/adiponectin axis in perivascular adipose tissue in obese mice

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Background and aims: Irisin is secreted by myocytes and has been proposed to improve endothelial function in obesity. However, most of the studies about endothelial function are concerned with the conditions of removed perivascular adipose tissue (PVAT). With regard to PVAT that could exacerbate endothelial dysfunction in obese subjects, it is important for us to know whether irisin also has a protective effect on endothelial function by regulating the PVAT function. Therefore, the objective of this study was to determine whether irisin could improve endothelial dysfunction via regulation of the heme oxygenase-1(HO-1)/adiponectin axis in PVAT in obesity.

Materials and methods: C57BL/6 mice were fed with a regular or a high-fat diet (HFD) with or without treatment with irisin. Endothelial function was determined by measuring endothelium-dependent vasodilatation of the thoracic aorta with or without PVAT (PVAT+ or PVAT−). Nitric oxide (NO) in the aorta was determined. Protein levels of HO-1 and adiponectin were detected by western blot. UCP-1, Cidea, and TNF-α gene expression in PVAT were tested by real-time PCR.

Results: Treatment of HFD mice with irisin improved glucose and lipid metabolism (glucose, 98.5 ± 4.6 mg/dl vs. 108.1 ± 4.7 mg/dl; triglycerides, 109.48±11.35 mg/dl vs. 132.13±8.86 mg/dl; NEFA 0.90 ±0.18 mmol/L vs. 1.20 ± 0.20 mmol/L; $P < 0.01$), reduced plasma levels of TNF-α (29.02 ±2.56 pg/ml vs. 40.22±4.87 pg/ml, $P < 0.01$), malondialdehyde (5.14±0.33 ± 0.60 umol/L vs. 6.63±0.76 umol/L, $P < 0.01$) and enhanced plasma adiponectin levels (15.86±1.04 ug/ml vs. 12.60±1.68 ug/ml, $P < 0.01$). The presence of PVAT significantly impaired endothelial function in the HFD mice (maximum vasodilatation rate, 38.3% ± 3.3% vs. 46.3% ± 3.5%, $P < 0.05$). Treatment of HFD mice with irisin restored this impairment with a similar response to both the PVAT+ ring and the PVAT− ring in vivo (60.2% ± 3.9% vs. 65.3% ± 4.0%, $P > 0.05$) or ex vivo (59.1% ± 3.6% vs. 60.7% ± 3.6%, $P > 0.05$). Incubated aortic rings (PVAT-) with PVAT-derived conditioned medium (CM) from HFD mice impaired endothelial function in control mice (40.5% ± 3.4% vs. 86.4% ± 4.3%, $P < 0.05$). This impairment was prevented by incubating the aortic rings (PVAT-) from HFD mice with PVAT-derived CM from irisin (59.1% ± 4.2% vs. 45.6% ± 3.6%, $P < 0.05$). However, the beneficial effects above were partly attenuated in the presence of HO-1 inhibitor (stannous protoporphyrin) and adiponectin receptor blocking peptide (ACRP-30 N-20). Treatment of HFD mice with irisin significantly increased NO levels, protein levels of HO-1 and adiponectin, mRNA expressions of UCP-1 and Cidea, and decreased superoxide production and TNF-α expression in PVAT ($P < 0.05$).

Conclusion: Irisin improved endothelial function by modulating HO-1/adiponectin axis in PVAT in HFD-induced obese mice. These findings suggest that regulating PVAT function may be a potential mechanism by which irisin improves endothelial function in obesity.

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Disclosure: **X. Sun:** None.

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Identification of the transcription factors ZBTB7A and MAZ as novel regulators of gene expression in endothelial cells of diabetic origin

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Background and aims: Endothelial dysfunction is a critical early step in the development of atherosclerotic plaques in type I diabetes (T1D). While protein

level changes in this transition to a pro-inflammatory state have been well characterised, the transcription events involved, including histone modifications, remain poorly defined. Histone deacetylase (HDAC) inhibitors, which paradigmatically activate gene expression via pharmacological inhibition of HDAC enzymes (acetylation writers), are protective against the development of T1D in animal models. Recent evidence shows anti-inflammatory effects of HDAC inhibitors in healthy primary human aortic endothelial cells (HAECs), though the effects in diabetic HAECs have yet to be defined. We compared and contrasted genome-wide trends in transcriptional regulation in HAECs derived from healthy and T1D individuals following HDAC inhibition.

Materials and methods: We studied genome-wide gene expression (RNA-seq) and histone acetylation (chromatin immunoprecipitation, ChIP-seq) profiles of HAECs derived from healthy and T1D individuals, before and after exposure to the HDAC inhibitor suberanilohydroxamic acid (SAHA, n=3, conc. 2 μM, for 12 or 16h).

Results: SAHA induced potent increases in histone acetylation at gene promoters in diabetic HAECs, consistent with the paradigmatic action of HDAC inhibitors. In contrast, SAHA mediated unexpected increased and decreased promoter acetylation in healthy HAECs, which correlated with gene expression. Transcription factor (TF) analysis identified that gene promoters that are deacetylated in healthy cells, but acetylated in diabetic cells by SAHA, were enriched for the TFs ZBTB7A and MAZ, suggesting altered activities of these proteins in diabetic HAECs. Though not previously linked to diabetes, *ZBTB7A* deletion in some cancers is implicated in the expression of glycolytic genes, while MAZ regulates the expression of insulin via its interaction with the IDDM2 locus. Furthermore, analysis of TF binding sites from the ENCODE projects shows that up to 90% of ZBTB7A binding sites intersect with MAZ genome-wide, suggesting that the two proteins work either in synergy or in opposition to regulate gene expression. Validation by RNA-seq of healthy and diabetic HAECs cultured in hyperglycaemic conditions for 15 days identified suppression of multiple genes regulated by ZBTB7A and MAZ, including *TRIB1*, an inflammatory regulator implicated in atherosclerosis and hepatic glucose homeostasis. *TRIB1* expression was reactivated by SAHA in diabetic HAECs. Furthermore, re-analysis of public data identified strong binding of ZBTB7A at the promoter of *TRIB1* that correlated with low gene expression, and reactivation *TRIB1* expression following *ZBTB7A* deletion in human erythroblasts. This implicates suppression of *TRIB1* mediated by increased ZBTB7A binding at the gene promoter in diabetic cells and on exposure to hyperglycemic conditions.

Conclusion: Our data predicts a role for the transcription factors ZBTB7A and MAZ in altered gene expression following chronic hyperglycaemic. Further work, including ZBTB7A and MAZ ChIP and deletion studies in hyperglycaemic cells, are in progress to validate these findings.

Disclosure: **H. Rafehi:** None.

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Strict glycaemic control improves arterial stiffness, left ventricular myocardial deformation, endothelial glycocalyx and oxidative stress in type 2 diabetic patients

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Background and aims: Poor glycaemic control affects myocardial function even subclinically. We investigated whether changes in arterial stiffness, left ventricular (LV) myocardial deformation and endothelial glycocalyx, an index of the microvasculature function, relate to changes of oxidative stress in patients with poorly controlled type-2 diabetes before and after strict glycaemic control.

Materials and methods: In 100 poorly controlled diabetic patients (age:51 ±12 years) and 25 controls matched for age and sex and no atherosclerotic risk factors, we measured at baseline and 6 months after strict glycaemic control: a)pulse wave velocity(PWVc-Complior), central systolic blood pressure(cSBP), augmentation index(AI), b) LV longitudinal strain(GLS), systolic(LongSr)and diastolic(LongSrE)strain rate, peak twisting(pTw), peak twisting(pTwVel)and peak untwisting velocity(pUtwVel)using speckle tracking echocardiography. The degree of LV untwisting was calculated as the percentage difference between peak twisting and untwisting at mitral valve opening(MVO) (%dpTw−Utw_{MVO}),at peak(%dpTw-Utw_{PEF})and end of early LV diastolic filling(%dpTw-Utw_{EDF}), c)perfused boundary region(PBR) of the sublingual arterial microvessels(ranged from 5-25μm) (Sideview,Darkfield imaging,Glycocheck). Increased PBR equals reduced endothelial glucocalyx thickness, d)Flow mediated dilatation(FMD) of the brachial artery and percentage difference of FMD(FMD%), e) malondialdehyde(MDA) and protein carbonyls(PCs) plasma levels.

Results: Diabetic patients had higher AI(26±15 vs. 19.4±14.7%), PWV(12±3 vs. 8.8±1.3m/sec), cSBP(133±20 vs. 119±18mmHg), PBR(2.1±0.3 vs 1.89 ±0.1μm)and lower GLS(-15,4±3 vs. -18±3%), LongSr(-0.8±0.1 vs. -0.96 ±0.2 l/sec), LongSrE(0.83±0.3 vs. 1.2±0.3 l/sec)vs. controls ($p<0.05$ for all comparisons). Intensive 6-months antidiabetic treatment reduced HbA1c(8.8 ±2 vs. 7±1.3%, $p<0.05$), PWV(12±3 vs. 11.1±2.7m/sec), PBR(2.1±0.2 vs. 2 ±0.2μm, $p<0.05$), MDA(1.29±0.8 vs. 1.1±0.9nM/L)and PCs(0.015±0.08 vs. 0.012±0.05nmol/mg protein) while increasing pUtwVel(-92±40 vs. -102 ±36deg/sec), %dpTw−Utw_{MVO}(28±9 vs. 37±11), %dpTw-Utw_{PEF}(46±19 vs. 59±18) and FMD%(8.1±5 vs. 12.8±8)($p<0.05$). Post treatment, reduced PWV positively correlated with PBR(r=0.50)and negatively with Longsr E(r=-0.60) and %dpTw-Utw_{EDF}(r=-0.56) respectively. PBR negatively correlated with pUtwVel(r=-0.43)($p<0.05$). PCs levels at baseline were negatively related to the difference of GLS(r=-0.53).MDA difference correlated with the difference of PWV(r=0.52) ($p<0.05$).

Conclusion: Intensified glycaemic control for a six-months period improves arterial stiffness, LV myocardial strain, twisting-untwisting velocity and glycocalyx thickness in previously poorly-controlled diabetic patients, probably by reducing oxidative stress. Strict glycaemic control can reverse early subclinical myocardial and endothelial dysfunction.

Clinical Trial Registraion Number: NCT03010956

Disclosure: **G. Pavlidis:** None.

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Effects of a 6-month liraglutide treatment on arterial stiffness, left ventricular myocardial deformation and oxidative stress in newly diagnosed type 2 diabetic patients

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Background and aims: Glucagon-like-peptide-1 analogs (GLP-1R) have recently exhibited a beneficial cardioprotective effect in high cardiovascular risk type-2 diabetes patients possibly by reducing the burden of atherosclerosis. We investigated whether liraglutide modifies arterial stiffness and left ventricular (LV) myocardial deformation, in relation to changes in oxidative stress after a short-term 6-month treatment period

Materials and methods: We examined 60 newly diagnosed, treatment-naive patients with type 2 diabetes. 30 patients were randomized to receive liraglutide, and 30 subjects received metformin for 6 months. We measured at baseline and after 6-months of treatment: a) carotid-femoral pulse wave velocity (PWV-Complior ALAM), central systolic blood pressure (cSBP) and augmentation index (AI), b) LV longitudinal strain (GLS), and strain rate (GLSR), peak twisting (pTw), peak twisting velocity (pTwVel) and peak untwisting velocity (pUtwVel) using speckle tracking echocardiography. The degree of LV untwisting was calculated as the percentage difference between peak twisting and untwisting at mitral valve opening (MVO) (%dpTw −UtwMVO), at peak (%dpTw-UtwPEF) and end of early LV diastolic filling

(%dpTw-UtwEDF) c) Flow mediated dilatation (FMD) of the brachial artery and percentage difference of FMD (FMD%) after hyperaemia d) malondialdehyde (MDA) and protein carbonyls (PCs) plasma levels for the assessment of oxidative stress status.

Results: After a short, six-month treatment period, subjects on liraglutide achieved a reduction in PWV (11.8±2.5 vs. 10.3±3.3m/sec) and MDA (1.47 ±0.2 vs. 0.89±0.2nM/L) in parallel with an increase of pUtwVel (-97±49 vs. -112±52deg,), %dpTw-UtwMVO (31±10 vs. 40±14), %dpTw-UtwPEF (43±19 vs. 53±22) and FMD% (8.9±3 vs. 13.2±6), ($p<0.05$ for all comparisons). There were no statistically significant differences in subjects that received metformin. At baseline, reduced GLS related positively to elevated HbA1c (r=0.39), PWV (r=0.47) and negatively to FMD% (r=-0.29). GLSR was inversely related to HbA1c (r=-0.35) and PWV (r=-0.50) while cSBP was associated with %dpTw−UtwMVO (r=0.29) ($p<0.05$ for all associations). In all subjects, PCs levels at baseline were negatively related to the difference of GLS (r=-0.53) post treatment and the difference of MDA was associated with the difference of PWV (r=0.52) ($p<0.05$ for all associations) after 6-month treatment

Conclusion: Six-month treatment with liraglutide improves arterial stiffness, LV myocardial strain, twisting and untwisting likely by reducing oxidative stress in newly diagnosed treatment-naive type-2 diabetic patients

Clinical Trial Registration Number: NCT03010683

Supported by: Research Grant Authority of Athens University

Disclosure: **V.A. Lambadiari:** None.

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A pilot study of the effect of relaxing and active music on attention and short term memory in patients with type 2 diabetes compared to control

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Background and aims: There is emerging evidence, that music, beside its stress lowering effect, has an impact on cognition, memory or attention in healthy subjects, in elderly, and in patients with cardiovascular disease. Diabetes is well known to be associated with lower cognitive performances, higher degree of dementia. Our aim was to study the effects of music in type 2 diabetes patients on short term memory and attention, a field not yet investigated

Materials and methods: We conducted a two-day, interventional, within-subject design study comparing the effect of relaxing and active music on metabolic parameters, attention and short term memory. 89 patients with only Metformin treated type 2 diabetes and 67 age and gender matched control subjects were included. We used Pierrot Toulouse test, and word recognition test (40 words read during music, recognized afterwards from a list of 80 words) to evaluate attention and short term memory. The music listened was 20 minutes allegro part of the K448 sonata, and 20 minutes andante part of the K331 sonata of Mozart. Participants performed the attention and short term memory test in two different days. Cognitive tests, blood pressure and blood glucose measurement were performed before and after each 20 minutes music intervention, having 30 minutes period between the two types of music

Results: Mean age was 62.06 ± 10.96 years in the control group and 63.82 ± 9.716 years in the type 2 diabetes group. Baseline attention performance was better in control group compared to type 2 diabetes (50.45±15.89% vs. 39.48±13.96%, p = .000). There was no difference in short term memory at baseline. Attention performance improved significantly in type 2 diabetes, under both music interventions (active and relaxing music: 46.92 ± 12.65% vs. 39.48 ± 13.96%, p =.042 and 55.04 ± 14.62% vs. 42.77± 13.71%, p = .002). Improvement was the same in the two groups (performance difference 7.44±8.51 % and 12.27 ± 6.75% in type 2 diabetes vs 8.32 ± 12.64% and 10.68 ± 10.81% in control). Female participants had a better attention performance improvement under active music then male (9.9 ± 8.4% vs. 2.5 ± 8.6%, p=.002). Neither of the music interventions had any effect on short term memory, except in the female participants, were under relaxing music remembered word count was better (62.5 ±10.1 vs.57 ±10.2, p=.04). Neither of the music intervention did influence blood glucose levels. Relaxing music lowered systolic blood pressure significantly more in the type 2 diabetes group (-9.06 ± 3.6 vs. 3.44 ± 4.3, p=.000)

Conclusion: In our study attention was improved, but not short term memory, by both type of music in type 2 diabetes group, and female patients were more sensitive to the effect of music.

Disclosure: **M.I.M. Szabo:** None.

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Altered olfactory-induced brain activation as an early manifestation of cognitive decline in patients with type 2 diabetes

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Background and aims: Although type 2 diabetes mellitus (T2DM) has been reported to be associated with olfactory dysfunction and increased risk of cognitive decline, it is still unknown whether olfactory neural circuit abnormalities involve cognitive impairment in T2DM. This study thus aimed to examine the comprehensive association of olfactory-induced brain activation with cognitive and metabolic parameters in patients with T2DM.

Materials and methods: Seventy-seven subjects with normal cognition including 41 patients with T2DM (24 males, age: 51±9 years, education degree:14±3 years) and 36 non-diabetic control (NC) subjects (18 males, age: 50±7 years, education degree: 14±4 years) were enrolled. Detailed clinical and biochemical information were collected and neuropsychological assessment was performed. Meanwhile, a computerized battery of olfactory function tests assessing odor threshold, identification and memory was investigated. Neural activation intensity in response to lavender odor stimuli was assessed with high-resolution functional magnetic resonance imaging (fMRI) scanning. Regions of interest (ROIs) were extracted from one-sample t-test of NC group in task fMRI for resting-state functional connectivity (RSFC) analysis between two groups.

Results: The olfactory threshold score was significantly lower in patients with T2DM than NC group (8.7±3.2 vs. 11.0±2.5, p=0.001). Task fMRI scanning demonstrated the olfactory-induced bilateral brain activation of the olfactory circuit in all subjects. Between-group analysis revealed the activation in bilateral orbitofrontal cortex (OFC) and the reduced activation in left hippocampus and left parahippocampus in patients with T2DM. Interestingly, we observed decreased RSFC between the OFC and all ROIs of the olfactory circuit including amygdala, entorhinal cortex and hippocampus in patients with T2DM compared to NC group. Further correlation analysis showed the disrupted connectivity in the olfactory circuit was positively associated with impairment in cognitive domains including memory, attention and executive functions accessed by neuropsychological tests in patients with T2DM. Remarkably, patients with poor pancreatic islet function, as measured by fasting and 2h postprandial C-peptide and insulin, had significantly lower olfactory and cognitive test scores. The 2h postprandial insulin of patients also positively correlated with the RSFC between left hippocampus and left OFC (r = 0.378, p = 0.027).

Conclusion: This is the first study to indicate that the alteration of olfactory-induced brain activation is present before clinical symptoms of cognitive decrements in patients with T2DM. Our study also raises the importance that the improvements of pancreatic islet function might also be beneficial for preserving cognition in diabetes.

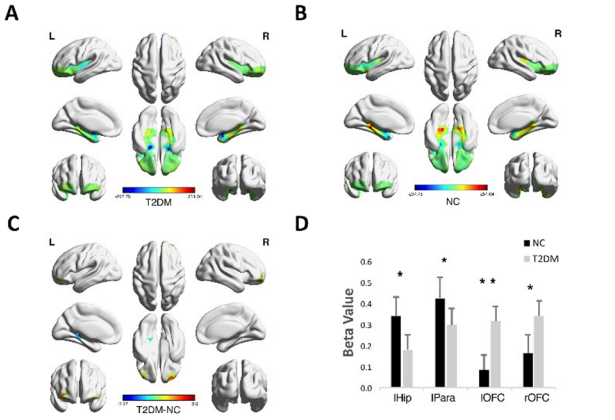

Figure: Olfactory-induced brain responses of patients with T2DM (A) and normal control(B). Independent two-sample T-test result (C) indicating significant altered brain activation in patients with T2DM compared to NC group. (T maps threshold at P<0.05 with AlphaSim corrected). Beta value differences between two groups (D) of the left hippocampus, left parahippocampus, left and right orbitofrontal cortex (lHippo: left hippocampus; lPara: left parahippocampus; lOFC: left orbitofrontal cortex; rOFC: right orbitofrontal cortex).

Clinical Trial Registration Number: NCT02738671

Disclosure: **Z. Zhang:** None.

OP 22 Animal models of metabolic disease

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Genetically modified human Mesenchymal Stromal Cells (MSCs) help improve glucose homeostasis by reducing inflammation and promotes browning of visceral fat

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Background and aims: MSCs are multipotent cells that can home-in to a site of inflammation. Upregulation of specific antioxidants in MSCs reduces intracellular inflammation and ROS formation in a hyperglycemic condition. Hypothesis: Antioxidant over-expressed MSCs will reach fat depots, reduce local & systemic inflammation and improve glucose homeostasis in diet-induced obese (DIO, 60% and 45% fat diet) hyperglycemic mouse models.

Materials and methods: We used GFP-containing adenoviral constructs to upregulate intracellular (SOD1, SOD2, Catalase) and extracellular (SOD3) antioxidants in human adipose-derived MSCs. GFP-Null transduced MSCs were used as control. The modified MSCs were delivered intraperitoneally in 60% and 45% DIO mice.

Results: In-vitro, SOD2 (mitochondrial anti-oxidant) upregulation showed reduced inflammatory markers IL6 and TNFa mRNA while PCG1A mRNA (a gene upstream of UCP1), upregulated. SOD2 upregulated MSC delivery in both DIO models demonstrated improved glucose tolerance test (GTT) at week 4 compared to SOD1-MSC and Null-MSC (control). Catalase-MSC delivery not only improved GTT but also improved insulin tolerance test (ITT) in 60% DIO mice. Interestingly, RT-PCR of pericardial fat showed significant increases in mRNA expression of both UCP1 (25-100,000-fold) and PRDM-16 (2-10-fold) in both DIO mice models that received antioxidants upregulated MSCs, compared to mice receiving Null MSCs. For omental fat, an increase in mRNA expression of UCP1 was observed in 60% fat DIO mice (1,000-6000 fold) for SODs 1-3 and catalase, while for 45% DIO mice only those receiving SOD1 & SOD2 upregulated MSCs presented UCP1 mRNA upregulation (1,000 to 11,000-fold). Omental Fat histology showed less hyperplastic fat with SOD2 and Catalase-MSCs. UCP1 staining of omental fat was also positive with SOD2-MSC. Inflammatory molecules such as IL-6 and TNF alpha levels by ELISA, were reduced with SOD2-MSC in 60% DIO mice model.

Conclusion: We conclude that delivery of antioxidant upregulated MSCs to the inflamed adipocyte depots in diabetic DIO models appear to upregulate UCP1, PGC1 alpha and PRDM-16 in visceral fat while reducing systemic inflammatory markers. This synergestic action via reduction of inflammation and "browning of white fat", may explain improvements noted in GTT and ITT. Delivery of modified MSC is a novel & robust therapeutic tool that may help improve glucose homeostasis in diabetes and obesity.

Disclosure: **S. Sen:** None.

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The novel obesity gene Ifi202b induces Zfp423 and reduces browning and body temperature

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Background and aims: Obesity results from a constant and complex interplay between environmental stimuli and predisposing genes. Recently, we identified the transcriptional modulator Ifi202b as putative obesity gene by positional cloning. Ifi202b is expressed in insulin-sensitive tissues of New Zealand

Obese mice - predominantly in white adipose tissue - but is absent in the lean C57BL/6J strain. This study aimed to validate Ifi202b as obesity gene and to clarify how it induces fat mass expansion.

Materials and methods: To investigate the impact of Ifi202b on adipocyte function and metabolism, it was overexpressed in 3T3-L1 preadipocytes and in C57BL/6J mice. B6-Tg(Ifi202b) and B6-wt mice were kept on a high-fat diet and body composition, adipose tissue function and insulin sensitivity were analyzed.

Results: Ifi202b overexpression in 3T3-L1 preadipocytes resulted in accelerated adipogenesis as marked by higher expression levels of Pparg, Fabp4, Glut4 and Plin1 as well as by elevated triglyceride storage capacity. In vivo, Ifi202b expression led to increased body fat mass with hypertrophic adipocytes, reduced isoprenaline-inducible lipolysis, increased expression of the transcription factor Zfp423 and white-selective genes (Tle3, Tcf21), and decreased expression of thermogenic genes (e.g. Prdm16, Cidea, Ucp1). Furthermore, Ifi202b transgenic mice exhibited lower body temperature, elevated liver fat accumulation, and systemic insulin resistance.

Conclusion: In summary, our findings provided direct evidence for the crucial role of Ifi202b as obesity gene, as it induced adipocyte differentiation and hypertrophic adipose tissue expansion accompanied by increased whitening and decreased browning. We hypothesize that Ifi202b determines white adipocyte identity which is at least in part mediated via the induction of Zfp423.

Supported by: BMBF, DZD

Disclosure: **M. Stadion:** None.

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Circulating exosomal miRNAs mediate tissue cross-talk during development of glucose intolerance in mice

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Background and aims: Maintenance of glucose homeostasis is a complex task that requires the coordinated action of many tissues. In addition to hormones and other soluble factors, cells communicate by releasing exosomes, small vesicles enriched in microRNAs (miRNAs) that are detected circulating in blood and other biological fluids. Importantly, these circulating exosomes can be captured by acceptor cells, where the miRNAs they contain induce transcriptomic changes, thus mediating intercellular communication. We hypothesize that the profile of exosomal miRNAs found in blood represents the contribution of many tissues and depends on the physiological context of the organism. Therefore, we focused on determining the changes induced by an obesogenic diet in the profile of exosomal miRNAs, and exploring the role of circulating exosomes in the establishment of glucose intolerance in mice.

Materials and methods: C57BL6/J male mice were fed either standard chow or a high-fat diet (HFD) to render them glucose intolerant. Exosomes were isolated from plasma by centrifugation, and western blotting demonstrated enrichment of the exosomal marker CD63. Exosomal miRNAs were profiled by real-time RT-PCR. Control mice were injected intravenously with exosomes isolated from plasma of control and HFD mice. Glucose tolerance and insulin sensitivity were determined by IGTT and ITT respectively. Blood and tissues were extracted for posterior RNA analysis.

Results: The profiling of 378 miRNAs shows increased levels of liver-enriched microRNAs such as *miR-122* and *miR-192* (19-fold and 7-fold respectively, $p<0.05$) in exosomes isolated from plasma of HFD mice as compared with control mice, thus indicating that development of glucose intolerance is associated with a modification of the population of circulating exosomes. Surprisingly, continued injection of exosomes isolated from HFD mouse plasma into control mice being fed standard chow results in induction of glucose intolerance and insulin resistance. Bioinformatics analyses using IPA software identified the PPAR family of transcription factors and other regulators of lipid metabolism as the main targets of the miRNAs significantly increased in HFD exosomes. We studied gene expression of candidate genes in insulin-sensitive tissues. Accordingly, the liver of exosome-treated mice showed decreased expression of genes associated with *de novo* lipogenesis including *pparg* and *ppara*, as well as the igf-1 signaling pathway. Moreover, the white adipose tissue (WAT) of exosome-treated mice displayed increased expression of adipogenic genes and no changes in the expression of inflammatory mediators, in contrast with the expression profile observed in WAT of HFD mice. Finally, addition of a short-term HFD to exosome-treated mice resulted in an exacerbation of the phenotype of glucose intolerance.

Conclusion: Our data shows that an obesogenic diet causes glucose intolerance and modifies the miRNA content of circulating exosomes. Transmission of the phenotype of glucose intolerance by exosomes suggests that circulating exosomes are not only a reflection of the pathology, but also active players on its development. Therefore, directed manipulation of circulating exosomes and their miRNA content emerges as a novel avenue for the treatment of diabetes.

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Disclosure: **C. Castaño:** None.

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Increasing GLP-1 levels is a route to improve adipose tissue angiogenesis, contributing to better metabolic outcome

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Background and aims: Vascularization is a crucial process in adipose tissue expansion and in its metabolic and secretory functions, improving insulin sensitivity and the metabolic outcome. Incretin-based therapies are known to improve the metabolic homeostasis and we hypothesized that such effect could include the modulation of adipose tissue angiogenesis. Thus, using animal models of increased glucagon-like peptide-1 (GLP-1) levels and the adipose tissue angiogenic assay, our aim was to assess the pro-angiogenic effects of GLP-1 in adipose tissue.

Materials and methods: We used two different animal models of increased GLP-1 levels: rats treated with the GLP-1 mimetic Liraglutide and rats submitted to metabolic surgery. For Liraglutide in vivo studies, 14 week male Wistar and non-obese type 2 diabetic Goto-Kakizaki (GK) rats were injected twice a day with liraglutide (200μg/Kg s.c.) during 14 days. For sleeve metabolic surgery studies Wistar and GK were used as control groups and high-fat diet-fed GK rats divided in 3 groups: GK without surgery intervention (GKHFD), GKHFD with sleeve gastrectomy (GKHFDSl) and GKHFD with sham surgery (GKHFDSh). Besides tested for fasting glycemia (6h) and insulin tolerance [intraperitoneal insulin tolerance test (IPITT)], both animal models were also analysed for the angiogenic mechanisms in the periepididymal adipose tissue. For the adipose tissue angiogenic assay (ex vivo studies), adipose tissue from 4 week old normal Wistar rats was collected and cut into very small pieces (~1mm^3). Explants were then embedded in collagen matrix and incubated for 5 days in EGM- 2 MV, BulletKit (Lonza, Switzerland) with or without liraglutide (50nM) and exendin-3 (GLP-1R antagonist 300nM).

Results: Both animal models showed improved insulin sensitivity and fasting glycemia. Liraglutide treatment upregulated adipose tissue angiogenic pathways in diabetic rats by increasing the levels of VEGFR2, Tie-2 and FGFR, as well as HIF2alpha levels and eNOS, which are involved in the activation of angiogenic pathways and modulation of the vascular function. Moreover, sleeve metabolic surgery also improved adipose tissue angiogenic markers (CD31, FGFR, VEGF and Ang-2) and vascular function (eNOS). In the adipose tissue angiogenic assay, liraglutide did not result in significantly higher area of vascularization, but dramatically increased vessel density in a GLP-1R-independent manner.

Conclusion: Therapies leading to increased GLP-1 levels result in improved metabolic profile, possibly resulting from better adipose tissue vascular remodelling and function. Future studies will reveal the mechanisms of GLP-1-induced angiogenesis and vascular remodelling in adipose tissue.

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Disclosure: **T. Rodrigues:** None.

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Reduced insulin secretion in a diet-induced glucose intolerance susceptible mouse model is coupled with increased CD36 and triglycerides in the beta cells

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Background and aims: To study the polygenic etiology and pathophysiology of type 2 diabetes, we recently established two mouse lines with high and low susceptibility to high fat diet (HFD)-induced glucose intolerance by selective breeding; designated *s*electively bred *d*iet-induced *g*lucose intolerance-*p*rone (SDG-P) and -*r*esistant (SDG-R), respectively. We previously demonstrated that the pancreatic islets from SDG-P mice showed lower glucose- and KCl-stimulated insulin secretion (GSIS/KSIS) when compared to those from SDG-R mice. Meanwhile, the islets from SDG-P mice showed higher gene expression of Cd36 than those from SDG-R mice. CD36 (also referred to as fatty acid translocase) is known as a key molecule in fatty acid influx in various types of tissues, while lipid accumulation-induced dysfunction (so-called "lipotoxicity") is known to contribute to the decline of islet insulin secretion in type 2 diabetes. Thus, CD36 is a candidate factor to explain the observed differential GSIS/KSIS response between the islets from SDG-P and SDG-R. We therefore aimed to investigate CD36 expression and lipid accumulation in the islets of SDG-P and SDG-R mice.

Materials and methods: Pancreatic islets were isolated from SDG-P and SDG-R mice by collagenase digestion. Gene and protein expression levels in the islets were evaluated by quantitative PCR and Western blotting, respectively. To determine the intracellular triglyceride (TG) content, total lipid was extracted from the islets and the fatty acid content in the TG fraction was analyzed by gas-liquid chromatography. Values of $p<0.05$ were considered as statistically significant by Student's *t* test.

Results: We found elevated Cd36 mRNA level in the SDG-P mouse islets compared to those from SDG-R (1.7-fold, n=6, p=0.002) even at 5 weeks of age, without HFD feeding. This was confirmed at the protein level, in which CD36 protein was 2.2-fold higher in the islet of SDG-P mice (vs SDG-R, n=6, p<0.001). The higher Cd36 gene expression in SDG-P mouse islets was sustained up to 10 weeks of age, including 5-week HFD feeding in the end of the period. Flow cytometric analysis revealed that CD36 protein was predominantly expressed in the β cells of the islets. Concomitant with the high CD36 expression, intracellular TG content was increased in the islets of SDG-P mice (1.6-fold vs SDG-R, n=6, $p=0.02$).

Conclusion: Our results strongly support the involvement of CD36 in lipid accumulation in islet β cells. Excessive fatty acid influx and the consequent lipid accumulation in pancreatic islets via hereditary higher CD36 expression may play an important role in the lower insulin secretion response and the pathogenesis of impaired glucose tolerance in SDG-P mice.

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Disclosure: **A. Asai:** None.

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The effect of dicarbonyl stress in the development of kidney dysfunction in metabolic syndrome

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Background and aims: Dicarbonyl stress plays an important role in the pathogenesis of microvascular diabetic complications through the development of renal dysfunction preceding advanced glycation end products formation. In renal cells, toxic dicarbonyl metabolites like methylglyoxal inhibit electron respiratory chain leading to mitochondrial dysfunction, protein structure modifications which in turn affects their function. Methylglyoxal can also affect different signaling pathways associated with vascular complications in kidney on transcriptomic levels. In our study, we investigated the effect of methylglyoxal on metabolic, transcriptomic and proteomic profile in the context of the development of kidney impairment in an experimental model of metabolic syndrome.

Materials and methods: Dicarbonyl stress was induced by intragastrical administration of methylglyoxal (3x/ week in dose 0.5 mg/kg b.wt.for 4 weeks) in a strain of hereditary hypertriglyceridaemic rats with insulin resistance and fatty liver (HHTg). Transcriptome assessment in kidney cortex was performed using microarray, urine proteome and metabolome were evaluated by mass spectrometry methods.

Results: Methylglyoxal administration aggravated glucose intolerance ($AUC_{0\text{-}120}$ $p<0.05$), increased plasma glucose (8.8±0.2 vs 7.1±0.2 mmol/l, $p<0.01$) and insulin (0.515±0.030 vs 0.246±0.026 μmol/l, $p<0.05$). Compared to controls, methylglyoxal-treated rats exhibited significantly higher levels of microalbuminuria (42.8±7.3 vs 13.8±2.9 mg/g creatinine, $p<0.01$) and urine lactate ($p<0.05$). Targeted proteomic analyses revealed increase in urine secretion of proinflammatory parameters (MCP-1, IL-6, IL-8), specific collagen IV fragments (endostatin), alpha1-antitrypsin and extracellular matrix proteins (heparan sulphate). Urine metabolomic biomarkers in methylglyoxal-treated rats were mainly associated with impairment of membrane phospholipids (MDA, 8-isoprostane, 4-hydroxynonenal). Decreased level of reduced glutathione (-30%, $p<0.01$) together with decreased activity of glutathione-dependent antioxidant enzymes worsened oxidative and dicarbonyl stress and can contribute to the impairment of kidney function. Methylglyoxal administration elevated relative expression of glyoxalase 1 ($p<0.05$), which is involves in methylglyoxal degradation. Comparative transcriptomic analysis in kidney cortex identified 96 genes differentially expressed (FDR < 0.05) after methylglyoxal administration. Network analysis revealed overrepresentation of genes in excessive oxidative stress and proinflammatory signaling pathways and inhibition of angiogenesis suggesting its contribution to renal fibrosis.

Conclusion: Our results support a key role of dicarbonyl stress in the pathogenesis of renal microvascular complications in early phases of development. At transcriptomic level methylglyoxal activated oxidative and proinflammatory pathways and inhibited angiogenesis. Its proinflammatory effects were further supported by urine metabolomic analysis.

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Disclosure: **H. Malinska:** None.

OP 23 The importance of psychological studies in improving diabetes care

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Aberrant brain functional connectivity linking insulin resistance to depression

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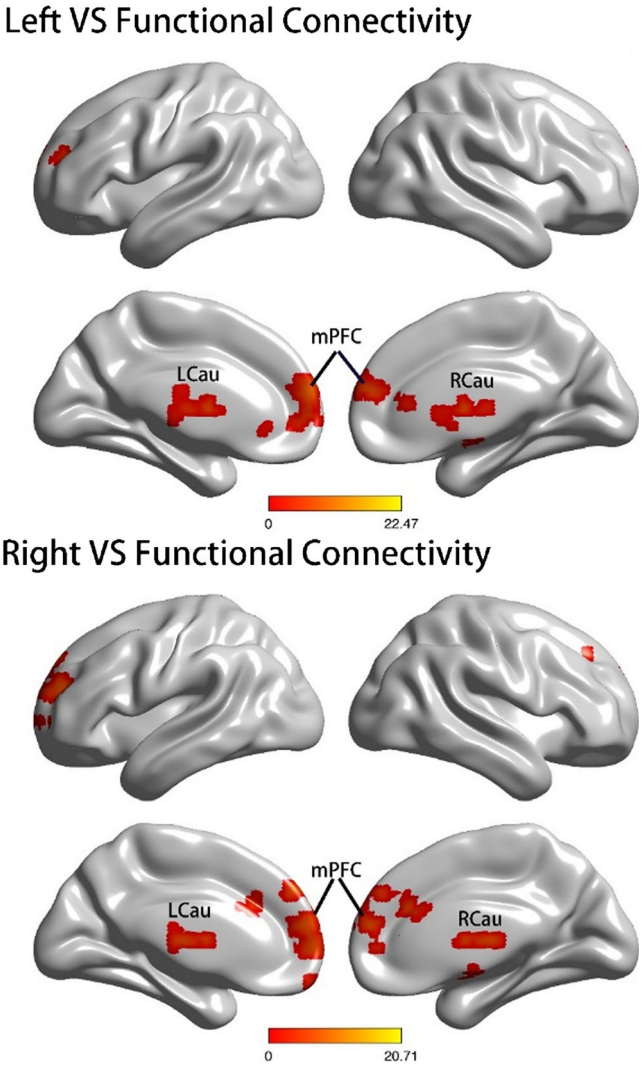
Background and aims: Type 2 diabetes mellitus (T2DM) often comorbid with major depressive disorder (MDD), but how T2DM and MDD are pathologically connected at the neural network level is poorly understood. A reasonable starting point regarding this field is the common pathophysiological mechanism shared by the two diseases, and brain insulin resistance (IR) reprents a promising target. Wihtin the pathway of IR, the importance of *mTOR* has been demonstrated. Furthermore, ventral striatum (VS), a region which is sensitive to the insulin levels, shows altered function in the context of both IR and depression, thus the VS network represents a promising phonetype to reveal the interactions between T2DM and MDD.

Materials and methods: Forty-nine MDD patients and 37 well-matched healthy control (HC) subjects were recruited and underwent a resting-state functional magnetic resonance imaging (R-fMRI) scan. Whole exome sequencing of *mTOR* gene were conducted, and 56 single nucleotide polymorphisms (SNPs) entered the further gene association analyses after quality control. Socres of Hamilton Depression Rating Scale (HAMD-24) were used as phenotypes to select *mTOR* SNP with behavioral significance, and the selected SNPs were used in the next genetic-imaging analyses. Seed-based connectivity analyses were conducted to construct ventral striatum functional connectivity (VSFC) network. Disease-by-genotype interaction analysis was performed to investigate the influence of *mTOR* on VSFC to illustrate how the genetic variation modified the performance of depression.

Results: Indicated by the gene association analyses, rs2275527 showed significantly influence on the performance of HAMD-24 (P = 0.013). Compared with HC participants, the VSFC network was widely decreased in patients with MDD. The further genetic-imaging analyses suggested the main effect of rs2275527 on VSFC network was located in left orbital frontal cortex, anterior insular and bilateral superior temporal gyrus. The rs2275527-by-disease interactions on VSFC network were mainly detected in regions including medial prefrontal cortex (mPFC) and bilateral caudates. Interesting, the VSFC in *mTOR* GG carriers was similar between HC and MDD group, but the VSFC was significant decreased in MDD compared to HC subject with *mTOR* A+ carriers. Furthermore, left VSFCs in mPFC and left caudate were positively related to the severity of depression in MDD group (mPFC, r = -0.289, P = 0.007, P_{FDR} = 0.042; Caudate, r = -0.5, P = 0.0001, P_{FDR} = 0.0006). The results were similar on the right VSFC network.

Conclusion: The IR risk gene jointed with depression influence the VSFC network. The alteration of VSFC in mPFC and caudates would predict the severity of depression in IR population. These findings extended our understanding about bidirectional associations between T2DM and depression. More importantly, this study provide new insight for further exploration of IR risk on brain function and may become useful for early identification of depression in T2DM patients.



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Disclosure: **C. He:** None.

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Depressive symptoms and related factors in elderly diabetic patients at a national geriatric hospital

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Background and aims: Depressive symptoms and diabetes are both increasing and worse in the elderly population. Diabetes complicates depressive symptoms which are conversely harmful to diabetic outcomes. The aim of the study was to evaluate the presence of depressive symptoms and the clinical variables associated with depressive symptoms in elderly diabetic patients.

Materials and methods: A cross-sectional study was conducted among diabetic patients aged ≥60 years old admitted to a National Geriatric Hospital from October 2015 to October 2016. Depressive symptoms were assessed by using the Geriatric Depression Scale (GDS).We obtained information on

socio-demographic, medical history, glycaemic control (fasting plasma glucose and HbA_{1c}), daily activities (Activities of Daily Living - ADL and Instruments Activities of Daily Living - IADL scale) and fall risks (the Time Up and Go test). Chi-square (χ^2) statistics and logistic regression were used to analyse the collected data.

Results: 412 diabetic patients were recruited with ages ranging between 60 and 91 years (average age 71.9 ± 7.63 years). 327 (79.4%) patients were categorized according to having depressive symptoms and the group aged 70 - 80 years old had the highest rate of depressive symptoms (141 patients, 43.1%). The proportion of participants with mild, moderate and severe depression symptoms were 62.9%, 14.6% and 1.9%, respectively. The average age, rates of secondary completion or lower, history of hypertension, and using insulin were higher in the depressive symptom group than those in the non-depressive symptom group ($p < 0.05$). The presence of depressive symptoms in the group with duration of diabetes ≥ 5 years was double in comparision with the group with duration of diabetes < 5 years (OR 2.4, 95% CI 1.27-4.66, p<0.01). The level of HbA_{1c} was significantly different between the depressive symptom group and the non-depressive symptom group (7.74 ± 1.57 % and 6.61±1.21 %, $p < 0.05$, respectively). Depressive symptoms increased risk of falls (OR: 2.93; 95% CI: 1.28-6.72, $p = 0.01$), uncontrolled fasting blood glucose (OR: 4.09, 95% CI: 2.1-7.9, $p < 0.001$) and impairment of IADL (OR: 7.12, 95% CI: 3.4-14.9, $p < 0.001$). Rate of decreased ADL in the depressive symptom group (56.6 %) were higher than that in the non-depressive symptom group (10.6%) (p< 0.05).

Conclusion: The prevalence of depressive symptoms was high and the presence of depressive symptoms was associated with increased poor glycemic control, fall risk and impairments of ADL, IADL among elderly diabetic patients.

Disclosure: **H.T.V. Vu:** None.

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Using sense of vitality as an independent marker for predicting risk for MACE in type 2 diabetes

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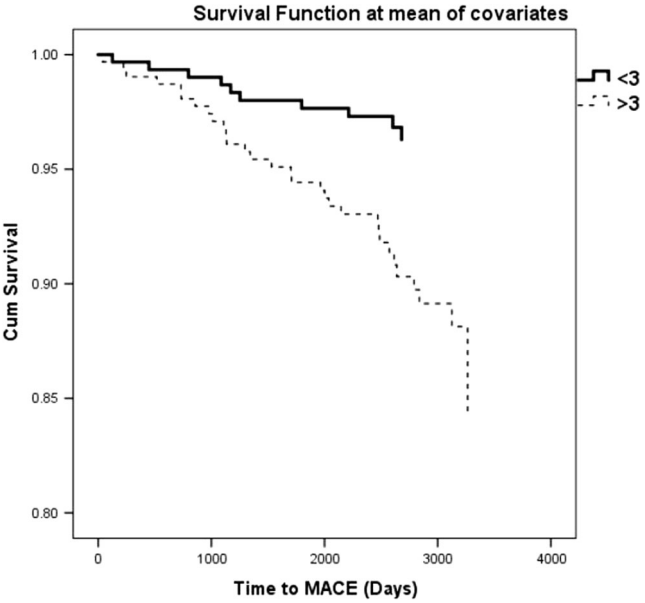
Background and aims: New and clinically useful markers of cardiovascular risk are of essence in type 2 diabetes since ischemic heart disease and stroke are a major causes of morbidity and death in these patients.

Materials and methods: We analyzed baseline data in relation to cardiovascular risk, from 476 men and 244 women who participated in "Cardiovascular Risk factors in Patients with Diabetes -a Prospective study in Primary care" study. All the participants had type 2 diabetes and were 55-66 years old at the recruitment that took place from 2005 to 2008. Except for traditional risk markers such as duration of diabetes, HbA1c, blood pressure and smoking, we also estimated vascular disease non-invasively by carotid-femoral pulse-wave velocity (PWV, with applanation-tonometry) and measured the sagittal abdominal diameter. A questionnaire was used at baseline in which the patients were asked questions about their sense of fatigue, anxiety and physical abilities. The scale for answers were from 1 to 6 in which 1 was "all the time" and then denoted lower frequencies to the figure 6 that corresponded to "never". Patients were followed for morbidity and mortality of ischemic heart disease and stroke (MACE) until December 31 2014, by obtaining data from the national Swedish Cause of Death and Hospitalization Registries.

Results: During the follow-up period of a mean of 7 years 59 patients died or were hospitalized for ischemic heart disease or stroke. To seldom " feel full of pep" predicted increased risk for MACE independently of diabetes duration, age, HbA1c, sagittal abdominal diameter, gender, smoking, systolic blood pressure and PWV (HR 1.321, p= 0.003, CI 1.098-1.589). Correspondingly, a sense of seldom having "lots of energy" related to an independently increased reduced risk with a HR of 1.442 (CI from 1.185 to 1.756, p<0.0001) in the corresponding analysis. In contrast, to feel "tired" or "worn out" were not independent risk markers in the same statistical analyses.

Conclusion: Our data support the use of questions on sense of vitality in type 2 diabetes in order to add prognostic information about risk for subsequent MACE that is independent of traditional risk markers and also of carotid-femoral PWV and sagittal abdominal diameter.



Clinical Trial Registration Number: NCT01049737

Disclosure: **M. Vergara:** None.

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The value-based emotion-cognition-focused educational programme to reduce diabetes distress in adults with type 2 diabetes: a cluster randomised controlled trial

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Background and aims: Type 2 diabetes mellitus (T2DM) patients experience many emotional disorders resulting in decreased self-care, quality of life and disease control. The purpose of this study was to evaluate the effectiveness of a brief value-based emotion-focused educational programme in adults with T2DM on diabetes distress (DD), depressive symptoms, illness perceptions, quality of life, self-efficacy, self-care and clinical outcomes.

Materials and methods: A cluster randomised controlled trial was conducted in 10 public health clinics in Malaysia. Patients' inclusion criteria: Malay, ≥ 18 years with T2DM for at least two years, on regular follow-up and HbA_{1c} or systolic blood pressure (SBP) or LDL sub-optimally controlled, and with a mean 17-item Diabetes Distress Scale (DDS-17) score ≥ 3. Depressive symptoms were measured with the 9-item Patient Health Questionnaire (PHQ-9), and diabetes self-care was measured with the 12-item Summary of Diabetes Self-Care Activities. The intervention (VG) consists of four sessions over a period of six weeks that provide information in proper perceptions of T2DM, managing emotions and goal-setting. The comparator was an attention-control group (AG) with two meetings over a similar period. Primary outcome: between groups difference in proportion of patients achieving a mean DDS-17 score < 3 (non-severe DD) post-intervention. Multilevel mixed-effects modelling were conducted with adjustment for life event (baseline imbalance), age and gender (scientific ground), time and time*group.

Results: A total of 124 participants were included (53 VG and 71 AG). The mean (SD) age in years for VG and AG were 55.6 (10.76) and 55.8 (8.82), respectively. The median diabetes duration was 7.0 and 8.0 years, respectively. At baseline, the mean (SD) DDS-17 score was significantly higher in the VG compared to the AG: 3.4 (0.61) vs. 3.1 (0.98). Reversely, mean (SD) PHQ-9 scores were higher in the AG [6.0 (4.75)] compared to the VG [4.3 (3.30)]. Levels of HbA_{1c} (9.9 vs 9.5%), SBP (137 vs 141 mmHg) and LDL (2.9 vs 3.0

mmol/L) were comparable between groups. Immediately after intervention, VG participants had a three times higher odds of having non-severe DD compared to those in the AG (adjusted OR 3.18 95% CI 1.004 to 10.085). However, VG participants spent on average half a day less to self-care activities per week (adjusted β -0.42 95% CI -0.77 to -0.06). No significant difference was observed between groups [6.2 (4.39) in VG vs 6.7 (5.08) in AG] in depressive symptoms. No differences in other outcomes.

Conclusion: A higher proportion of participants in the intervention group became less distressed but reported less diabetes self-care immediately after the intervention. At short term, our intervention was successful.

Table 1: Baseline characteristics of participants in the VEMOFIT trial

	Attention Control (n = 71)	VEMOFIT Group (n = 53)	Total (n = 124)
Male gender, n (%)	27 (38.0)	21 (39.6)	48 (38.7)
Age (year), mean (SD)	55.8 (8.82)	55.6 (10.76)	55.7 (9.66)
Diabetes Duration (year), median (IQR)	8.0 (9.00)	7.0 (6.50)	7.0 (8.00)
Marital status, n (%)			
Married/living with a partner	63 (88.7)	43 (81.1)	106 (85.5)
Widow/Divorced/separated/Single	8 (11.3)	10 (19.2)	18 (14.5)
Education, n (%)			
Primary	9 (12.7)	14 (26.4)	23 (18.5)
Secondary	54 (76.1)	32 (60.4)	86 (69.4)
Tertiary	8 (11.3)	7 (13.2)	15 (12.1)
Employment, n (%)			
Retired/home manager	46 (64.9)	33 (62.3)	79 (63.7)
Employed	24 (33.8)	19 (35.8)	43 (34.7)
Unemployed	1 (1.4)	1 (1.9)	2 (1.6)
Life event in the past 6 months, n (%)	23 (32.4)*	9 (17.0)*	32 (25.8)
Family history of psychiatric illnesses, n (%)	13 (18.3)	4 (7.5)	17 (13.7)
Hypertension, n (%)	55 (77.5)	43 (81.1)	98 (79.0)
Dyslipidaemia, n (%)	59 (83.1)	36 (67.9)	95 (76.6)
Any diabetes complication, n (%)	17 (23.9)	12 (22.6)	29 (23.4)

*p value < 0.05
VEMOFIT= Value-based **EMO**tion-cognition-**F**ocused educat**I**oral programme to reduce diabetes distress in adults with **T**ype 2 diabetes mellitus; LDL-C= low-density lipoprotein cholesterol; SD= standard deviation; IQR= interquartile range

Clinical Trial Registration Number: NCT02730078
Supported by: the Malaysian MOH-NIH Research Grant (MRG)
Disclosure: **B. Chew:** None.

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Association between improved diabetes-related distress and metabolic control and patient-centered indicators

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Background and aims: New approaches to cope with clinical and psychosocial aspects of type 2 diabetes (T2DM) are needed. We used data from the BENCH-D study to assess impact of changes in diabetes-related distress on clinical and patient-centered outcomes.

Materials and methods: Clinical data was derived from electronic medical records. Diabetes-related distress was measured through the Problem Areas in Diabetes Area-5 (PAID-5). Other nine self-administered validated questionnaires [SF-12 Health Survey; WHO-5 well-being index; Health Care Climate Questionnaire, Patients Assessment of Chronic Illness Care, Diabetes Empowerment Scale (DES-SF), Diabetes Self-care Activities (SDSCA-6), Global Satisfaction for Diabetes Treatment (GSDT), Barriers to Taking Medications (BM), Perceived Social Support (PSS)] were adopted as person-centered outcomes indicators. Association between changes in PAID-5 scores and changes in metabolic control and questionnaire scores (i.e. patient-centered indicators) was assessed by comparing outcomes reached by tertiles of PAID-5 changes (Kruskall-Wallis one-way ANOVA).

Results: Overall, 26 diabetes clinics enrolled 2,390 patients, of whom 1,634 had PAID-5 re-evaluated after 18 months. In the 3 tertiles of PAID-5 changes, PAID-5 score varied from baseline by -40.3±17.0, -8.8±5.9, and +21.0±17.1. Patients in the 3 tertiles did not differ in terms of age (65±10 years) and diabetes duration (13±12 years), but they differed for gender distribution (women represented 44.8%, 35.9%, and 37.0% in the 3 tertiles; p<0.0001). Reduction in PAID-5 score was associated with significant improvements in HbA1c levels, which were reduced by -0.5±1.6%, -0.2±1.3%, and -0.2±1.2% (p=0.003) in the 3 tertiles. Reduction in PAID-5 was also associated with significant improvements in psychological well-being (WHO-5) (score mean changes from baseline to 18 months in the 3 tertiles: +5.4±23.7, -2.2±20.2, -10.4±23.6, p<0.0001), physical well-being (SF-12 PCS) (+2.5±10.2, -0.7 ±8.8, -3.6±10.4, p<0.0001), patients empowerment (DES-SF) (+2.8±17.6, -0.1±16.7, -3.2±17.7, p<0.0001), barriers to medications (BM) (-0.4±11.6, +0.2 ±12.3, +3.2±11.9, p<0.0001), global satisfaction with diabetes treatment (GSDT) (+1.2±15.2, -0.7±12.4,-3.7±13.9, p<0.0001), adherence to pharmacological treatment (SDSCA-6 Drugs) (+0.3±1.8, +0.1±1.6, +0.1±1, p=0.03), perceived social support (PSS) (+0.3±18.3, +0.3±16.0, -2.7±16.3, p<0.0006).

Conclusion: We documented an association between decrease in diabetes related distress and improvement in metabolic control and several quality of life/satisfaction measures in people with T2DM. This suggests that diabetes-related distress might represent a key target of patient-centered diabetes education. Patient-centered outcomes can be regularly monitored with simple, validated questionnaires and should represent an integral component of quality of care evaluation.

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Disclosure: **M. Rossi:** None.

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Improvements in diabetes distress are associated with improvements in biomedical outcomes in type 1 diabetes

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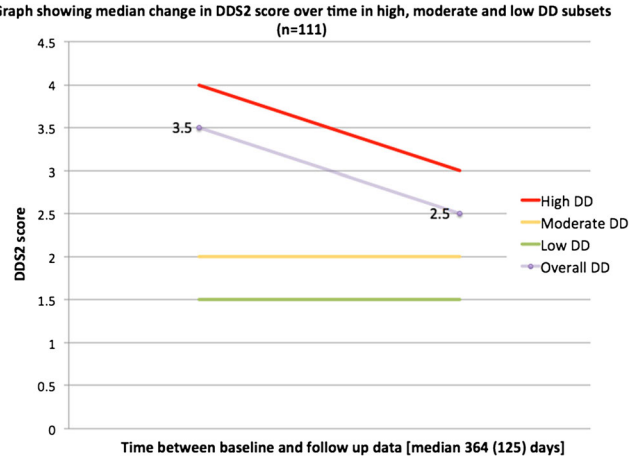
Background and aims: Diabetes distress (DD) refers to the emotional distress associated with ongoing worries, burdens and concerns over time when managing diabetes. High DD is associated with impaired self management and resultant high HbA1c and severe hypoglycaemia. This study aims to analyse the relationship between change in DD and change in biomedical outcomes, such as HbA1c and hypoglycaemia risk, over time. The relationship between DD and healthcare utilisation will also be investigated.

Materials and methods: Between September 2015 and March 2016, patients with Type 1 diabetes, attending diabetes specialist clinics at two teaching hospitals, used a tool to assess DD, HbA1c, and hypoglycaemia risk. DD was measured using the brief diabetes distress score (DDS2), and hypoglycaemia risk was measured using the Gold score, for hypoglycaemia awareness, and history of severe hypoglycaemia. Using patient records, patients were followed up for the same parameters a year later, and their use of healthcare services was measured. Wilcoxon signed rank test was used to analyse changes in DD over time. Spearman's rank correlation was used to evaluate the relationship between change in DD, HbA1c, Gold score and overall hypoglycaemia risk, and also baseline DD and healthcare utilisation.

Results: 111 patients were included in the study. The median follow up time was 364 (125) days. At baseline, mean age was 43.9 ± 5.5 years, 61% were female, mean BMI was 26.1 ± 4.8 kgm^{-2}, mean duration of diabetes was 24.8 ± 12.6 years and 75% used an insulin pump. None of these demographic parameters significantly changed over time. There was a significant reduction in overall DD [median DDS2 score 3.5 (2.5) to 2.5 (1.5), p<0.005], and HbA1c [8.3% (1.6) to 7.9% (1.5), p<0.005] over time. On sub analysis, those with high DD (DDS2 ≥ 3) had a significant reduction in DD [4 (1) to 3 (2), p<0.005], those with moderate DD (DDS2 2-2.9) did not significantly change [2 (0.5) to 2 (1), p=0.422], and those with low distress (DDS2 < 2) had a slight increase in DD [1.5 (0.5) to 1.5 (1), p=0.048] but stayed in the low DD category. There was a significant correlation between change in DD and change in HbA1c (r=0.238, p=0.012). There was no statistically significant

correlation between change in DD and change in Gold score (r=0.131, p=0.171) or change in overall hypoglycaemia risk (r=0.123, p=0.198). There was a significant correlation between baseline DD and number of diabetes-related hospital admissions, letters sent out, consultations attended, emails sent out and missed appointments (r=0.223, p=0.019; r=0.392, p<0.005; r=0.237, p=0.012; r=0.289, p=0.002; r=0.193, p=0.042, respectively).

Conclusion: These data demonstrate that reductions in DD are associated with improvements in biomedical control, and high DD is associated with high healthcare utilisation. Acknowledging high diabetes distress in consultations, and addressing it in management plans, may help to reduce DD and improve biomedical outcomes.



Disclosure: **S.K. Shah:** None.

OP 24 Control of beta cell function and viability

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Identification of Wisp-1 as a young blood-borne factor that promotes adult pancreatic beta cell proliferation

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Background and aims: Restoring functional β-cell mass is a current therapeutic goal in diabetes. One of the proposed strategies is to promote replication of remaining β-cells. It is well known that under normal physiological conditions, adult β-cells have a limited capacity to proliferate. The decline in β-cell replicative activity appears to occur around weaning by mechanisms that are still poorly understood. We hypothesize that blood borne factors present in early postnatal life but absent in adulthood have an important role in this age-related decay. Identification of these factors is important to uncover novel switchers of adult β-cell replication.

Materials and methods: In order to test whether circulating factors are important to maintain β-cell replicative activity, we performed transplants of 20-weeks old C57BL/6J mouse islets into the anterior chamber of 16-days and 20-weeks old mouse recipients. To compare serum from 14-days and 20-weeks old mice, we used commercially available antibody arrays (Proteome Profiler, Mouse XL Cytokine Array Kit, R&D Systems). Proliferation (percentage of ki67+insulin+/insulin+ cells) was studied by immunofluorescence staining of transplanted and cultured islets. Gene expression levels were determined by quantitative real time PCR. Wisp-1 serum levels were determined by ELISA (Quantikine ELISA, Mouse/Rat Wisp-1/CCN4 Immunoassay, R&D Systems). Wisp-1 recombinant protein was obtained from R&D (Recombinant Mouse Wisp-1/CCN4, R&D Systems).

Results: Adult β-cells exhibit a significantly higher proliferation rate when transplanted in young than in adult recipients (16-days recipients, 3.5 ±0.38%; 20-weeks recipients, 1.43±0.35%; p<0.005). Antibody arrays revealed Wisp-1/CCN4 (Wnt-1 inducible signaling pathway-1), as one of the circulating factors that are more abundant in young than in adult serum. We corroborated these data by ELISA (14-days old serum, 11613± 977 pg/ml; 20-weeks old serum, 1844±335 pg/ml; p<0.0001). We surveyed Wisp1 gene expression in several tissues in young mice and observed highest expression in bone. In contrast, levels of Wisp1 mRNA in islets were negligible, thus supporting that, if any, the role of Wisp1 on β-cells would be as an extrinsic factor. To directly test whether Wisp-1 impacts β-cell proliferation, we incubated adult islets in the presence of Wisp-1 recombinant protein for 48h and obtained a significant induction of β-cell proliferation (control, 0.38±0.09%; Wisp-1, 1.04±0.13%, p<0.001). Immunoblot analysis showed that Wisp-1 increases Akt/PKB phosphorylation (2.01 ±0.27 fold above control; p<0.05), thus revealing this kinase as a likely intracellular mediator of the pro-proliferative effect of this factor.

Conclusion: In summary, we provide evidence that Wisp-1 promotes proliferation of adult β-cells, hence supporting the idea that young blood borne factors may be a useful strategy to modulate the intrinsic ability of β-cells to proliferate later in life.

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Disclosure: **R. Fernández-Ruiz:** None.

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miRNAs transferred by exosomes from T lymphocytes to beta cells contribute to type 1 diabetes development

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Background and aims: During type 1 diabetes development, lymphocytes infiltrating the pancreatic islets cause β-cell dysfunction and death. Beside

cytokines, lymphocytes release small vesicles called exosomes containing miRNAs that can be transferred in active form to recipient cells. Since during insulitis β-cells and T cells are brought in close proximity and miRNAs are important regulators of β-cell functions, we hypothesized that an exosome-mediated transfer of miRNAs from T lymphocytes to β-cells may contribute to the death of the insulin-secreting cells in the initial phases of type 1 diabetes.

Materials and methods: To test this novel concept, exosomes were isolated from the culture media of Jurkat T cells or T effector cells of NOD mice. After analysis of their miRNA content, the exosomes were used to study the transfer of miRNAs from T cells to β-cells and to investigate their functional impact. Blockade of miR-142-3p/-5p and miR-155 in vivo was achieved by injecting an Adeno Associated Virus expressing, under the control of the insulin promoter, a transcript containing multiple binding sites for these miRNAs.

Results: We observed that T-cell specific miRNAs, such as miR-142-3p/-5p and miR-155, are highly upregulated in pancreatic islets of NOD mice during pre-diabetic insulitis. The increase of these miRNAs is not caused by pro-inflammatory cytokines, but results from an exosome-mediated cell-to-cell transfer. Indeed, exposure of islet cells to T-cell exosomes led to a rise in the level of miR-142-3p/-5p and miR-155 and caused apoptosis of β-cells but not of α-cells. This effect could be prevented by inhibiting the action of these miRNAs and was reproduced by direct overexpression of miR-142-3p, miR-142-5p or miR-155 in β-cells. To verify the relevance of this phenomenon in vivo, pre-diabetic NOD mice were injected with a viral construct capable of blocking specifically the miRNAs of interest in β-cells. Inactivation of miR-142-3p, miR-142-5p and miR-155 in β-cells of NOD mice led to a decrease in islet inflammation and T-cell recruitment, combined with an important reduction in the incidence of type 1 diabetes.

Conclusion: Taken together, our results suggest that a subset of miRNAs carried by exosomes released by T cells is transferred in active form to β-cells triggering apoptosis. Our results support the concept that the transfer of miRNAs constitutes a novel cell-to-cell communication mechanism contributing to β-cell failure in type 1 diabetes.

Supported by: SNSF, EFSD/JDRF/Lilly, DDF, FRM, FD

Disclosure: **C. Guay:** None.

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Glucose-stimulated cAMP formation in beta cells involves amplificaton of constitutive from the GLP-1 receptor

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Background and aims: The second messenger cAMP is a key amplifier of Ca^{2+}-triggered exocytosis in insulin-secreting β-cells. Apart from mediating the effects of various Gαs-protein-coupled receptor agonists, like glucagon and glucagon-like peptide-1 (GLP-1), cAMP is important for glucose-induced insulin secretion. Glucose consequently stimulates cAMP generation in β-cells, but the underlying mechanisms are incompletely understood. The aim of this study was to clarify if the GLP-1 receptor and Gαs protein are involved in glucose-stimulated cAMP generation in β-cells.

Materials and methods: Total internal reflection fluorescence microscopy and fluorescent reporters were used to record changes of the intracellular concentration of cAMP ($[cAMP]_i$) and insulin secretion from single MIN6-cells and primary β-cells within intact pancreatic islets.

Results: Elevation of the glucose concentration from 3 to 20 mM induced an increase of $[cAMP]_i$ in both MIN6-cells and mouse and human islet β-cells. $[cAMP]_i$ increased also in response to an elevation of the cytoplasmic Ca2+ concentration induced by membrane depolarization with 30 mM K+ in the presence of 3 mM glucose and the ATP-sensitive K+ channel opener diazoxide. Elevation of the glucose concentration under these conditions induced a further increase of $[cAMP]_i$, reflecting stimulation of cAMP production by cell metabolism. The metabolic component of the glucose-induced cAMP formation was reduced after shRNA-mediated knockdown of Gαs. Both the glucose- and K+-evoked $[cAMP]_i$ increases were inhibited by the GLP-1 receptor antagonist exendin-(9-39). The reduction of $[cAMP]_i$ in glucose-stimulated cells was paralleled by suppression of insulin secretion. The effect of exendin-(9-39) did not involve activation of the inhibitory G-protein Gαi, since pertussis toxin did not affect the $[cAMP]_i$-lowering effect of the antagonist in glucose-stimulated cells, while preventing $[cAMP]_i$-lowering induced by α_2-adrenoceptor activation with 100 nM clonidine. Since no GLP-1 was added to the cells, the findings are taken to reflect that exendin-(9-39) is an inverse agonist of the GLP-1 receptor.

Conclusion: Glucose-stimulated cAMP formation in β-cells involves distinct Ca^{2+}- and metabolism-dependent mechanisms. Glucose metabolism activates cAMP-generating adenylyl cyclases by amplifying constitutive signaling from the GLP-1 receptor via Gαs.

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Role of Gs/cAMP-dependent signalling pathway in the establishment of beta cell mass

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Background and aims: Several signaling pathways have been shown to regulate β-cell proliferation. However, the specific roles of individual pathways, as well as their crosstalk, that participate in the establishment of functional β-cell mass remain less understood. In this study we have investigated the role of the signaling pathway activated by the stimulatory protein Gs - acting through the second messenger cAMP in the regulation of postnatal β-cell mass.

Materials and methods: We generated β-cell Gsα deficient mice (β-GsαKO) by crossing *Ins1*Cre with *Gnas*$^{flox/flox}$ mice. Whole body glucose homeostasis was studied by glucose tolerance tests (IpGTT, OGTT), glucose-induced insulin secretion (GSIS) and insulin tolerance test (ITT). β-cell mass was measured with insulin immunofluorescence followed by morphometry analysis. Apoptosis was determined by TUNEL assay and β-cell proliferation was assessed by Ki67 and phospho-histone H3 staining. Isolated islets were used to study insulin and cAMP content by ELISA, protein levels by western blot and gene expression by real time PCR.

Results: At 4-weeks old (wo) β-Gsα KO mice are hyperglycemic, glucose intolerant, exhibit reduced GSIS and similar insulin sensitivity relative to control littermates. They also show lower islet insulin content and decreased fractional β-cell area and mass. This diminution in β-cell mass is associated with reduced β-cell proliferation (WT: 2.8±0.33%; KO: 1.04±0.22% ki67+ ins+/ins+ $p<0.001$) without signs of enhanced apoptosis. In view of these results, experiments were aimed at deciphering the molecular signatures responsible for the observed decreased β-cell growth. As an initial step, we measured cAMP levels and found an 85% reduction ($p<0.0005$) of this second messenger in 4-wo β-GsαKO as compared to control islets. In agreement with decreased β-cell proliferation, expression of the cell cycle genes *CcnA2* and *Ki67* is downregulated (50% of controls, $p<0.01$), whereas expression of the cell cycle inhibitor *cdkn1a* is upregulated (2.5 fold, $p<0.001$) in β-GsαKO islets relative to controls. We surveyed gene expression of several components of the Gs signaling pathway and found reduced islet mRNA levels for *Glp1r, Gipr, Prkarb1* and *Akap11*. However, genes coding for the cAMP effector transcription factor Creb were unaltered. Further, basal activation levels of the Creb protein were only marginally reduced (20%) in KO relative to control islets. These results led us to study other signaling pathways with established roles in β-cell proliferation and found that the gene encoding the IGF1 receptor (*Igf1r)* was severely downregulated in β-GsαKO islets relative to controls, from as early as postnatal day 7. In accordance to reduced IGF1-dependent signaling, 4-wo β-GsαKO islets present significantly reduced AKT (60% of controls, $p<0.001$) and S6K activation (40 % of controls $p<0.05$).

Conclusion: This study reveals the importance of cAMP-mediated signaling in the establishment of β-cell mass. Furthermore, it supports that the crosstalk between Gs and IGF1/AKT-mediated intracellular signaling in β-cells is

relevant for β-cell mass establishment. Further work is ongoing aiming to identify the molecular determinants of this crosstalk and their specific roles in the regulation of postnatal β-cell proliferation.

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Disclosure: **B. Serra-Navarro:** None.

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Multiple type 2 diabetes genes identified using single-cell RNA-seq of human islets

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Background and aims: The pancreatic islets are key regulators of glucose metabolism and deranged islet function is a culprit in type 2 diabetes (T2D). Currently, major focus is on assessing differential expression of genes in T2D vs. non-T2D whole-islet preparations. This strategy does, however, not take into account that the islets are composed of at least five different cell types. If a gene is upregulated in one cell type and downregulated in another, the net outcome may be neutral. Therefore, single-cell information on disease vs. control cells is warranted. Single-cell RNA-sequencing has been successfully performed in human islets, but the available data is contradictory, warranting an additional resource produced at the highest standards.

Materials and methods: Human islets from cadaver donors were provided by The Nordic Network for Clinical Islet Transplantation, Uppsala, Sweden. Islets (100/donor) were handpicked to minimize exocrine content and dissociated into single cell suspensions using an Accutase protocol. Cells were isolated by unbiased FACS and Smart-seq2 single-cell transcriptomics was used to sequence human pancreatic islets cells from 6 human non-diabetic donors and 4 Type 2 diabetic donors (obtaining the transcriptome of 3075 cells). Cell identity was determined using the two way unsupervised clustering algorithm BackSPIN and t-SNE technique. Gene-network analysis was performed using weighted gene co-expression network analysis (WGCNA).

Results: BackSPIN clustering resulted in a model with 12 distinct cell populations, including alpha, beta, delta, PP- and ghrelin cells. The remaining cell populations were exocrine cells, duct cells, stellate cells, endothelial cells, as well as cells from the immune system. Clustering was verified manually by comprehensive analysis of differential expression of established markers for each cell type. Furthermore, we identified subpopulations of beta- and alpha-, and exocrine cells and multiple subpopulation-specific genes. Using reads from 4 T2D donors (1352 cells) and 6 non-diabetic donors (1723 cells), we identified genes that are differentially expressed in T2D specifically in beta cells (972 genes) and alpha cells (857 genes). Most of these genes have not previously been associated with islet function and WGCNA shows that the top listed genes are co-expressed with important T2D associated islet genes, e.g. PDX1, INS, GCGR, and GLP1R. Select genes have been verified by immunohistochemistry in isolated islets, as well as pancreatic sections from the same donors.

Conclusion: We have created a complete roadmap of genes expressed in all islet cell types, as well as subpopulations of alpha and beta cells. We have identified hundreds of genes that are differentially expressed in T2D specifically in human alpha and beta cells. Functional follow up studies will likely generate increased understanding for T2D disease mechanisms and novel drug targets.

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Disclosure: **N. Wierup:** None.

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Short-chain fatty acids potentiate insulin secretion and protect against apoptosis in mouse and human islets; role of FFAR2

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Background and aims: Short chain fatty acids (ScFAs) originate from colonic fermentation of fibre and undigested carbohydrates. The main circulating forms are sodium acetate (SA) and sodium propionate (SP), which we have previously shown to increase insulin secretion and protect β-cells from apoptosis. The current study identified whether these beneficial effects are mediated via Gq-dependent signalin through FFAR2

Materials and methods: Islets were isolated from WT and FFAR2KO mice, and human organ donors. Insulin secretion was profiled by perifusion and RIA; real-time changes in [Ca2+]i in islets were determined by microfluorimetry; PLC was inhibited with U73122; PKC was down-regulated by 20h exposure of islets to 200nM PMA; levels of ATP, cAMP and caspases 3/7 (for apoptosis) were quantified by luminescence assays.

Results: Results: In human islets the selective PLC inhibitor U73122 impaired SA-induced potentiation of insulin release at 20mM glucose (20G) (0.1mM SA: 67.2±8.7 pg/islet/min, + 10μM U73122: 34.3±3.3, $p<0.05$). The effects of SA and SP to increase insulin secretion were PKC-dependent (control, 20G + 0.1mM SA: 44.5±12.6 pg/islet/min; PMA-treated: 19.7±1.1, $p<0.01$; control islets, 20G + 0.1mM SP: 18.7±0.8; PMA-treated: 5.6±0.4, $p<0.01$). Deletion of FFAR2 did not affect the peak amplitude of glucose-stimulated insulin secretion (GSIS) (WT: 10.5±0.9 pg/islet/min; KO: 10.2±1.4). However, the effects of SA and SP on GSIS were significantly reduced following FFAR2 knockout (WT, 20G + 1mM SA: 11.2±2.2 pg/islet/min; KO: 6.5±0.7, $p<0.05$; WT, 20G + 1mM SP: 14.8±1.2; KO: 6.6±0.9, $p<0.01$). In agreement with this, SA-induced elevation in [Ca2+]i was lost in islets from FFAR2KO mice (WT, 20G: 0.68 basal to peak ratio, 20G + 1mM SA: 0.74; KO, 20G: 0.72; 20G + 1mM SA: 0.72). Neither SA nor SP affected cAMP levels in islets from WT or FFAR2KO mice (WT, control: 108.6±12.8nM, +1mM SA: 135.0±10.8; +1mM SP: 91.6±32.4; KO, control: 150.3±28.5; +1mM SA: 137.3±7.8; +1mM SP: 117.7±7.0). Deletion of FFAR2 did not influence islet glucose-induced ATP generation (WT, 20G: 148.5±14.0% basal; 20G + 1mM SA: 127.5±6.8; + 1mM SP 154.6±11.9; KO, 20G: 135.1±14.5; + 1mM SA: 143.1±15.3; + 1mM SP: 143.0±12.9, $p>0.2$ FFAR2KO vs WT in all conditions). No differences in basal apoptosis were observed in FFAR2KO islets (caspase 3/7 activity, WT, basal: 0.96±0.04, KO: 1.06±0.08, $p>0.2$), but the ability of SA and SP to protect against cytokine-induced apoptosis was lost in islets from FFAR2KO mice (WT, + cytokines: 2.21±0.12; +1mM SA: 1.72 ±0.09; +1mM SP: 1.51±0.13, $p<0.01$; KO, +cytokines: 2.43±0.19, +1mM SA: 2.29±0.19; +1mM SP: 2.74±0.20, $p>0.2$). Similarly, the ScFAs did not protect islets against apoptosis induced by palmitic acid (PA) following FFAR2 deletion (WT, +0.5mM PA: 1.61±0.10; +1mM SA: 1.24±0.09; +1mM SP: 1.41 ±0.16, $p<0.05$; KO, +0.5mM PA: 1.45±0.16, +1mM SA: 1.38±0.09; +1mM SP: 1.64±0.13, $p>0.2$).

Conclusion: Although FFAR2 is reported to have dual Gi/Gq coupling, we have demonstrated that in islets SA and SP act solely via FFAR2/Gq to elevate [Ca2+]i, potentiate insulin secretion in a PKC-dependent manner and protect islets against cytotoxic and lipotoxic insults. All of these are hallmarks of beneficial effects in the treatment of type 2 diabetes and position FFAR2 as a potential pharmacological target.

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OP 25 Incretins: entry and actions

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Reduction of HbA_{1c} with dulaglutide in type 2 diabetes (T2D) patients negative, low positive or high positive for GAD antibodies (GADA): a post hoc analysis of AWARD -2, -4 and -5

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Background and aims: Dulaglutide is a weekly GLP-1 receptor agonist indicated for T2D. It enhances insulin secretion from β-cells in a glucose-dependent manner. We evaluated the effect of dulaglutide on HbA_{1c} in GADA negative and GADA positive patients with T2D in a post hoc analysis of 3 dulaglutide trials: AWARD-2, -4 and -5.

Materials and methods: GADA positive T2D, termed LADA (latent autoimmune diabetes of the adult), was defined as GADA ≥5 IU/mL (ELISA, Kronus Inc). Changes in HbA_{1c} were estimated using Mixed-Effect Model Repeat Measurement (MMRM) analysis, adjusted for baseline HbA_{1c}, grouped country, study, treatment, GADA status, visit, and treatment-by-visit, study-by-visit, GADA-by-visit, GADA-by-study and GADA-by-treatment interactions.

Results: Results: 2466/2893 patients (85.2%) were tested for GADA: 92.4% (N=2278) were GADA negative (T2D) and 7.6% (N=188) were GADA positive (LADA). Baseline characteristics were generally comparable between patients with T2D and LADA: mean age range across the trials 54.1-59.6 years and 56.4-57.6 years; BMI 31.2-32.6 kg/m^2 and 30.1-31.6 kg/m^2; diabetes duration 7.1-13.0 years and 6.1-10.9 years; HbA_{1c} 8.13-8.44% and 8.14-8.61%. Of all LADA patients, 58 were considered to be GADA high (>200 IU/mL) while 130 were GADA low (≤200 IU/mL). These subgroups were also generally comparable at baseline. Dulaglutide therapy resulted in similar reductions in HbA_{1c} in patients with T2D and LADA, with comparable results across all 3 studies (Table). Pooled analysis showed similar HbA_{1c} reductions at 6 months in dulaglutide-treated T2D (LS mean change [95% CI]: -1.25% [-1.31, -1.20]) vs. LADA patients (-1.12% [-1.31, -0.93]) and at 12 months (-1.09% [-1.15, -1.03] vs. -0.94% [-1.15, -0.72]). Reductions in HbA_{1c} were numerically larger but not statistically significant at 6 months in GADA low (-1.22% [-1.43, -1.01]) vs. GADA high LADA patients (-0.89% [-1.29, -0.48]) and at 12 months (-1.02% [-1.26, -0.78] vs. -0.72% [-1.21, -0.24]).

Conclusion: HbA_{1c} decreased to a similar extent in T2D and LADA at 6 and 12 months of dulaglutide treatment. Our results conflict with a recent study where GLP-1 analogs failed to reduce HbA_{1c} in GADA-positive patients.

Table: Summary of changes in HbA_{1c} levels at 6 and 12 months with dulaglutide in LADA and T2D patients per study (ITT population)

	AWARD-2 (dulaglutide vs. glargine in T2D patients on metformin and glimepiride)		AWARD-4 (dulaglutide vs. bedtime glargine, both in combination with prandial lispro)		AWARD-5 (dulaglutide vs. sitagliptin)	
LS mean (SE) change from baseline in HbA_{1c} (percentage points)	T2D	LADA	T2D	LADA	T2D	LADA
N	432	16	444	54	541	25
At 6 months	-1.00 (0.04)	-1.18 (0.20)	-1.71 (0.05)	-1.32 (0.12)	-1.11 (0.04)	-0.99 (0.17)
N	404	13	415	47	456	21
At 12 months	-0.84 (0.05)	-1.11 (0.24)	-1.48 (0.06)	-1.08 (0.15)	-1.00 (0.05)	-0.76 (0.20)

N = the number of patients with HbA_{1c} data at baseline that were eligible for the 6-month analysis or 12-month analysis.
All data shown are in the dulaglutide arm only, and not in the comparator arms.

Clinical Trial Registration Nubmer: NCT01075282, NCT01191268, NCT00734474

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Bone turnover in women with prior gestational diabetes after 52 week's treatment with liraglutide

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Background and aims: Liraglutide induces weight loss and as weight loss is associated with loss of bone mass, we investigated the effect of liraglutide-treatment in women with prior gestational diabetes mellitus (pGDM) on whole-body bone mineral density (BMD), whole-body bone mineral content (BMC), the bone resorption marker C-terminal telopeptide of type 1 collagen (CTX) and the bone formation marker procollagen type-1 amino-terminal propeptide (P1NP).

Materials and methods: Women with pGDM (n=102, age: 38±5 years (mean ±SD), BMI: 32±4 kg/m^2) underwent a 4-hour 75-g OGTT at baseline and after one year's randomised and blinded intervention with placebo (n=54) or liraglutide, 1.8 mg once-daily, (n=48). During the trial, 10 women withdrew from the liraglutide group and 9 from the placebo group leaving 83 women for analysis of the effect of the intervention. Controls matched for age and BMI were studied once (n=15, age: 39±4 years, BMI: 31±5 kg/m^2) with a 4-hour 75-g OGTT. Dual energy X-ray absorptiometry was used to assess whole-body BMD and whole-body BMC in both groups. Comparisons between the control group and the pGDM group at baseline were analysed using Mann-Whitney U test. Changes from baseline to 52 weeks were analysed with a constrained linear mixed model. Changes in BMD and BMC are tabulated as changes in means. CTX and P1NP tAUC data were log-transformed due to lack of normal distribution and changes are therefore expressed as percent change in mean.

Results: At baseline, there were no differences between controls and women with pGDM with respect to whole-body BMD (1.32±0.1 vs. 1.26±0.1 g/cm^2, p=0.149), whole-body BMC (2730±388 vs. 2656±279 g, p=0.492) and the total AUC (tAUC) during the OGTT for CTX (30±16 vs. 27±15 μg/L*min, p=0.548) and P1NP (7355±2243 vs. 8355±3368 μg/L*min, p=0.358). Compared to placebo, liraglutide-treatment induced a significantly greater weight loss after 52 weeks (-4.8 [-6.50; -3.00] vs. -1.5 [-3.13; 0.09] kg (mean [CI]), p=0.009). No changes in whole-body BMD (0.004 vs. 0.005 g/m^2, p=0.883), whole-body BMC (6.7 vs. 9.7 g, p=0.799), tAUC for CTX (9.2 vs. 11.5%, p=0.853) or tAUC for P1NP (-4.5 vs. -11.6%, p=0.254) were observed in the placebo vs. liraglutide group.

Conclusion: Liraglutide-treatment-induced weight loss in women with pGDM does not affect bone turnover, whole-body BMD and whole-body BMC respectively, suggesting that liraglutide treatment from a bone turnover perspective is safe to use in this population.

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Site of absorption of an oral formulation of semaglutide

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Background and aims: Absorption of oral peptide-based drug formulations is challenged by the limited permeability of the gastrointestinal (GI) epithelium and by the fact that peptides are degraded in the stomach due to low pH and proteolytic enzymes. A novel oral tablet formulation of the human glucagon-like peptide-1 analogue, semaglutide, has been developed by co-formulation with the absorption enhancer, sodium N-(8-[2-hydroxybenzoyl] amino) caprylate (SNAC). SNAC facilitates the absorption of semaglutide across the GI epithelium primarily via the transcellular route and protects against proteolytic degradation of semaglutide through a localised increase in pH. The site of absorption of oral semaglutide was investigated in a series of clinical and non-clinical studies.

Materials and methods: In a pharmacoscintigraphic clinical study, 24 healthy males in the fasted state received single-dose oral semaglutide (10 mg/300 mg SNAC) with 50 mL water followed by 4 hrs post-dose fasting. Tablet erosion kinetics were assessed by gamma scintigraphy and systemic absorption of semaglutide was assessed by pharmacokinetic blood sampling. In a food-effect clinical study, the pharmacokinetics of oral semaglutide were investigated in 25 healthy subjects receiving once-daily oral semaglutide in the fed state for 10 days (5 days of 5 mg/300 mg SNAC followed by 5 days of 10 mg/300 mg SNAC). Complementary non-clinical studies were conducted in Beagle dogs. Semaglutide exposure was compared between anaesthetised, pyloric ligated dogs (to prevent the tablet from exiting the stomach) receiving intragastric dosing (10 mg/300 mg SNAC), and awake, non-ligated dogs receiving oral dosing. In another study, semaglutide exposure was compared between the splenic vein (draining the gastric cavity) and the portal vein (draining the GI system) after intragastric dosing in anaesthetised, non-ligated dogs. Finally, using endoscopic technique in anaesthetised dogs, liquid from underneath an oral semaglutide tablet and 3 and 6 cm from the tablet was aspirated and assayed for semaglutide and SNAC.

Results: In the pharmacoscintigraphic study, complete tablet erosion (CTE) occurred in the stomach with an estimated mean time to CTE [95% CI] of 85 min [62;118]. Semaglutide plasma exposure confirmed early systemic absorption with median t_{max} of 90 min and mean $AUC_{0\text{-}24h}$ of 119 nmol·h/L [59;243]. In the food-effect study, when dosed in the fed state, limited (44% of subjects) or no (56%) semaglutide exposure was observed. In dogs, prevention of intestinal absorption by pyloric ligation resulted in semaglutide exposure comparable to that seen in non-ligated dogs (mean±SEM dose-normalised C_{1h} 4174±931 vs. 3764±1066 pmol/L/mg; p=0.83). Moreover, semaglutide exposure ($AUC_{0\text{-}0.5h}$) in non-ligated dogs was higher in the splenic vein than in the portal vein (ratio splenic/portal vein [95% CI] 2.2 [1.2;3.2]). Finally, endoscopy showed that high concentrations of semaglutide and SNAC in the gastric fluid were only observed close to the tablet, indicating that semaglutide absorption occurs in a localised environment and depends on the spatial proximity of semaglutide and SNAC.

Conclusion: Clinical and non-clinical data collectively suggest that orally administered semaglutide is absorbed in the stomach, and clinically relevant exposure requires administration of oral semaglutide in the fasting state.

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Evaluation of the effect of food on the pharmacokinetics of oral semaglutide

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Background and aims: Semaglutide, a glucagon-like peptide-1 (GLP-1) analogue, has been co-formulated with the absorption enhancer, sodium N-(8-[2-hydroxybenzoyl] amino) caprylate (SNAC), to allow administration as an oral tablet. Oral semaglutide is absorbed in the stomach, where SNAC facilitates absorption and protects against proteolytic degradation of semaglutide through a localised increase in pH. The aim of this trial was to investigate the effect of food on the pharmacokinetics of oral semaglutide.

Materials and methods: In an open-label, randomised, parallel-group trial design, 78 healthy subjects (39 females/39 males; mean±SD age 55±14 years, body weight 73.5±12.2 kg, body mass index 24.8±2.6 kg/m^2) were allocated 1:1:1 to once-daily treatment (5 mg for 5 days followed by 10 mg for 5 days) using 3 different dosing conditions: 1) Fed (high-caloric, high-fat breakfast ingested during the last 30 minutes pre-dose; 240 mL water taken with dosing; 4 hours post-dose fasting), 2) Fasting (fasting overnight for at least 10 hours; 240 mL water with dosing; 4 hours post-dose fasting) or 3) Reference (fasting overnight for at least 6 hours; 120 mL water with dosing; 30 minutes post-dose fasting). In the Fed and Fasting arms, no further water or liquid was allowed from 2 hours pre-dose until 4 hours post-dose, while there were no restrictions on water, liquid or food intake from 4 hours post-dose. In the Reference arm, no further water or liquid was allowed from 2 hours pre-dose until 30 minutes post-dose, while there were no restrictions on water, liquid or food intake from 30 minutes post-dose. Fed and Fasting arms were designed according to guidelines on the investigation of food effect. The Reference arm reflected the dosing conditions used in the ongoing oral semaglutide clinical development program and was included in order to be able to bridge to the guideline defined dosing conditions.

Results: In the Fed arm, limited semaglutide exposure (11 of 25 subjects) or no semaglutide exposure (14 of 25 subjects) was observed. Semaglutide exposure appeared to be approximately 40% greater for Fasting vs. Reference for both 24-hour exposure ($AUC_{0\text{-}24h,Day\ 10}$; estimated means 383 vs. 272 nmol*h/L; estimated ratio [95% confidence interval] 1.41 [0.96;2.07], p=0.082) and maximum concentration ($C_{max,Day\ 10}$; 20.1 vs. 14.2 nmol/L; 1.41 [0.96;2.08], p=0.080), although not statistically significant. Time to maximum concentration ($t_{max,Day\ 10}$) appeared longer for Fasting vs. Reference (median of 1.75 vs. 1.00 h), while there was no apparent difference in half-life ($t_{½,Day\ 10}$) between Fasting and Reference (geometric mean 160 vs. 152 hours). Overall, the safety profile was as expected for the GLP-1 receptor agonist drug class. In this trial, the most frequently reported adverse events were headache (15%, 38% and 35% of subjects in Fed, Fasting and Reference arms, respectively) and gastrointestinal disorders (19%, 50% and 27%).

Conclusion: Sufficient semaglutide plasma exposure is only achieved when oral semaglutide is administered in the fasting state. Administration with 120 mL water and 30 minutes post-dose fasting is expected to be acceptable to patients and results in clinically relevant semaglutide plasma exposure.

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Risk of pancreatic diseases by second-line anti diabetes drug class: real world based evidence

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Background and aims: Recent clinical observational studies have raised concerns on the possible association of treatment with incretin-based therapies, particularly with DPP-4 inhibitor (DPP-4i), and the risk of acute pancreatitis (AP) or cancer of pancreas (COP) in patients with type 2 diabetes (T2DM). However, a holistic risk analysis in patients receiving different classes of anti-hyperglycaemic agents (AHA) is scarce. The aims of this study were to evaluate the risk of AP, COP, and other disease of pancreas (ODP) in patients with T2DM following the addition of a second-line AHA to metformin therapy.

Materials and methods: Patients with T2DM, aged 18-80 years and diagnosed after 2004, who received metformin plus a second line AHA for at least 3 months, were identified from a large Electronic Medical Record database from the USA. Sulfonylurea (SU), thiazolidinedione (TZD) or insulin (INS) users who had ever received a DPP-4i or GLP-1 receptor agonist (GLP-1RA) were excluded, as were patients with any disease of pancreas or any type of

cancer that occurred prior to initiation of second line AHA therapy (baseline). The rates of events per 1000 person-years were estimated. With DPP-4i as the reference group, the risk analyses were conducted balancing on age and follow-up time, and adjusting for appropriate confounders.

Results: Baseline characteristics varied among SU (n=110,747), DPP-4i (n=50,095), INS (n=34,805), TZD (n=17,597), and GLP-1RA (n=12,654) treatment groups with mean age: 53-60 years; male proportion: 34-52%; mean weight: 98-108 Kg; median HbA1c: 7.2-8.9%; and mean follow-up: 2.8-4.6 years.The rates/1000 person-years of AP were similar in the DPP-4i, GLP-1RA and SU groups (Table 1). Compared to DPP-4i group, patients treated with INS had significantly higher AP rate and those treated with TZD had significantly lower AP rate. Except for INS group, the rate/1000 person-years for ODP were similar across the treatment groups, while there was no significant difference in the rate of COP/1000 person-years among all groups. In the DPP-4i group, the adjusted mean time to AP was 2.63 (95% CI: 2.38, 2.88) years; time to COP was 2.70 (95% CI: 2.19, 3.21) years; and time to ODP was 2.73 (95% CI: 2.33, 3.12) years. Compared to the DPP-4i group, INS patients developed AP 0.48 years ($p<0.01$) earlier and GLP-1RA patients (n=12,654) developed COP 3 years later ($p<0.01$). However, with the constraint of no event within 6 months of INS initiation, the risk of AP in INS group was insignificant. No other significant differences in mean times to events were observed between groups.

Conclusion: This real-world study based on large number of patients with reasonable follow-up time suggests no significant difference in the risk of developing pancreatic diseases in patients treated with different classes of anti-hyperglycaemic agents.

Table 1: Event rates (95% CI) per 1000 person-years; Adjusted Mean Time to Events (95% CI) in DPP-4 group and Adjusted Difference in Time to Events in other treatment groups with DPP-4i as a reference.

		Acute Pancreatitis (95% CI)	Cancer of Pancreas (95% CI)	Other Disease of Pancreas (95% CI)
DPP-4	Rate per 1000 person-years	1.38 (1.21, 1.59)	0.46 (0.36, 0.59)	0.93 (0.78, 1.10)
	Mean Time to Event (years)	2.63 (2.38, 2.88)	2.70 (2.19, 3.21)	2.73 (2.33, 3.12)
GLP-1RA	Rate per 1000 person-years	1.49 (1.16, 1.92)	0.17 (0.08, 0.36)	0.78 (0.55, 1.10)
	Time Difference (years)	-0.18 (-0.72, 0.37)	3.00 (0.84, 5.16)*	0.52 (-0.60, 1.65)
INS	Rate per 1000 person-years	2.01 (1.75, 2.31)	0.59 (0.46, 0.77)	1.48 (1.26, 1.75)
	Time Difference (years)	-0.48 (-0.90, -0.06)*	-0.70 (-1.56, 0.17)	-0.49 (-1.01, 0.03)
SU	Rate per 1000 person-years	1.45 (1.33, 1.58)	0.55 (0.47, 0.63)	1.04 (0.94, 1.15)
	Time Difference (years)	-0.01 (-0.51, 0.50)	-0.57 (-1.26, 0.11)	-0.43 (-1.13, 0.28)
TZD	Rate per 1000 person-years	0.89 (0.70, 1.12)	0.36 (0.25, 0.52)	0.85 (0.67, 1.08)
	Time Difference (years)	-0.25 (-0.56, 0.05)	-0.09 (-0.74, 0.56)	-0.28 (-0.74, 0.18)

*p<0.01

Disclosure: **S. Paul:** Employment/Consultancy; Novartis, GI Dynamics, Roche, AstraZeneca, Guangzhou Zhongyi Pharmaceutical, Amylin Pharmaceuticals LLC. Grants; Merck,, Novo Nordisk, AstraZeneca, Hospira, Amylin Pharmaceuticals, Sanofi-Avensis, Pfizer. Lecture/other fees; University of Melbourne.

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Efficacy and safety of liraglutide in insulin pump treated people with type 1 diabetes: the lira pump trial

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Background and aims: Glucagon-like peptide-1 receptor agonists reduce body weight and risk of hypoglycaemia with moderate effect on HbA1c in people with type 1 diabetes (T1D) using basal-bolus insulin therapy. The evidence in people with insulin pump treated T1D is sparse.

Materials and methods: This randomised, double-blinded, placebo-controlled, 26-week trial evaluated the efficacy and safety of liraglutide 1.8 mg added to insulin pump therapy in overweight people with T1D and insufficient glycaemic control. In total 44 persons with insulin pump treated T1D, HbA1c >7.5 % and BMI >25 kg/m^2, were randomised to liraglutide 1.8 mg or placebo once daily added to insulin pump therapy.

Results: Baseline characteristics were similar between groups (mean (SD) or median (interquartile range)) HbA1c 8.2 (0.5) %, diabetes duration 20 (15;35) years, daily insulin dose 51 (17) IU/day and body weight 87 (12) kg. After 26 weeks of treatment, liraglutide reduced HbA1c and body weight compared with placebo (table). No differences were found in changes in daily insulin dose, time spent in hypoglycaemia (<3.9 mmol/l), heart rate or blood pressure between groups. Treatment satisfaction evaluated by the Diabetes Treatment Satisfaction Questionnaire version c (DTSQc) increased more with liraglutide than with placebo. Gastrointestinal adverse events occurred more frequently with liraglutide than placebo, e.g. nausea (39% vs. 20%). Finally, one event of severe hypoglycaemia was reported during the trial in a person treated with placebo.

Conclusion: Liraglutide 1.8 mg once daily added to insulin pump treatment in overweight people with T1D and insufficient glycaemic control reduces HbA1c, body weight and increases treatment satisfaction with no effect on daily insulin dose or time spent in hypoglycaemia compared with placebo after 26 weeks of treatment.

Key Results: 26 weeks of liraglutide 1.8 mg vs. placebo once daily added to insulin pump treatment

	Liraglutide, n=22 [95% CI]	Placebo, n=22 [95% CI]	Difference	*P* value
Change in HbA1c (%)	-0.4 [-0.7; -0.2]	0.2 [0.0; 0.5]	-0.6 [-1.0;-0.3]	<0.001
Change in body weight (kg)	-6.4 [-7.9; -4.9]	-0.7 [-2.1; 0.8]	-5.7 [-7.9;-3.6]	<0.001
Change in daily insulin dose (IU/day)	0.5 [-2.1; 3.1]	2.5 [0.1; 5.0]	-2.0 [-5.6; 1.6]	0.270
Change in time spent in hypoglycaemia (%)*	0.3 [-2.6; 3.2]	2.0 [-0.7; 4.7]	-1.7 [-5.6;2.2]	0.397
Change in systolic blood pressure (mmHg)	-3.7 [-9.2; 1.8]	-3.1 [-8.3; 2.1]	-0.6 [-8.2; 7.0]	0.880
Change in diastolic blood pressure (mmHg)	-0.1 [-2.7; 2.5]	0.1 [-2.4; 2.5]	-0.2 [-3.8; 3.4]	0.921
Change in heart rate (beats/min)	5.4 [1.1; 9.7]	0.3 [-3.8; 4.3]	5.1 [-0.7; 11.0]	0.086
Change in Diabetes Treatment Satisfaction Questionnaire version c	11.7 [8.6; 14.8]	6.1 [3.1; 9.0]	5.6 [1.3; 9.9]	0.010

*Glucose <3.9 mmol/L assessed by 1 week of blinded continuous glucose monitoring at baseline, 3 weeks, 13 weeks and 26 weeks.

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Olfactory function is associated with the intensity of self-reported physical activity in adults with type 1 diabetes

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Background and aims: Diabetes mellitus contributes to the central nervous system degeneration and cognitive decline. Olfactory dysfunction is related to the presence of diabetic neuropathy and suggested to be a clinical manifestation of central diabetic neuropathy. Regular physical activity is known to have beneficial effects on the metabolic control of diabetes, prevention of chronic neurovascular complications and cognitive function. The aim of this study was to assess olfactory function in relation to self-reported physical activity in adults with type 1 diabetes (T1D).

Materials and methods: We included 89 patients with type 1 diabetes (43 men), median age 35 (IQR 29-42) years, disease duration 17 (IQR 13-26) years, HbA1c 8.2 (IQR 7.3-9)%. The control group consisted of 22 healthy people matched for age and gender. The patients underwent ENT examination with nasal endoscopy to exclude other factors disabling sense of smell. Olfactory function was assessed with "Sniffin' Sticks. For the assessment of odor identification 12 pens with different odors were used and patient should select 1 of 4 presented items which best described each odor for every pen (score 0-12, normosmia: score 11-12). The short version of International Physical Activity Questionnaire (IPAQ) was used to assess self-reported physical activity. Higher IPAQ result reflects higher intensity of physical activity and is presented as the time spent on activities of different intensity in the last 7 days. We assessed the metabolic control of diabetes.

Results: Hyposmia was found in 70% of patients with T1D compared to 45% in control group (p=0.03). No differences in self-reported physical activity were found between T1D and control group (median score 1588 [IQR 975-3291] vs. 1968 [IQR 1257-3573], respectively; p=0.2). In T1D group we found a positive correlation between olfactory identification scores and IPAQ results (Rs 0.26; p=0.01) and a negative correlation of olfactory identification scores with body mass index (Rs -0.23, p=0.03) and triglycerides (Rs -0.22; p=0.04). Stepwise multiple linear regression analysis indicated IPAQ result (beta 0.26, p=0.01) and BMI (beta -0.24, p=0.03) as predictors of olfactory function independently from age, HbA1c and triglycerides.

Conclusion: Better olfactory function is observed in adults with type 1 diabetes who self-report higher intensity of physical activity. This study confirms the beneficial role of physical activity in type 1 diabetes within the structures of the central nervous system.

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Somatosensory cortical plasticity determines clinical presentation in diabetic neuropathy

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Background and aims: Cortical plasticity is a fundamental property of the human CNS that enables adjustment to nerve injury but it can have maladaptive consequences resulting in chronic pain. We have previously demonstrated significant brain volume loss localised to the primary somatosensory cortex (S1) in diabetic neuropathy (DN). More recently, we reported functional reorganisation of S1 in patients with painful DN who were also insensate. This study aims to examine the relationship between the degree of functional reorganisation and intensity of neuropathic pain.

Materials and methods: Clinical, neurophysiological and magnetic resonance imaging (MRI) data were compared for 35 Type 1 diabetes subjects [n=9 No-DN (male 6, mean age 45.9±10.1), 9 painless DN (5, 46.3±12.0), 9 painful DN sensate (4, 48.4±12.0) and 8 painful DN insensate (6, 44.5±12.1)] and 9 healthy volunteers (4, 51.5±7.9)]. 3T MRI system (Acheiva, Phillips) and cortical reconstruction and volumetric segmentation by FreeSurfer software were used. Blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) was used to examine functional organisation of the S1 cortex in response to noxious thermal stimulation of the right foot. Linear regression analysis was performed to examine the contribution of brain structural changes (S1 cortical thickness), pain (neuropathic and evoked pain) and clinical parameters (age, neuropathy composite score, diabetes and pain duration) on observed S1 plasticity.

Results: Painful DN insensate subjects had significantly lower mean S1 cortical thickness [$F(4,39)=5.78$, $p=0.001$] compared to other study cohorts. No significant difference emerged between subjects in cortical thickness in the control region [frontal cortex $F(4,39)=1.23$, $p=0.31$]. On average somatosensory cortical thickness was 12.2% lower in painful DN insensate subjects compared to healthy volunteers. S1 plasticity was significantly correlated with overall neuropathic pain score (NTSS-6, r=-0.45, p=0.04), evoked foot pain score (r=-0.46, p=0.03) and S1 cortical thickness (r=-0.57, p=0.01). The most parsimonious regression model relied solely on neuropathy composite score as the independent variable ($R^2=0.54$, $F(1,15)=17.9$, $p=0.01$; adjusted $R^2=0.51$) that explained most of the variance in S1 plasticity.

Conclusion: Subjects with painful DN who were also insensate had the greatest reduction in S1 cortical thickness. FMRI demonstrated greater S1 cortical neuronal plasticity which was significantly associated with pain intensity and the severity of neuropathy. We have demonstrated in these experiments how structural brain changes are related to functional reorganisation of the S1 cortex which ultimately determines the clinical presentation of DN. This may provide clues to the pathogenesis of different sensory phenotypes of DN.

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Nerve and vascular biomarkers in skin biopsies differentiate painful from painless advanced diabetic peripheral neuropathy

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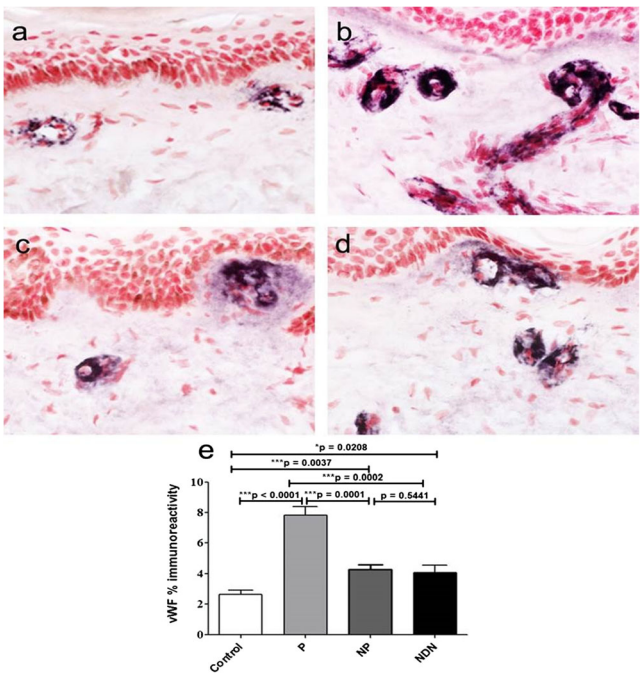
Background and aims: Painful diabetic peripheral neuropathy (painful-DPN) can be intractable with major impact, yet the underlying pain mechanisms remain uncertain. While distal leg intra-epidermal nerve fibre density is recommended for the diagnosis of small fibre neuropathy, we found that intra-epidermal nerve markers may not differentiate between advanced painful-DPN and painless-DPN. The relationship of neuronal and vascular biomarkers was therefore investigated in painful-DPN and painless-DPN.

Materials and methods: 61 T2DM subjects and 19 healthy volunteers (HV) underwent detailed clinical and neurophysiological assessments and were subsequently divided into three groups based on the neuropathy composite score of the lower limbs [NIS(LL)] plus 7 tests (23 Painful-DPN, 19 Painless-DPN and 19 No-DPN). All subjects underwent calf skin punch biopsy, and immunohistochemistry was used to quantify intra-epidermal (IENF) and sub-epidermal (SENF) nerve fibres with structural marker PGP9.5, regenerating fibres with GAP43, sensory fibres with neuropeptide CGRP, and the dermal blood vessels with von Willebrand Factor (vWF).

Results: IENF density was severely decreased (p<0.001) in both DPN groups, with no differences for PGP9.5, GAP43, CGRP, or GAP43/PGP9.5 ratios. There was significant increase of vWF in Painless-DPN and no-DPN groups compared to controls, but this was markedly greater for painful-DPN, and significantly higher than painless-DPN (p<0.0001). The ratio of SENF CGRP:vWF showed a significant decrease in painful-DPN vs Painless-DPN (p =0.014).

Conclusion: In advanced neuropathy increased dermal vasculature and its ratio to nociceptors may differentiate painful-DPN from painless-DPN. Increased blood vessels following tissue ischaemia/hypoxia associated with disproportionate and abnormal nerve fibres (irritable nociceptors) may lead to a "painful vaso-neuropathy".



Clinical Trial Registration Number: STH15701
Supported by: Sheffield Teaching Hospitals Charitable Fund
Disclosure: **P. Shillo:** None.

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Corneal confocal microscopy detects improvements in small nerve fibres in subjects with latent autoimmune diabetes in adults with enhanced glycaemic control

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Background and aims: Latent Autoimmune Diabetes in Adults (LADA) is often misdiagnosed, and a prolonged period of poor glycaemic control may increase the risk of microvascular complications. We aimed to evaluate a cohort of subjects with LADA over 1 year and undertake detailed assessment of measures of large and small fibre neuropathy to assess the natural history of diabetic neuropathy.

Materials and methods: We have undertaken a detailed assessment of neuropathy in control subjects (C) (n=14), subjects with type 2 DM (T2DM) (n=14) and LADA (n=14) by quantifying: neuropathy disability score (NDS), peroneal and sural nerve conduction velocity and amplitude (PMNCV, PMNAmp, SSNCV, SSNAmp), cold threshold (CT), warm threshold (WT), corneal nerve fibre density (CNFD), branch density (CNBD) and fibre length (CNFL) at baseline and 1 year follow up.

Results: There was no significant difference in age (50.0±2.9 vs 54.0±6.5 vs 50.1±13.7 years, P=NS), duration of diabetes (T2DM: 5.3±3.9 vs LADA: 12.5 ±10.6 years, P=NS), blood pressure, total cholesterol, HDL, triglycerides and eGFR. HbA1c (39.7±2.8 vs 53.4±7.3 vs 83.8±25.3 mmol/mol, P<0.0001) was higher and BMI (29.4±5.2 vs 31.0±4.5 vs 26.5±4.6 kg/m^2, P=0.01) was lower in LADA compared to T2DM. There were no significant differences in NDS (0.3±0.6 vs 1.6±1.7 vs 3.8±3.3, P=NS), PMNCV (49.2±4.2 vs 44.3±8.8 vs 40.7±7.6 m/s, P=NS), PMNAmp (5.5±1.9 vs 4.0±2.1 vs 3.2±2.2 mV, P=NS), SSNCV (50.5±3.5 vs 47.8±5.1 vs 43.4±5.6 m/s, P=NS), SSNAmp (17.7±7.1 vs 12.6±7.6 vs 11.0±5.8 μV, P=NS), WT (37.0±3.1 vs 40.9±3.6 vs 42.4±5.0 °C, P=NS), CT (28.9±1.2 vs 27.0±3.2 vs 24.1±4.7 °C, P=NS) between LADA and T2DM. However, CNFL (16.9±4.6 vs 17.1±4.7 vs 11.7±3.3 mm/mm^2, *P*=0.003) and CNBD (37.8±18.0 vs 45.5±26.4 vs 21.0±10.6 no/mm^2, *P*=0.008) were significantly lower with no difference in CNFD (29.0±9.1 vs 28.8±9.8 vs 18.8±7.1 no/mm^2, *P*=NS) between LADA and T2DM. Comparing baseline to 1 year follow up, there were no changes in BMI, lipids, NDS, CT, WT and electrophysiology in C, T2DM and LADA. HbA1c, CNFD, CNBD and CNFL did not differ in C and T2DM. However, HbA1c was lower and approached significance (83.8±25.3 vs 70.0±17.4 mmol/mol, P=0.07) with a higher CNBD (21.0±10.6 vs 34.8±16.3 no/mm^2, *P*=0.005) and CNFL (11.7±3.3 vs 14.3±4.0 mm/mm^2, *P*=0.02) with no change in CNFD (18.8±7.1 vs 22.3±5.9 no/mm^2, *P*=NS) in LADA at 1 year follow up.

Conclusion: Subjects with LADA have a significant small fibre neuropathy due to poorer glycaemic control and enhanced glycaemic control improves neuropathy measured using CCM. CCM not only detects but also monitors progression of small fibre neuropathy and provides the ideal surrogate marker in therapeutic trials of diabetic neuropathy.

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Disclosure: **U. Alam:** None.

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Genome-wide association study of neuropathic pain in individuals with type 1 diabetes

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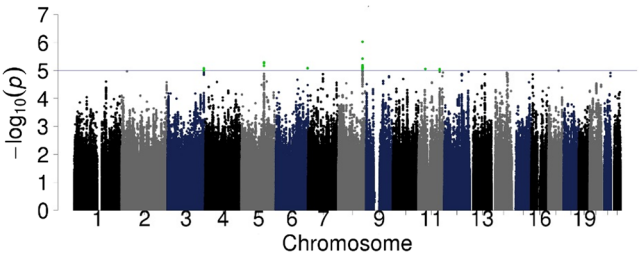
Background and aims: Neuropathic pain is one of the most common complications in individuals with diabetes, yet little is known about the genetic risk factors of the complication. Previous studies have suggested that part of the disease risk can be explained by genetic factors. We aimed to investigate the association of genetic factors with neuropathic pain in individuals with type 1 diabetes (T1D).

Materials and methods: This study included 2,292 individuals with T1D from the Finnish Diabetic Nephropathy Study (FinnDiane). The patients were required to have an age at onset of diabetes below 40 years and insulin treatment initiation within one year of diagnosis. Individuals were divided based on neuropathic pain into cases (N=677) and controls (N=1,615). Individuals filled a questionnaire and neuropathic pain was considered present if the individual was using drugs prescribed for neuropathic pain or if the individual had experienced burning or prickling pain in the foot or the leg. Controls were not using any of the pain medications and had not experienced burning or prickling pain in the feet or the legs after a minimum diabetes duration of 15 years. Genotyping was perfomed with the Illumina Human CoreExome chip. The genotype data was imputed using the 1000 Genomes Phase 3 version 5 reference panel. After quality control the imputed genotype data included approximately 7 million single nucleotide variants (SNVs). The association of the

genotype to neuropathic pain was tested using a score test with an additive model implemented in SNPTest.

Results: After adjusting for sex, age, diabetes duration, the 4 first genetic principal components and genotyping batch, 6 loci were suggestively associated with neuropathic pain with a p-value $\leq 10^{-5}$. The strongest association signal with a p-value of $9.5*10^{-7}$ was observed on chromosome 8q24.21 in an intergenic region between two uncharacterized non-coding RNA genes *LOC101927657* and *LOC105375749*.

Conclusion: We performed to date the largest GWAS study of neuropathic pain in T1D individuals. Suggestive association to neuropathic pain was observed for 6 loci. Further investigation of these variants in independent cohorts is required to confirm these associations.



Supported by: Folkhälsan Research Foundation, Wilhelm and Else Stockmann Foundation

Disclosure: **E. Valo:** Grants; Folkhälsan Research Foundation, Wilhelm and Else Stockmann Foundation.

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Impairment of proopiomelanocortin-mu opioid receptor antinociceptive pathway contributes to painful diabetic neuropathy

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Background and aims: One-third of diabetic patients experience hyperalgesia and/or allodynia. Current treatments are inefficient in reversing these symptoms and can have adverse side effects. Understanding the mechanisms underlying increased pain sensitivity will help design better treatment strategies. Pro-opiomelanocortin (POMC; endogenous opioid precursor) is cleaved to produce the opioid peptide β-endorphin, which binds to mu opioid receptor (MOR) resulting in alleviation of pain. This study examines the significance of POMC-MOR axis in context of painful diabetic periperhal neuropathy (DPN).

Materials and methods: Streptozotocin (STZ) induced C57Bl/6 mice were used to study changes in POMC-MOR. POMC and MOR expression was studied in the dorsal root ganglia (DRG), sciatic nerves(SN) and feet using ELISA, western blot and immunoflourescence. mRNA levels were determined by qPCR. Promoter studies were carried in AtT20 cells and in vivo using EMSA and ChIP techniques, while MOR regulation was studied in primary DRG culture and in vivo using live cell staining and kinase activity assay. Viral constructs of POMC and MOR were overexpressed in lumbar DRG of diabetic mice using direct DRG injections. Pain sensitivity was measured using hotplate, Hargreaves and Frey-filament technique. Lastly, POMC and MOR expression in human DRG were analysed by immunohistochemistry.

Results: Immune cells, previously identified to be opioid sources in injured nerves, were studied in the diabetic sciatic nerves. Although a two fold increase ($p<0.001$) in CD68+macrophages was observed in the sciatic nerves, an expression of opioids was not observed in these cells. Instead, the opioids were majorly present in the axons of sciatic nerves and neuronal cell bodies in lumbar DRG. At 12 weeks post STZ, opioid peptide level for the other classical opioids (proenkephalin and prodynorphin) were unchanged, but a significant downregulation was observed for POMC in DRG (46%; $p<0.05$), SN (62%; $p<0.01$) and feet (46%; $p<0.05$) compared to healthy controls. The downregulation in POMC was reflected in the decreased ß-endorphin (ng/mg protein) in SN (3.7 ± 1.17 vs 0.94 ± 0.79; $p<0.05$) and the feet (6.55 ± 0.02 vs 1.35 ± 0.55; $p<0.05$). High glucose induced NF-kB activation and its binding to POMC promoter was responsible for promoter suppression, and hence, the decreased POMC mRNA and peptide level. Surprisingly, MOR protein level, too, was decreased by 50% in DRG ($p<0.001$), SN ($p<0.05$) and feet ($p<0.05$). Downregulation of MOR was due to its lysosomal degradation induced by chronic PKC activation. Remarkably, the decreased POMC and MOR were associated with increased thermal and mechanical hypersensitivity in the diabetic mice, which was reversed upon overexpression of both genes. A decrease in POMC and MOR content was also observed in the lumbar DRG of diabetic patients.

Conclusion: This study reports for the first time, the importance of POMC-MOR antinociceptive pathway in the pathogenesis of DPN. Therefore, treatment strategies including stimulating the endogenous POMC levels and preventing the lysosomal degradation of MOR could help counter the pain.

Supported by: DIAMICOM(DFG)

Disclosure: **D.D. Deshpande:** Grants; DFG.

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Pharmacological autonomic blockade during experimental hypoglycaemia exacerbates cardiac repolarisation abnormalities

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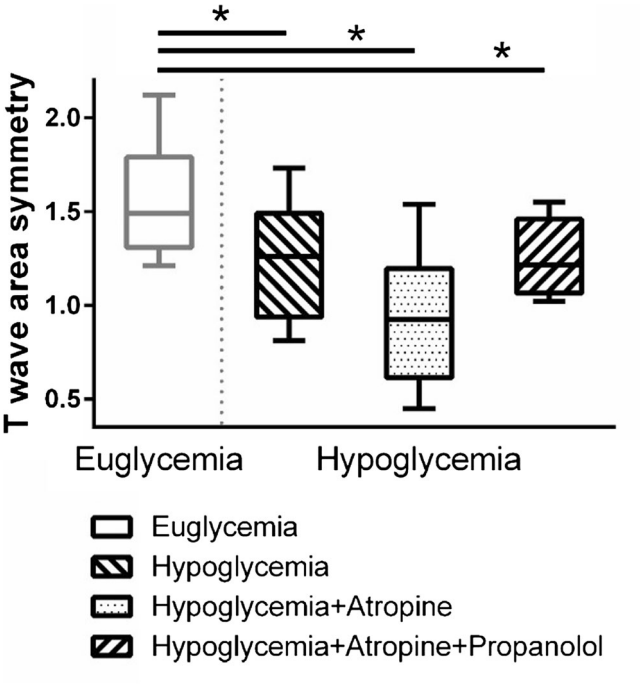
Background and aims: Hypoglycaemia may contribute to sudden death in patients with diabetes by provoking cardiac arrhythmias. Potential mechanisms include abnormal cardiac electrophysiology and autonomic neuropathy. We hypothesised that cardiac autonomic dysfunction may interact with direct effects of hypoglycaemia to increase arrhythmic risk. The aim of the study was to investigate the effect of hypoglycaemia and coincident pharmacological parasympathetic and sympathetic blockade on cardiac repolarisation and actaion potential duration.

Materials and methods: Six healthy subjects underwent sequential hyperinsulinaemic euglycaemic (5mmol/l) and hypoglycaemic (2.5mmol/l) clamp studies with parasympathetic (atropine) and sympathetic (propranolol) blockade given sequentially during hypoglycaemia. Twelve lead surface ECG and intracardiac electrograms at right ventricle apex and free wall were recorded. Corrected QT (QTc), parameters of ventricular repolarisation (T wave amplitude and area symmetry), complexity of repolarisation (PCA ratio) and intracardiac activation-recovery intervals (ARI) were calculated in all four conditions.

Results: During hypoglycaemia, mean adrenaline increased to 1.49 ±1.70nmol/l, noradrenaline to 2.31±0.84nmol/l and potassium fell to 3.38±0.22mmol/l (all $p<0.05$). Heart rate increased (Δ7±16bpm) and QTc prolonged (Δ29±35ms) during hypoglycaemia vs euglycaemia. Parasympathetic blockade under hypoglycaemia caused further increases in heart rate (Δ48±12bpm, $p<0.001$ vs euglycaemia) and QTc prolongation (Δ78±38ms, $p=0.004$). T wave symmetry significantly decreased (Δ-0.62±0.57, $p=0.04$, see figure), resulting in more symmetric waves and complexity of repolarisation increased (Δ11.6 ±10, $p=0.04$). ARIs shortened with atropine but non-uniformly across the right ventricle (Δ-33ms at apex, $p=0.004$ vs Δ-49ms, $p=0.001$ at free wall). After sympathetic blockade with propranolol all parameters were reversed to hypoglycaemia levels.

Conclusion: Blocking parasympathetic activity exacerbates abnormal cardiac repolarisation during experimental hypoglycaemia. This mechanism could contribute to arrhythmias that have been reported during clinical hypoglycaemic episodes. It is particularly relevant to diabetic autonomic dysfunction since diminished parasympathetic activity is an early manifestation.



Data: median and maximum range. * $p<0.05$

Supported by: National Institute for Health Research (NIHR)

Disclosure: **A. Bernjak:** Employment/Consultancy; SRH: Sanofi Aventis, SRH: Eli Lilly, SRH: Takeda, SRH: NovoNordisk, SRH: Astra Zeneca.

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Severe hypoglycaemia, cardiovascular outcomes and death: the LEADER experience

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Background and aims: In the LEADER cardiovascular outcomes trial (N=9340), the risk of cardiovascular and hypoglycaemia events was reduced with liraglutide treatment vs placebo, when added to standard of care, in patients with type 2 diabetes and high risk for cardiovascular disease. This post hoc analysis examines the potential associations between severe hypoglycaemia and cardiovascular events and death.

Materials and methods: We analysed the time to first major adverse cardiovascular event (MACE) (cardiovascular death, non-fatal myocardial infarction or non-fatal stroke), cardiovascular death and all-cause death among patients with/without severe hypoglycaemia, and adjusted for different periods of follow-up and randomised treatment.

Results: During the trial, 267 patients experienced severe hypoglycaemia (liraglutide n=114, placebo n=153; rate ratio, 0.69; 95% CI: 0.51; 0.93). These patients were more likely than those without severe hypoglycaemia to experience MACE, cardiovascular death and all-cause death, with a considerably higher risk up to 60 days after the hypoglycaemic episode (Table), irrespective of treatment group. The protective effect of liraglutide on risk of MACE was unchanged when patients with severe hypoglycaemia were excluded from the analysis (patients with severe hypoglycaemia accounted for 5% of all MACE in the trial).

Conclusion: In conclusion, patients experiencing severe hypoglycaemia were at greater risk of cardiovascular events and death, particularly early after the hypoglycaemic episode. Reducing severe hypoglycaemia remains a cornerstone of diabetes management.

	Risk of outcome in patients with versus without severe hypoglycaemia, hazard ratio [95% CI], *p*-value				
Outcome	Severe hypoglycaemia at any time*	Time-dependent: event after severe hypoglycaemia†	Time-dependent: within 15 days†	Time-dependent: within 30 days†	Time-dependent: within 60 days†
MACE	1.9 [1.5;2.5], p<0.0001	2.2 [1.6;3.0], p<0.0001	5.4 [1.7;16.8], p<0.01	5.8 [2.6;13.0], p<0.0001	3.1 [1.4;7.0], p<0.01
CV death	2.2 [1.5;3.2], p<0.0001	3.7 [2.6;5.4], p<0.0001	9.5 [2.4;38.1], p<0.01	12.6 [5.2;30.5], p<0.0001	6.7 [2.8;16.1], p<0.0001
All-cause death	2.2 [1.7;3.0], p<0.0001	3.6 [2.7;4.9], p<0.0001	8.6 [2.8;26.8], p<0.001	12.2 [6.1;24.5], p<0.0001	8.9 [4.9;16.2], p<0.0001

*Time to first MACE, CV death or all-cause death, using Cox regression with severe hypoglycaemia (yes/no) at any time as a factor. †Severe hypoglycaemia episodes leading to MACE, CV death or all-cause death, using a time-dependent covariate Cox regression: all events (follow-up until last contact date), follow-up within 15, 30 and 60 days. CI, confidence interval; CV, cardiovascular; MACE, major cardiovascular event

Clinical Trial Egistration Number: NCT01179048

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Disclosure: **B. Zinman:** Other; Consultant and/or Grant support and/or Author: Novo Nordisk A/S, Boehringer Ingelheim GmbH, AstraZeneca, Eli Lilly and Company, Janssen Biotech, Inc., Sanofi, Merck & Co., Inc.

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Efficacy and safety of insulin degludec/insulin aspart vs biphasic insulin aspart 30: an international randomised trial in adults with type 2 diabetes fasting during Ramadan

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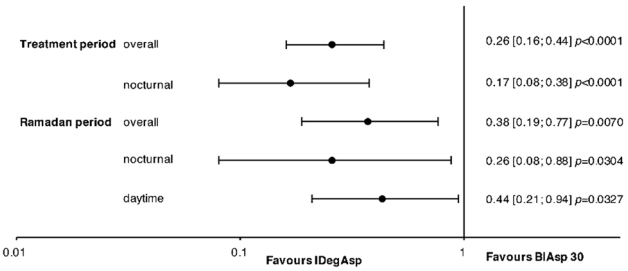
Background and aims: Muslims with type 2 diabetes (T2D) on twice daily (BID) mixed or basal-bolus insulin are at high risk of dehydration, hypoglycaemia and overt hyperglycaemia during Ramadan fasting (abstain from food or drink, dawn-sunset). Some are thus given medical and religious advice not to fast during Ramadan but many participate against this advice. Trials on insulin use in Ramadan are scarce. IDegAsp, a co-formulation of insulin degludec and insulin aspart is associated with reduced hypoglycaemia risk vs biphasic insulin aspart 30 (BIAsp 30) due to the flat pharmacokinetic profile and long duration of action of insulin degludec. This international, randomised, treat-to-target trial aimed to compare the efficacy and safety of IDegAsp vs BIAsp 30 during Ramadan.

Materials and methods: Adults with T2D who intended to fast and were on basal, pre- or self-mixed insulin ± oral antidiabetic drugs for ≥90 days, were randomised (1:1) to IDegAsp BID or BIAsp 30 BID. Treatment was for 16-28 weeks (treatment initiation: 8-20 weeks pre-Ramadan; Ramadan: 4 weeks; post-Ramadan: 4 weeks). Insulin doses were titrated to achieve a plasma glucose (PG) target of 4-5 mmol/L (72-90 mg/dL) during initiation and 5-7 mmol/L (90-126 mg/dL) during Ramadan. At Ramadan start, a 30-50% reduction in insulin dose taken at Suhur (pre-dawn meal) was recommended. Hypoglycaemia was recorded as severe (requiring third-party assistance), or confirmed (severe and/or PG <3.1 mmol/L) and analysed as overall, nocturnal (00:01-05:59), or daytime (2 h after Suhur-pre-Iftar [pre-sunset meal]; post hoc).

Results: Patients were randomised to IDegAsp (n=131) or BIAsp 30 (n=132). There was no significant difference with IDegAsp vs BIAsp 30 in HbA_{1c} reduction from baseline to end of Ramadan (end of treatment difference [ETD]: 0.02% [95% CI -0.20; 0.24], p=0.8426). During treatment, rates of overall and nocturnal hypoglycaemia were significantly lower with IDegAsp vs BIAsp 30 (Fig). During Ramadan, pre-Iftar self-measured PG (SMPG) was significantly lower with IDegAsp vs BIAsp 30 (ETD: -0.54 mmol/L [95% CI -1.02; -0.07], p=0.0247, post hoc). There were significantly lower overall, nocturnal and daytime hypoglycaemia rates with IDegAsp vs BIAsp 30 during Ramadan (Fig).

Conclusion: This trial in high-risk adults with T2D who fasted in Ramadan showed that IDegAsp had similar glycaemic efficacy to BIAsp 30 before and during Ramadan, but significantly lower overall and nocturnal hypoglycaemia rates during the treatment period. During Ramadan, despite achieving significantly lower pre-Iftar SMPG, IDegAsp had significantly lower overall, nocturnal and daytime hypoglycaemia rates vs BIAsp 30.

Figure. Hypoglycaemia rates of IDegAsp vs. BIAsp 30 during 28-week treatment period and 4-week Ramadan period



Data are presented as treatment rate ratios [95% CI]
CI, confidence interval; IDegAsp, insulin degludec/insulin aspart; BIAsp 30, biphasic insulin aspart 30

Clinical Trial Registration Nubmer: NCT02648217

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Disclosure: **M. Hassanin:** Honorarium; Novo Nordisk, Sanofi, Lilly, MSD, Novartis. Lecture/other fees; Novo Nordisk, Sanofi, Lilly, MSD, Novartis.

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Weight gain is associated with mild to moderate hypoglycaemia in patients with type 1 diabetes: results from the DCCT

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Background and aims: In type 1 diabetes (T1D), the intensive glucose control has shown to prevent chronic complications of diabetes. However, this beneficial effect is associated with an increased rate of hypoglycemia and weight gain which can led to metabolic abnormalities similar to those found in the insulin resistance syndrome. Whether the higher weight gain observed during intensive glycemic control is associated with hypoglycemia is not clear. The aim of our study was to analyse the association between mild to moderate hypoglycemia and weight gain in patients with T1D from DCCT.

Materials and methods: We included the 1441 patients of DCCT. Mild to moderate hypoglycemia has been assessed by a score (hypo-score) calculated by dividing the number of self-monitoring blood glucose (SMBG) values <70 mg/dl by the total number of measurements done during all the DCCT quarterly visits. Weight was measured every 3 months and to calculate the annual weight gain we divided the total weight gain during the study by the years of follow up.

Results: The mean weight gain for all patients in DCCT was 1.19±1.37 kg/years. Mean number of SMBGs during the follow-up was 248±16 per patient. Mean hypo-score was 9.3±7.0. There was a significant association between the hypo-score and weight gain (p<0.001). The annual weight gain by tertile of hypo-score was 0.82±1.22 kg/years (T1), 1.32 ±1.47 kg/years (T2) and 1.42±1.32 kg/years (T3). There was a significant difference between the weight gain of tertiles T2 and T3 compared

with T1 (p<0.0001 for both comparison). After adjustment for age, sex, duration of diabetes, HbA1c at baseline and treatment arms, these differences remained significant (p<0.0001 for both comparison). Similarly, considering the weight gain from baseline during follow-up, a higher weight gain was observed in T2 and T3 compared with T1. We analyzed the risk of weight gain according to the hypo-score by dividing all the study population of DCCT in patients who gained more (25%) or less than 1.8 kg/years. The odd ration (OR) for a weight gain >1.8 kg/years was 2.14 (95%CI, 1.56-2.93) for T2 and 2.53 (95%CI, 1.85-3.45) for T3. After adjustment for the factors described above, the OR was 1.71 (95%CI, 1.19-2.47) for T2 and 1.57 (95%CI, 1.04-2.37) for T3.

Conclusion: In our study we observed that a higher rate of mild to moderate hypoglycemia, extrapolated from quarterly SMBG measurements, was significantly associated with a higher weight gain.

Disclosure: **L. Potier:** Grants; Sanofi, Eli Lilly. Honorarium; Sanofi, Eli Lilly, Novo Nordisk. Non-financial support; Eli Lilly.

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DPP-4 inhibition: preserved glucagon counterregulation during hypoglycaemia in elderly subjects with type 2 diabetes

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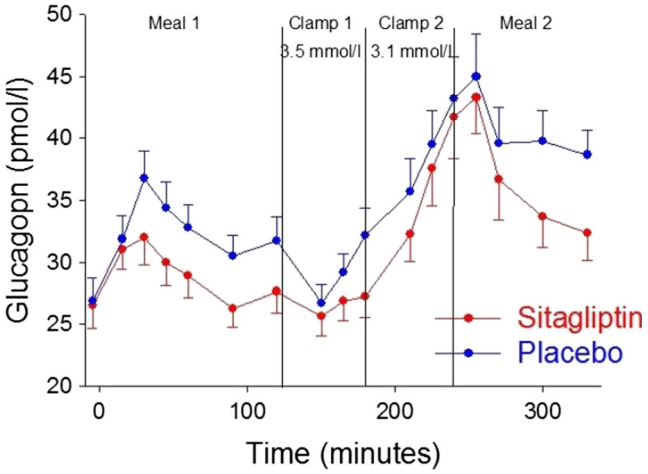
Background and aims: Dipeptidyl peptidase-4 (DPP-4) inhibition is associated with a low risk of hypoglycemia when used as glucose-lowering therapy in type 2 diabetes. This is partially due to its glucose-dependency in stimulating insulin secretion but a sustained glucagon response if glucose levels decline may contribute. However, the glucose threshold for a glucagon counterregulation during DPP-4 inhibition is not known. In this study, we therefore evaluated the glucagon response to two-step lowering of glucose in patients treated with the DPP-4 inhibitor sitagliptin in combination with metformin. The study was undertaken in elderly subjects with mild type 2 diabetes, since DPP-4 inhibition is a common treatment in this group.

Materials and methods: The study was a single-center, double-blind, randomized, placebo (PBO)-controlled crossover study involving 28 subjects with type 2 diabetes (17 males, 11 females) with mean age 74 yrs (inclusion critera ≥65 yrs), diabetes duration 10 yrs, baseline HbA1c 51.5 mmol/mol and BMI 30 kg/m^2 who were treated with metformin (mean 1.7 g/day). Subjects received sitagliptin (100 mg OD) or placebo as add-on therapy for four weeks in random order with a four-week washout in-between. After each of the four week periods, the subjects underwent an initial meal test (to raise levels of incretin hormones), followed by a two-step hyperinsulinemic hypoglycemic clamp (target values of 3.5 and 3.0 mmol/l were preserved for 30 min), in turn followed by a lunch.

Results: After four weeks therapy, HbA1c was reduced by 2.3±0.6 mmol/mol during sitagliptin (P=0.001) vs. a non-significant increase of 0.6±0.4 mmol/mol during placebo. Glucose excursion after breakfast was lower after sitagliptin than after placebo (area under the 120 min curve 1.16±0.04 vs. 1.31±0.04 mmol/lxmin; P<0.001) and, similarly, the glucagon response to breakfast was lower after sitagliptin than after placebo (suprabasal area under the curve 260 ±119 vs. 649±111 pmol/lxmin; P=0.001). The glucagon response during clamp at 3.5±0.1 mmol/l was lower after sitagliptin than after placebo (1.6±1.0 vs. 5.5±1.5 pmol/l; P=0.021). In contrast, the glucagon response to further lowering glucose to 3.1±0.1 mmol/l was higher after sitagliptin than after placebo (14.5±2.3 vs. 11.0±2.6 pmol/l; P=0.007), resulting in similar glucagon values at end of clamp (see Fig.).

Conclusion: In elderly subjects with mild metformin-treated type 2 diabetes, the glucagon response to meal and the glucagon response to lowering glucose to 3.5 mmol/l are reduced by sitagliptin whereas the glucagon response to hypoglycemia at 3.1 mmol/l is sustained by sitagliptin. Hence, the glucose threshold for alleviating the inhibition by DPP-4 of glucagon secretion is between 3.5 and 3.1 mmol/l, assuring a sustained glucagon response to mild hypoglycemia. This may contribute to the low risk of hypoglycemia during DPP-4 inhibition in elderly subjects.



Clinical Trial Regsitration Number: EudraCT 2014-002685-70
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Disclosure: **J.H. Farngren:** Grants; Merck & Co.

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Glycaemic variability: a significant predictor of hypoglycaemia in type 1 and type 2 diabetes

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Background and aims: Hypoglycaemia is a major impediment to intensification of diabetes therapy. Whilst HbA1c is universally accepted as a marker of hyperglycaemia, it is a poor predictor of hypoglycaemia. To date, no specific marker for hypoglycaemia exists. We hypothesized that glycaemic variability (GV), measured by coefficient of variation (CV), is a significant predictor of hypoglycaemia, independent of HbA1c.

Materials and methods: HbA1c and blinded continuous glucose monitoring (CGM) over 4-6 days were performed in 100 type 2 diabetes (T2D) and 60 type 1 diabetes (T1D) subjects. CV: Standard deviation / Mean glucose (from CGM: CV-CGM, from self-monitored blood glucose: CV-SMBG). Hypoglycaemic episode = sensor glucose < 3mmol/L for ≥ 20 minutes (m). Outcomes: frequency of hypoglycaemic episodes / 24 hours (h) and duration of interstitial sensor glucose (ISG) < 3mmol/L (m / 24h). Statistical analyses included T tests, Mann Whitney U Test, multiple linear regression and ROC analysis.

Results: T1D patients were younger (35 ± 13y vs 57 ± 10y, p<0.001), leaner (BMI 22.3 ± 2.6 vs 28.9 ± 5.4 kg/m^2, p<0.001), had lower HbA1c [8.0 ± 1.6 vs 8.6 ± 1.7% (63.9 vs 70.5mmol/mol), p=0.015] and total daily dose (TDD) of insulin (0.71 ± 0.24 vs 1.0 ± 0.56 u/kg/d, p<0.001) compared to T2D. 94% of T2D were on sulphonylureas (SU) / insulin. Frequency of hypoglycaemia and duration of ISG <3mmol/L were higher in T1D than T2D (T1D vs T2D: episodes / 24h: 0.53 ± 0.69 vs 0.08 ± 0.17, m / 24h: 43.3 ± 69.3 vs 7.9 ± 27.3, both p<0.001). In T1D (Model r^2 =0.52, 0.55), CV was the only predictor of frequency of hypoglycaemia and duration of ISG <3mmol/l (β=0.68, β=0.70, both p<0.001). In T2D (Model r^2 =0.21, 0.27), CV was the main predictor of frequency of hypoglycaemia and duration of ISG <3mmol/l (β=0.49, β=0.45, both p<0.001) and was more predictive than HbA1c (β= -0.29, β= -0.08, both p<0.05). These were independent of age, gender, diabetes duration, race, insulin / SU use and TDD insulin. Mean CV was higher in T1D than T2D (0.4 + 0.1 vs 0.3 + 0.1, p<0.001). Hypoglycaemia episodes were differentiated by a significantly higher CV in both T1D and T2D, and a lower HbA1c only in T2D (Table 1). In T1D, a CV >0.37 predicted any hypoglycaemia with sensitivity 75%, specificity 75%, AUC 0.85. In T2D, a CV >0.30 predicted any hypoglycaemia with sensitivity 75%, specificity 65%,

AUC 0.80. CV-CGM correlated well with CV-SMBG in both T1D and T2D (both r=0.76, p<0.001).

Conclusion: CV of glucose is a major predictor of hypoglycemia in both T1D and T2D. HbA1c did not predict hypoglycaemia in T1D and was less predictive than CV in T2D. CV-SMBG correlated well with CV-CGM and could be used to target those at risk of hypoglycaemia for further intervention. Measures of glycaemic status and quality should go beyond HbA1c to incorporate CV.

Table 1: Characteristics of those with and without hypoglycaemia during study period

	Type 1			Type 2		
	No hypo (n=28)	≥1 hypo episodes (n=32)	p value	No hypo (n=76)	≥1 hypo episodes (n=24)	p value
Age (y)	35±15	35±11	NS	56±11	59±7	NS
BMI (kg/m²)	22.9±2.8	21.9±2.5	NS	29±5.6	28.3±4.6	NS
DM duration (y)	15±11	17±8	NS	12±8	16±9	NS
HbA1c (%) mmol/mol	8.1±1.6 65	7.8±1.6 61.7	NS	8.8±1.8 72.7	8.0±1.3 63.9	0.038
CV-CGM	0.33±0.06	0.46±0.10	<0.001	0.27±0.07	0.36±0.08	<0.001
CV-SMBG	0.37±0.07	0.52±0.11	<0.001	0.29±0.08	0.35±0.10	0.006

Hypo-hypoglycaemia, y-year, DM-Diabetes mellitus, CV-Coefficient of variation, CGM-Continuous glucose monitoring, SMBG- Self monitored blood glucose, NS-Not significant (p>0.05).

Disclosure: **S. Rama Chandran:** None.

OP 28 CV risk prediction and potential intervention

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Physical activity and premature mortality in patients with type 1 diabetes

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Background and aims: To assess whether baseline Leisure-Time Physical Activity (LTPA) is associated with mortality in patients with type 1 diabetes (T1D) with and without CKD.

Materials and methods: Prospective and observational study as part of the on-going nationwide multi-centre Finnish Diabetic Nephropathy (FinnDiane) Study. Mean follow-up time was 11.4 ± 3.5 years. The study included altogether 2369 patients with T1D (1633 normoalbuminuric, 312 microalbuminuric, 271 macroalbuminuric, and 153 with ESRD). Out of these patients 48.5% were men, mean age was 40.1±12.6 years, BMI 25.2±3.6 kg/m^2, SBP 135±19 mmHg, HbA_{1c} 8.3±1.4% and duration of diabetes 23.3±12.8 years. A total of 310 patients had chronic kidney disease (CKD) defined as eGFR ≤60 ml/min/1.73m^2. The primary end point was death from any cause. LTPA was assessed by a validated self-report questionnaire. The relationship between all-cause mortality and baseline total LTPA, as well as between all-cause mortality and exercise duration, frequency, and intensity were assessed.

Results: Of the patients with CKD, 127 died during follow-up. The total amount of LTPA (HR 1.47, 95%CI 1.02-2.12) and exercise frequency (1.90, 1.26-2.87) were associated with lower risk of mortality in these patients with CKD when adjusted for sex, diabetic nephropathy, duration of diabetes, age at onset of diabetes, systolic blood pressure, triglycerides, BMI and HbA_{1c}. Notably, all exercise components were associated with reduced premature mortality in the group of patients without CKD (N=2059, 143 events) adjusted for the previous covariates. In a separate analysis excluding patients with ESRD, there were altogether 200 deaths during follow-up. The total amount of LTPA (1.47, 1.10-1.96) and all exercise components (intensity [1.60, 1.17-2.17], duration [1.47, 1.01-2.16], frequency [2.09, 1.50-2.90]) were associated with reduced premature mortality also when adjusted for the same covariates as above.

Conclusion: Exercise is associated with lower risk of premature mortality in patients with type 1 diabetes. Frequent physical activity reduces the risk of premature mortality also in patients with type 1 diabetes and CKD.

Supported by: Folkhälsan Research Foundation, Wilhelm and Else Stockmann Foundation

Disclosure: **H. Tikkanen-Dolenc:** None.

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Both type 2 diabetes and prediabetes are associated with low heart rate variability: the Maastricht Study

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Background and aims: Autonomic dysfunction is a risk factor for cardiovascular morbidity and mortality in type 2 diabetes (T2D). Heart rate variability (HRV) is a marker of autonomic dysfunction and is known to be 'low' in individuals with T2D with established cardiovascular disease. Population-based data on the association between T2D and HRV is scarce. In addition, there is scarce data on the association between prediabetes and HRV, despite the fact that low HRV may explain prediabetes-associated increased

cardiovascular morbidity and mortality. We therefore investigated the association between glucose metabolism status and HRV in the population-based Maastricht Study with the use of 24hECG registrations.

Materials and methods: 2506 participants (mean age: 59±8 years; 52% men; 1421 with normal glucose metabolism (NGM), 383 with prediabetes and 702 with T2D (oversampled)) underwent an extensive phenotyping protocol including 24hECG registrations. HRV was defined in both the time and frequency domain (individual Z-scores for both, based upon 7 and 6 parameters, respectively). Linear regression analyses (with NGM as reference category) were used and adjusted for age, sex, BMI, alcohol use, smoking, physical activity (model 1), prior cardiovascular disease, hypertension, antihypertensive medication, estimated glomerular filtration rate, lipid profile and lipid-modifying medication (model 2).

Results: After adjustment for the variables of model 1, the time domain Z-score (β(95%CI)), as compared to NGM, was -0.28(-0.37;-0.18) in T2D and -0.11(-0.21;-0.01) in prediabetes. After full adjustment (model 2), the time domain Z-score was -0.24(-0.35;-0.14) in T2D and -0.08(-0.19;0.02) in prediabetes. After adjustment for the variables of model 1, the frequency domain Z-score, as compared to NGM, was -0.27(-0.35;-0.20) in T2D and -0.10(-0.18; -0.01) in prediabetes. After full adjustment (model 2), the frequency domain Z-score was -0.22(-0.30;-0.13) in T2D and -0.07(-0.15;0.01) in prediabetes. Further analyses showed independent and continuous associations between fasting and post-load OGTT glucose levels and HbA1c with both HRV domains (P<.001 for all).

Conclusion: In this population-based study both T2D and prediabetes are independent determinants of HRV. This is further substantiated by the independent and continuous associations between HRV and glucose and HbA1c. These data thereby strongly support, for low HRV, the 'ticking clock hypothesis' with its central role for hyperglycemia before the onset of T2D.

Disclosure: **C. Coopmans:** None.

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Metabolic profile of cardiovascular disease risk in Chinese people with diabetes: the Da Qing Diabetes long-term follow-up study

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Background and aims: Diabetes is a major risk factor for cardiovascular morbidity and mortality, but it is hard to predict early cardiovascular diseases (CVD) in type 2 diabetes. The aim of this study was to find potential biomarkers of CVD caused by hyperglycemia through metabonomics analysis in the cohort population of the China Da Qing Diabetes Study (CDQDS).

Materials and methods: UPLC-MS/MS analysis was performed in 120 subjects were new diagnostic diabetes mellitus (NDM) and 120 subjects were normal glucose tolerance (NGT) who were diagnosed by oral glucose tolerance test in 1986. The plasma samples were collected during the follow-up study in 2009 and selected the patients including 50% with CVD and without CVD in each group respectively. Principal component analysis and orthogonal partial least squares discriminant analysis were performed. Potential biomarkers were selected on the basis of variable importance in the project value and S-plot. The specific metabolites related to CVD caused by diabetes were obtained by subtracted the impact of diabetes and CVD to the metabolites.

Results: The clinical characters of the CDQDS cohort in 2009 showed more risk factors in NDM with CVD than in NGT with CVD, including higher Systolic blood pressur (152.2±24.8 vs. 143.6±22.0 mmHg, p<0.05), HbA_{1c} (8.2±1.2 vs. 6.2±1.0 %, p<0.001), Serum Total Cholesterol (5.6±1.5 vs. 4.8 ±1.0 mmol/L, p<0.01), Low density lipoprotein cholestero (129.2±45.8 vs. 110.2±32.0 mg/dL, p<0.01) and urinary albumin creatinine ratio [median(IQR): 57.0(17.0, 302.5) vs. 18.0(9.0, 51.5), p<0.001]. By UPLC-MS/MS analysis and subtraction, 72 specific metabolites related to diabetic CVD were obtained. The results of metabolic network analysis showed that eight metabolic pathways with impact value > 0.1 were considered as the most pertinent in the process of disease. According to the order of the impact value (from high to low), the pathways were glycerophospholipid, arachidonic acid, sphingolipid andglycerolipid metabolism, glycosylphosphatidylinositol-anchor biosynthesis, lysine degradation, fructose and mannose metabolism, and tyrosine metabolism. Further compare to NGT without CVD, thirty-four metabolites increased at least 1.0 fold in NGT with CVD and increased at least 2.4 folds in NDM with CVD that indicate they were closely related to CVD with diabetes. Eight out of the 34 metabolites increased1,000-8,000 folds in NDM with CVD compare with NGT with CVD. Four of the 8 metabolites were identified as phosphatidylglycerol (PG, 16:1(9Z)/18:1(9Z), phosphatidylcholine (PC, 18:3/P-18:1), phosphatidylcholine (PC,20:1/18:1) and 20-Hydroxyeicosatetraenoic acid, three of them belong to glycerophospholipid metabolism pathway which were the important metabolites with atherosclerosis and CVD risk.

Conclusion: Seventy-two specific metabolites and eight metabolic pathways were found and associated with diabetic CVD. The increase changes of plasma glycerophospholipid metabolites were the important markers for cardiovascular diseases risk, these metabolites increased more in diabetes with CVD. It suggests that the further studies will be focused on the glycerophopholipid metabolites.

Supported by: Novo Nordisk

Disclosure: **Y. Chen:** None.

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Interactive changes in cardiovascular risk factors and the long-term cardiovascular risk differ by adiposity levels in incident type 2 diabetes patients: real world study

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Background and aims: Patterns of interactive changes in cardiovascular risk (CVR) factors at different adiposity levels and their long-term consequences on CVR in patients (pts) with incident type 2 diabetes (T2DM) have not been studied at population level. Aim of this study was to evaluate the changes in HbA1c, body weight (WT), systolic blood pressure (SBP) and LDL-cholesterol (LDL) over 2 yrs post diagnosis of T2DM, and the association of interactions of such changes with long-term CVR.

Materials and methods: From UK primary care database, 10040 normal weight, 35941 overweight and 88391 obese pts at diagnosis of T2DM (after 1999), aged 18-70 years, without history of CV disease (CVD), kidney disease, cancer or bariatric surgery, and a minimum 2 yrs of follow-up, were identified. CVD was defined as first occurrence of heart failure, stroke or ischemic heart diseases. Adjusted CVR for different categories of risk factor reduction, compared to those who did not reduce any risk factor at 2 yrs from diagnosis, were estimated.

Results: Patients were on average 51 yrs old, 44% female, and 53% ever smokers. Among obese pts with mean HbA1c 8.6%, WT 101 Kg, SBP 140 mmHg and LDL 3.3 mmol/L at diagnosis: 24, 47 and 26% had reductions in WT ≥ 5%, SBP ≥ 5 mmHg and LDL ≥ 1 mmol/L at 2 yrs respectively. While 38% pts did not achieve reduction in any risk factor, 11% reduced both WT and SBP, and 1.6% reduced all 3 factors simultaneously. Among those with HbA1c ≥ 7.5% at diagnosis, 33% achieved HbA1c ≤ 7% at 2 yrs. Among obese, compared to those who did not reduce any CVR factor by above categories, those who reduced WT and SBP, and all risk factors simultaneously had 18% (CI of HR: 0.74, 0.91) and 55% (CI of HR: 0.33, 0.61) significantly reduced CVR (Table 1). Among overweight pts, only simultaneous reduction in SBP and LDL was associated with 38% significant reduction in CVR (CI of HR: 0.48, 0.80). Among normal weight pts with mean HbA1c 9.1%, WT 67 Kg, SBP 134 mmHg and LDL 3.3 mmol/L at diagnosis: 15, 44 and 29% reduced WT ≥ 5%, SBP ≥ 5 mmHg and LDL ≥ 1 mmol/L respectively at 2 yrs. While 44% pts did not achieve reduction in any risk factor, only 7 / 1.2% reduced WT and SBP / all factors simultaneously. Among those with HbA1c ≥ 7.5% at diagnosis, 36% achieved HbA1c ≤ 7% at 2 yrs. In normal weight pts, WT reduction alone or in combination with other risk factor reductions, did not significantly reduce CVR. Glycaemic control with HbA1c ≤ 7% was

independently associated with 19, 15 and 10% reduction in CVR in normal weight, over weight and obese patients respectively ($p < .05$).

Conclusion: This real-world study with 8 yrs of median follow-up suggests significant differences in the synergy between CV risk factors control in patients under different adiposity levels. While the association of WT and SBP reduction (independently or in combination with reduction in LDL) with CVR were significant among obese patients, significance of such dynamics was not clear among normal and overweight patients.

Table 1: Hazard Ratio (95% CI) for composite cardiovascular risk in different categories of risk factor control, compared to those who failed to reduce body weight, blood pressure and LDL cholesterol. Hazard Ratio (95% CI) for those who reduced HbA1c below 7% at 2 years from diagnosis of diabetes, compared to those who failed to achieve glycaemic control. Separately for BMI categories at diagnosis of diabetes; adjusting for age, sex, baseline weight, systolic blood pressure, LDL cholesterol, HbA1c; stratified by smoking status, quantiles of age and appropriate medications; and accounting for immortality bias.

Reduction in Risk Factors	Normal Weight (N=10,040)	Overweight (N=35,941)	Obese (N=88, 391)
Weight Reduction ≥ 5%	0.83 (0.59, 1.18)	1.25 (1.09, 1.44)	0.86 (0.78, 0.96)
SBP Reduction > 5 mmHg	0.79 (0.63, 0.98)	0.91 (0.82, 1.01)	0.92 (0.86, 0.98)
LDL Reduction ≥ 1 mmol/l.	0.74 (0.48, 1.15)	0.81 (0.64, 1.04)	0.77 (0.64, 0.92)
Weight and SBP Reduction	0.82 (0.59, 1.13)	1.00 (0.86, 1.16)	0.82 (0.74, 0.91)
Weight and LDL Reduction	0.24 (0.03, 1.75)	0.77 (0.46, 1.27)	0.57 (0.39, 0.84)
SBP and LDL Reduction	0.57 (0.36, 0.90)	0.62 (0.48, 0.80)	0.66 (0.55, 0.79)
Simultaneous Reduction in all Factors	0.36 (0.11, 1.13)	0.72 (0.48, 1.08)	0.45 (0.33, 0.61)
HbA1c ≤ 7%	0.81 (0.68, 0.97)	0.85 (0.79, 0.93)	0.90 (0.85, 0.96)

Disclosure: **E. Adjah:** Grants; National Health and Medical research Council, AstraZeneca.

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Development and validation of a decision support tool for individualising lipid, blood pressure and aspirin treatment in patients with type 2 diabetes

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Background and aims: Patients with type 2 diabetes (DM2) are at high risk of cardiovascular disease (CVD). Therefore, CVD prevention is vitally important in patients with DM2. However, preventive treatment effects differ between patients depending on their baseline CVD-risk as constituted by their risk factor levels. Our aim was to develop and geographically validate in different continents a treatment decision support tool for individualizing lipid, blood pressure and aspirin treatment in patients with DM2 by estimating treatment effects on 10-year CVD-risk and CVD-free life-expectancy.

Materials and methods: The prediction algorithm was developed based on a random 75% of patients of the Swedish National Diabetes Registry included after 2002 (SNDR; n=295,614). The algorithm includes predetermined clinical patient characteristics that are commonly available in practice (figure 1). It consists of two complementary competing risk adjusted Cox proportional hazards models with left truncation (i.e. using age as the time-scale) and right censoring for prediction of the survival without CVD-events (vascular mortality, stroke or myocardial infarction) and non-vascular mortality. This algorithm can be used for the prediction of CVD-free life-expectancy and 10-year CVD-risk for individual patients. When combined with hazard ratios from randomized trials or meta-analyses, individualized preventive treatment effect can be estimated. Validation was performed in patients with DM2 enrolled in the ADVANCE-trial (n=11,139), ACCORD-trial (n=10,251), ASCOT-trial (n=4,646), ALLHAT-trial (n=3,903), SMART-cohort (n=1,910) and EPIC-NL-cohort (n=524), pooled by geographical origin.

Results: An example of an individual life-expectancy estimation is shown in figure 1 for a 60-year old man. The estimated effect of statin use for this patient is shown, assuming a 20% relative risk reduction for CVD. Similarly, the effects blood pressure-lowering and aspirin use or a combination of treatments can be estimated, both for immediate or postponed initiation. Predicted and observed survival without myocardial infarction or stroke showed good agreement in all external validation sets. The c-statistic, as a measure of model discrimination, was in Western Europe 0.65 (95% CI 0.64-0.67), Eastern Europe 0.67 (95% CI 0.64-0.70), Asia 0.66 (95% CI 0.63-0.68), North America 0.64 (95% CI 0.63-0.65), and Oceania 0.62 (95% CI 0.58-0.66).

Conclusion: CVD-free life expectancy can be estimated for individual patients with DM2 by using a validated prediction algorithm. When combined with hazard ratios from trials, the effect of lipid-lowering, blood pressure-lowering and aspirin treatment can be calculated for individual patients. This may facilitate personalized medicine and shared decision-making.

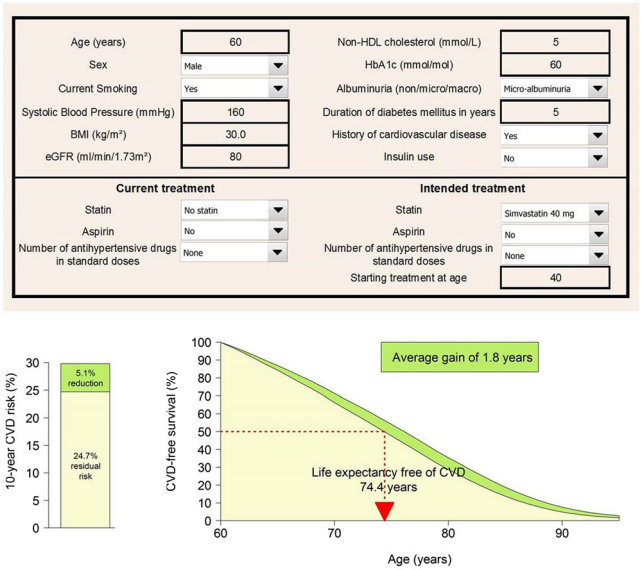

Figure 1: Screenshot of the decision-making support tool: example of an individual treatment effect estimation for a 40-year old patient, considering starting statin treatment in 20 years at age 60.

Disclosure: **G.F.N. Berkelmans:** None.

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Lower risk of cardiovascular disease with higher refill adherence to lipid-lowering therapy in patients with type 2 diabetes

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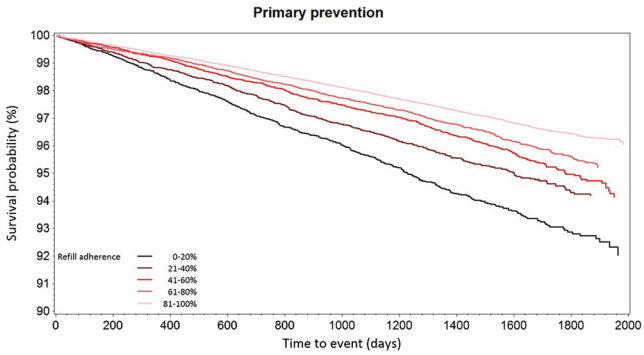
Background and aims: Lipid-lowering therapy (LLT) has shown to reduce the risk of cardiovascular disease (CVD) in patients with type 2 diabetes (T2D). The aim of this study was to analyze the risk of CVD in relation to refill adherence level in patients with T2D initiating LLT as primary or secondary prevention.

Materials and methods: Patients ≥ 18 years, registered with T2D in the Swedish National Diabetes Register, who filled at least one prescription for LLT between 1 Jan 2007 and 31 Dec 2010 were identified in the Swedish Prescribed Drug Register. Patients with filled LLT prescriptions within a year prior to inclusion were excluded to obtain new users. Patients with CVD prior to inclusion were defined as receiving LLT as secondary prevention; otherwise patients received primary prevention. Refill adherence was estimated by the medication possession ratio (MPR) during an 18-month exposure period following inclusion. MPR represent the proportion of days with LLT at hand and was divided into five levels; 0-20%, 21-40%, 41-60%, 61-80% and 81-100%. Risk of CVD was analyzed by MPR level from the day after the exposure period until migration, CVD, death, or 31 Dec 2013 using Cox proportional hazard regression and survival plot generated, adjusting for concurrent medicines, socioeconomic and clinical factors.

Results: In total, 86 568 patients were included, 70% were born in Sweden and 57% were men. The mean age was 63 years, average diabetes duration was 5 years and mean HbA1c was 54 mmol/mol. The mean BMI was 30 and 22% were physically active less than once a week. Mean MPR was 77% in primary prevention patients (n=74 909) and 83% in secondary prevention patients (n=11 659). compared to patients with MPR 81-100%, the hazard ratios for CVD increased with lower MPR level, from 1.33 to 2.36 (p<0.001) for primary prevention, and from 1.19 to 1.58 (p<0.001) for secondary prevention. The figure shows the survival probability at different MPR levels in patients with LLT as primary prevention.

Conclusion: The risk of CVD increased with lower refill adherence to LLT in primary and secondary prevention patients with T2D. This indicates that a high level of adherence to LLT is important to optimize the cardiovascular prevention in T2D.



Supported by: FORTE

Disclosure: **S.A. Karlsson:** None.

OP 29 Metabolism and the brain

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Glucose uptake in skeletal muscle, brain and visceral adipose tissue assessed with PET/MR strongly predicts whole body glucose uptake during hyperinsulinaemia

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Background and aims: Type 2 diabetes (T2D) is characterized by impaired glucose homeostasis, associated with beta-cell failure and insulin resistance. The current study aimed to assess: 1) in which main metabolic tissues glucose uptake (GU) has the highest predictive value for whole body insulin sensitivity, and 2) glucose uptake rates in various region of the brain in relation to whole body insulin sensitivity.

Materials and methods: 12 non-diabetic control (Con) and 12 T2D subjects ($HbA1_c$: Con: 33.7±0.6 mmol/mol; T2D: 57.1±3.4 mmol/mol,) matched for age (Con: 62±2 y; T2D: 62±2 y), sex (6 M /group) and BMI (Con: 30.1±1.3 kg/m^2; T2D: 30.4±1.1 kg/m^2) were recruited. Rate of glucose uptake (rGU) was measured in individual tissues using dynamic whole-body ^{18}FDG PET combined with MR during a hyperinsulinemic (approx. 110 mU/L) euglycemic clamp. This allowed for concomitant measurement of whole body insulin sensitivity and rGU in selected tissues; brain, visceral abdominal fat (VAT), subcutaneous abdominal fat (SAT), liver, heart, and the thigh muscle. To analyze the association of rGU of the different cortical regions within the brain to whole-body insulin sensitivity a voxel-wise analysis was performed.

Results: Subjects with T2D had significantly lower whole body glucose uptake (M-values in Con: 9.06 ± 1.15 mg/kg LBM/min; T2D: 5.12 ± 0.69 mg/kg LBM/min, p<0.01). In T2D, rGU was significantly lower in the thigh muscle, liver, VAT and SAT, but higher in the brain compared to con (all p<0.05). There were positive correlations between the M-value and rGU in SAT (r=0.524, p<0.05), VAT (r=0. 789, p<0.001) and muscle (r=0.871, p<0.001). In contrast, there was an inverse correlation with brain rGU (r=-0.678, p<0.05). There was a tendency (p= 0.053) for correlation with liver rGU, whereas there was no correlation with myocardial rGU. In multivariate regression analysis rGU in the muscle, VAT and brain had the highest predictive value for the M-value (r^2 for model= 0.88, p<0.001; muscle: Std.β Coeff. = 0.51, p<0.01; VAT: Std.β Coeff. = 0.28, p<0.05; brain: Std. β Coeff. = -0.34, p<0.01). Adding rGU in liver, SAT or heart did not improve the model, and this was also true for BMI, body fat percentage, sex, age, waist/hip ratio or $HbA1_c$. Using a voxel-wise analysis approach we observed the strongest inverse correlation in the brain rGU vs M-value in the associative visual cortex. The secondary visual, the somatosensory association and the premotor cortex and the angular and the inferior prefrontal gyrus also showed significant correlations (all p<0.001).

Conclusion: We showed that rGU in brain and peripheral organs displayed opposite associations with whole body GU and insulin sensitivity during hyperinsulinemia. Multivariate modeling suggests that skeletal muscle, brain and VAT rGU together strongly predict whole-body GU and insulin sensitivity. Thus, altered metabolic function of these tissues is of importance for impaired glucose turnover in T2D. Skeletal muscle quantitatively dominates in insulin-mediated glucose disposal. In contrast, dysregulated glucose handling in VAT and brain may be related to altered signaling from these tissue to muscle and other organs.

Supported by: AstraZeneca, EXODIAB, the Swedish Diabet

Disclosure: **G.J. Boersma:** None.

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Endogenous glucose production is independently associated to brain glucose uptake in morbidly obese subjects

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Background and aims: In preclinical studies, direct cerebral insulin action suppresses endogenous glucose production (EGP). Recent imaging studies during euglycaemic hyperinsulinemia have shown that brain glucose uptake is increased in subjects with impaired glucose tolerance. Our aim was to investigate whether brain insulin-stimulated glucose uptake (GU) is associated with EGP in healthy and morbidly obese insulin resistant subjects.

Materials and methods: Twenty morbidly obese (BMI 43.7±0.8 $kg \cdot m^{-2}$; 6 with T2D) and 14 age-matched healthy controls were recruited. Whole body and brain GU were measured during euglycemic hyperinsulinemic clamp with [^{18}F]FDG-PET. The obese group was studied before and 6 months after bariatric surgery. EGP was measured during clamp with [^{18}F]FDG. Brain GU parametric images were calculated using the Gjedde-Patlak plot. Voxel-based mapping analysis was done using SPM12. A p value <0.05 at cluster level, corrected for false discovery rate (FDR) was considered statistically significant.

Results: As compared to controls, morbidly obese were insulin resistant (M-value 11.5±1.4 vs. 36.4±2.6 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, p<0.0001). Insulin-stimulated brain GU and EGP were significantly higher in obese when compared to controls (21.0± 3.0 vs 19.3± 1.7 $\mu mol \cdot 100g^{-1} \cdot min^{-1}$, p=0.01, and 2.80±0.66 vs -0.49±1.12 $\mu mol \cdot kg \cdot min^{-1}$, p=0.01, respectively). Brain GU associated inversely with M-value in the whole dataset and in the obese group alone (p<0.001, in both). Only in the obese group, brain GU correlated positively with EGP in the whole brain (p<0.0005) and separately in gray and white matter (p<0.001, both). The EGP-brain GU correlation remained significant after accounting for M-value or age (p=0.02 and p<0.0005 respectively). After surgery, BMI decreased by 24% (p<0.0001) and whole-body insulin sensitivity improved by 113% (p<0.0001). The positive correlation between insulin-stimulated brain GU and EGP persisted (p<0.0001) and so did the negative correlation between M-value and brain GU (p<0.001). When accounting for M-value or for age, the correlation between EGP and brain GU remained (p<0.001, for both). Interestingly, insulin-stimulated brain GU before surgery was associated with an increase in fasting glucose at 3 years of follow-up (p<0.001), but not with changes in body weight.

Conclusion: In the present study, independently from whole-body insulin sensitivity, insulin-stimulated brain GU correlated positively with EGP in morbidly obese subjects. This association persists after bariatric surgery-induced marked weight loss and improvement in insulin sensitivity. This study suggests an independent interrelation between liver and brain glucose metabolism and that enhanced brain GU might be a predictor of worse glycemic control in the future.

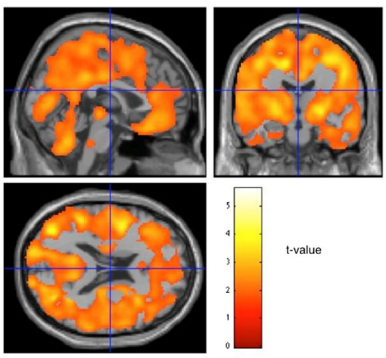

Fig. 1 Brain glucose uptake during insulin stimulation is positively associated with EGP in obese subjects (n=20) (p-value < 0.001, FDR-corrected)
*Brighter colours denote higher significance. Threshold p <0.05 (uncorrected)

Clinical Trial Registration Number: NCT00793143
Disclosure: **E. Rebelos:** None.

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GLP-1 receptor agonism preserves parahippocampal glucose metabolism in Alzheimer´s disease

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Background and aims: T2D increases the risk of Alzheimer's disease (AD) and vice versa, but the understanding of underlying mechanisms is poor. Animal models point to hippocampus (HC) and parahippocampus (PHC) as sites of insulin resistance (IR) leading to cognitive dysfunction in T2D and AD. A major feature of AD is the steady decrease of cerebral metabolic rate of glucose (CMRglc). The decline of CMRglc is closely related to cognitive impairment as a marker of neuronal activity and disease progression. In experimental models, GLP-1 receptor agonism alleviates several features of neurodegeneration. We predicted that treatment with liraglutide would raise CMRglc measured with PET in AD patients.

Materials and methods: In this 26-week, double-blind, placebo-controlled trial, we randomized 38 patients with AD to treatment with liraglutide (n=18) or placebo (n=20). We mapped HC and PHC values of CMRglc and volume from regional radioactivity, and we scored changes of cognition with the WMS-IV scale during the observation period.

Results: With placebo, CMRglc declined significantly in PHC (P=0.048) and insignificantly in HC (P=0.17) but remained unchanged with liraglutide. Overall cognition did not change in either group, with significant impairment of orientation with placebo vs liraglutide (P =0.041). In PHC at baseline, we confirmed the predicted positive correlations between CMRglc and cognition (P=0.008, r=0.5), CMRglc and volume (P=0.039, r=0.37), and volume and cognition (P=0.008, r=0.5). We also confirmed the negative correlation between values of CMRglc and duration of AD (P=0.004, r=-0.5). PHC volume decreased with increasing BMI (P=0.01, r=-0.44), increasing fasting plasma glucose (P=0.02, r=-0.42) and increasing age (P=0.04, r=-0.36). Volumes remained constant within and between groups.

Conclusion: In AD, liraglutide prevented the decline of CMRglc in PHC seen with placebo, signifying cognitive impairment and disease evolution. As sites of imaging, the cognitively relevant PHC and HC regions are easily identified targets of determination of cerebral glucose metabolism.

Clinical Trial Registration Number: NCT01469351
Disclosure: **M. Gejl:** None.

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Cerebral effects of ketone bodies in humans: reduced glucose uptake, unchanged oxygen consumption, and increased blood flow by positron emission tomography

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Background and aims: High levels of ketone bodies have experimentally and clinically been shown to be effective in a number of neurological disorders and these neuroprotective effects have been linked to increased blood flow, decreased glucose utilization and - theoretically - improved oxygen efficiency. Here, we tested whether 3-hydroxybutyrate (3OHB) beneficially affects CNS blood flow and glucose and oxygen consumption in human subjects.

Materials and methods: Five men and four women were included in a randomized, controlled, crossover study. All subjects were investigated twice after an overnight fast during 1) saline infusion (CTR) or 2) ketone body infusion (KET) with Na-3-hydroxybuturate (3-OHB (75g/l)). We used positron emission tomography to estimate cerebral metabolic rate of glucose

(CMR_{glu} - by ^{18}F-labelled FDG) and oxygen (CMR_{oxygen} by ^{15}O-labelled O_2) consumption and blood flow (CBF by ^{15}O-labelled H_2O) in the brain in the two conditions.

Results: 3OHB was elevated during KET (5.5±0.4 mmol/l) compared with CTR (0.2±0.02 mmol/l), p<0.001. Ketosis resulted in 15% lower CMR_{glu} during KET (19.6±0.8 mmol/100g/min) compared with CTR (22.8±1.3 mmol/100g/min), p=0.02. CBF increased 30% during KET (52.0±2.0 ml/100 g /min) compared with CTR (40.9±2.1 ml/100 g/min), p<0.001, whereas CMR_{oxygen} was unaffected by 3-OHB. Paired two-tailed t-test was used to test for differences.

Conclusion: We conclude that acute ketone body infusion reduces CMR_{glu} and increases CBF, without detectable effects on CMR_{oxygen}. Increased oxygen supply in the presence of unchanged oxygen utilisation and - possibly - a favourable oxygen-ATP ratio may explain the neuroprotective effects of ketone bodies.

Clinical Trial Registration Number: NCT02357550

Supported by: DCSR (grant no.0603-00479B)

Disclosure: **M. Svart:** None.

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The role of maternal obesity on short-term modulation of offspring neural circuits

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Background and aims: Maternal obesity is associated with significant changes in the metabolism of the offspring, which can have impact on weight gain, glucose homeostasis, adiposity and other physiological conditions during adulthood. Most studies have focused on the effects of maternal obesity on the functional regulation of metabolically relevant peripheral tissues such as the liver, muscle, adipose tissue and pancreatic islets. Very little is known about the effects of maternal obesity on the hypothalamic activity of the offspring. The hypothalamus responds to nutrients and hormones controlling caloric intake and energy expenditure, which impact on whole body energy homeostasis. Therefore, the aim of the present study was to determine if maternal obesity can influence development of hypothalamic POMC neurons, microglia and astrocytes and to elucidate potential underlying mechanisms.

Materials and methods: We used a mouse model of maternal diet-induced obesity. Female C57Bl/6J mice were fed either a control diet or an obesogenic diet from three weeks prior to mating and during pregnancy and lactation. Male offspring were weaned at 21 days and immediately studied. Distribution of POMC neurons, microglia and astrocytes was measured by fluorescence microscopy and zoometric parameters were determined at same point. In parallel, metabolic parameters related to dams were investigated at both pregnancy and lactation periods in order to define the maternal programming factors.

Results: Dams fed a high fat diet presented increased body weight before (t-test, p<0.05) and during (p<0.0001) pregnancy. Furthermore, there was increased adiposity (p<0.01 both), hyperglycemia (p<0.01 and p<0.05, respectively) and hyperinsulinemia (p<0.01 and p<0.05, respectively) at both stages, pregnancy and lactation. Male offspring born from obese dams presented decreased body weight at birth (p<0.01) and at third (p<0.05) week of age. At weaning, male offspring from obese dams presented decreased ACTH and IBA1 (microglia) staining whereas GFAP (astrocytes) staining was considerably increased in the arcuate nucleus.

Conclusion: Together, our findings revealed an impaired development of hypothalamic neural circuits modulated by maternal obesity even at premature stage of offspring life**.** As ACTH is a product of POMC neurons, our results suggest the offspring from obese dams presented losses in an important anorexigenic component at arcuate nucleus short-term. In addition, microglia and astrocytes, which control inflammation, gliosis, and neuronal stress were found modulated by maternal obesity at the same hypothalamic region. As arcuate nucleus provides many physiological roles involved in feeding and metabolism, the imbalance in the expression of its different neuronal types may be related to the development of metabolic diseases in offspring adult life. The evaluation of young adult offspring from lean and obese dams will be undertaken in future.

Supported by: FAPESP 2016/07361-7

Disclosure: **J.A. Faria:** None.

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Exenatide modulates visual cortex response to food images

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Background and aims: GLP1 enhances metabolic activity in neural tissue and modulates food-related behavioral responses. We investigated whether this effect may involve changes in early sensory processing of stimuli such as visual appreciation of food images.

Materials and methods: The effect of Exenatide (Ex) on fMRI responses to food and non-food pictures was evaluated in 10 normal weight (NW: age 40.2±6.9 yrs; BMI 24.8±0.9 Kg/m^2) and 10 NGT obese subjects (OB: 49.4±6.1 yrs, 34.2±1.1 Kg/m^2). fMRI responses to images were evaluated before and after i.v. EX (0.05 μg/min). BOLD signal at rest (BSR) was calculated as the average of the raw fMRI signal in the last 3 volumes of the rest blocks. Visual activations (BSV) were computed as % signal change relative to BSR.

Results: Under baseline condition OB had comparable plasma glucose (93±3 vs. 93±2 mg/dl) and higher insulin (11±1 vs 6±1 μU/mL; p=0.002). BSR was higher in NW vs. OB in the parieto-occipital cortex (899±12 vs 837±13 a.u.) with no effect on temporal and frontal cortex. Across occipital cortex, there was a negative correlation between BSR and BMI (r= -0.43; p<0.001). With EX infusion, there was no difference in plasma glucose (94±2 vs 92±3 mg/dl) and insulin concentrations (32 ±9 vs 30±6 μU/mL) in the two groups. BSV increased in a parallel manner in both groups over most of the cortex, extra-striate areas (lateral-occipital, sparing V1) and prefrontal cortex so that BSV was higher in absolute terms in NW but with similar percent increase (+15 vs +17%). BSV was reduced in temporal pole in both groups.

Conclusion: BSR is higher in NW. EX modulated BSV responses to food images in a similar manner in OB and NW, though the former always had higher numerical BSV values. These preliminary results support a role for GLP-1 in food image brain processing

Disclosure: **G. Daniele:** None.

OP 30 Beta cell signal transduction and insulin secretion

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The Rab27 effector, exophilin-8, assembles insulin granules for exocytosis in the actin cortex via interaction with RIM-binding protein and myosin-VIIa

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Background and aims: Secretory granules generated at the *trans*-Golgi network must somehow pass through the actin cortex before they fuse with the plasma membrane. However, the precise mechanism by which they anchor and prepare for exocytosis is unknown. Exophilin-8 (also known as MyRIP and Slac2-c) is suspected to play a role in this process, due to its direct binding activities to Rab27 on the granule membrane and to F-actin and its motor protein, myosin-Va.

Materials and methods: We generated exophilin-8-knockout mice to examine its in vivo function in glucose tolerance and insulin secretion. We further identified exophilin-8-interacting proteins, using the tandem affinity purification approach followed by a liquid chromatography-tandem mass spectrometry analysis, and investigated their roles in insulin secretion in beta cells.

Results: Exophilin-8-null mouse pancreatic islets lose polarized granule localization at the beta-cell periphery and exhibit impaired insulin secretion. We further show that exophilin-8 accumulates granules in the cortical F-actin network not by direct interaction with myosin-Va, but by indirect interaction with myosin-VIIa through its previously unknown binding partner, RIM-binding protein (RIM-BP2). RIM-BP2 also associates with pivotal exocytic machinery, such as Cav1.3, RIM, and Munc13-1. Disruption of the exophilin-8-RIM-BP2-myosin-VIIa complex by ablation or knockdown of each component markedly decreases both the peripheral accumulation and exocytosis of granules.

Conclusion: The newly identified, exophilin-8-RIM-BP2-myosin-VIIa complex acts as a physical and functional scaffold and provides a mechanism supporting a releasable pool of granules within the F-actin network beneath the plasma membrane.

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Disclosure: **T. Izumi:** None.

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Glucocorticoids re-programme the beta cell signalling cassette to preserve functional identity and insulin secretion

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Background and aims: Excessive glucocorticoid exposure is considered to be deleterious for pancreatic beta cell function and insulin release. However, glucocorticoids at physiological levels are essential for many homeostatic processes, including normal glycemic control. Here, we set out to investigate the mechanisms by which glucocorticoid might directly affect beta cell function.

Materials and methods: Islets from wild-type and 11β-hydroxysteroid dehydrogenase type 1 (11B-HSD1) knockout animals were treated for 48 hrs with corticosterone or the less active 11-dehydrocorticosterone (11-DHC). Insulin secretion was measured using HTRF assay. Beta cell gene expression was determined with RT-PCR. Intracellular calcium (Ca^{2+}), ATP/ADP and cyclic AMP (cAMP) fluxes were monitored using fluo8/Fura2, Perceval and Epac2-camps, respectively. Insulin granule distribution was mapped in 3D using structured-illumination microscopy. Analogous studies were repeated in human islets but using instead cortisol and cortisone.

Results: Corticosterone (cort) and 11-DHC impaired beta cell Ca^{2+} responses to glucose but not incretin via effects on voltage-dependent Ca^{2+} channel function (amplitude = 5.01 vs. 3.21 AU, control vs. cort; P<0.01). However, beta cell identity was unaffected, with normal Ins1, Pdx1 and Nkx6.1 expression and ATP/ADP responses to fuel. Moreover, insulin content and glucose- and KCl-stimulated insulin secretion remained intact (2.55 vs. 3.28 ng/ml, control vs. cort; non-significant). Strikingly, glucocorticoid increased the number of membrane-proximal granules, and boosted glucose-induced cytosolic cAMP signals (AUC = 17.41 vs. 30.90 AU, control vs. cort; P<0.01). Notably, insulin secretory failure was induced using lipotoxicity to restrain cAMP responses to glucose (P<0.05). Effects of 11-DHC could be prevented by glucocorticoid receptor antagonism using RU486 (P<0.01) (amplitude = 2.23 vs. 2.51 AU, 11-DHC vs. 11-DHC + RU486; P<0.05), and were associated with pre-receptor glucocorticoid availability, since deletion of 11B-HSD1 rescued Ca^{2+} responses and normalized cAMP generation (11-DHC) (P<0.01). Similar results were seen in human islets, with both cortisone and cortisol impairing Ca^{2+} fluxes, while potentiating cAMP rises and maintaining insulin secretion.

Conclusion: We have identified a feedback loop whereby steroid boosts cAMP to maintain insulin secretion in the face of perturbed ionic signals in rodent and human islets. This is important from a translational viewpoint, since failure of this protective feedback loop may contribute to impaired beta cell function, insulin release and diabetes risk during states of glucocorticoid excess (e.g. Cushing's), which are associated with profound dyslipidemia.

Supported by: DUK EFSD/JDRF/Novo Nordisk MRC ERC

Disclosure: **N.H.F. Fine:** None.

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Beta-arrestin2 plays a key role in GLP-1 receptor signalling in pancreatic beta cells

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Background and aims: The scaffold protein β-arrestins (ARRB1 and ARRB2) are ubiquitously expressed and are known to uncouple G protein coupled receptors (GPCR) from G protein and to recruit new signaling pathways (such as ERK1/2) to the activated GPCR. We have recently shown the involvement of ARRB2 in the regulation of pancreatic β-cell mass and identified a defect of insulin signaling. Nevertheless, the role of ARRB2 in the signaling of the GLP-1 receptor (GLP-1R), a GPCR, remains to be elucidated. Our study aims to determine the molecular mechanism(s) involving ARRB2 in the signaling of GLP-1R in living β-cells by dynamic imaging measurements.

Materials and methods: The experiments were carried out in mouse β-cells from $Arrb2^{+/+}$ or $Arrb2^{-/-}$ mice. Four-month-old or twelve-month-old male mice were used. cAMP production, endogenous PKA and endogenous ERK1/2 activation were measured after adenoviral infection of mouse β-cells with FRET-based sensors of interest (CAMP-epac, AKAR3, EKAR; respectively) by live microscopy. CREB phosphorylation was determined by immunofluorescence. Expression of ARRB2 and GLP-1R were measured by qPCR and western blot. The islet mass was determined by morphometric analysis of pancreatic sections stained with eosin/hematoxylin.

Results: In β-cells from $Arrb2^{+/+}$ mice, GLP-1 (10pM/100pM/1nM/10nM) lead to cAMP production and rapid activation of PKA with a maximum reached between 100pM-1nM. Surprisingly, PKA activation under GLP-1 remained sustained (10nM) or persisted at least 25min (1nM) after stopping the stimulation in contrast to the effect of 1μM Forskolin (reversibility <2min), which produced more cAMP than 10nM GLP-1. In β-cells from $Arrb2^{-/-}$ mice, cAMP production and PKA recruitment were higher than in $Arrb2^{+/+}$ cells in the presence of pM range (1-100pM) of GLP-1 (p<0.05), but not at the nM range, suggesting that ARRB2 contributes to a partial uncoupling of the GLP-1R for the production of cAMP. Conversely, GLP-1-induced ERK1/2 activation was strongly decreased (~50%, p<0.001), indicating a major recruitment of ERK1/2 by ARRB2 to GLP-1R. Finally, the activation of CREB, a

transcription factor involved in β-cell survival, was also significantly decreased ($p<0.05$). Interestingly, during aging, the increase in pancreatic islet mass was correlated with an increase in the expression of GLP-1R and ARRB2, while Arrb2-/- aged mice were unable to fully compensate their pancreatic islet mass.

Conclusion: Our study revealed for the first time in living mouse β-cells, a significant role of ARRB2 in the signaling of GLP-1-R, involving not only inhibition of the cAMP/PKA pathway but also the recruitment of ERK1/2 kinases and activation of CREB. Most importantly, it occurs in the pM range of GLP-1, which is the physiological circulating concentration of the incretin. Thus, any variation in the expression of ARRB2 should affect the signaling of GLP-1R.

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Disclosure: **M.A. Ravier:** None.

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A TRPV1-to-secretagogin regulatory axis controls beta cell survival by modulating protein turnover

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Background and aims: Ca^{2+} sensor proteins are generally implicated in insulin release through SNARE interactions. Secretagogin is an EF-hand Ca^{2+} sensor protein which is amongst the most abundant proteins in pancreatic β cells.

Materials and methods: Here, we combined bulk mRNA expression profiling of pancreatic islets obtained from a human cohort and single-cell RNA-seq from diabetic donors to establish a correlation between secretagogin expression and the incidence of type 2 diabetes. We then used pharmacological probing, *in silico* models, secretagogin promoter assays, biochemistry and mouse genetics to establish receptor-to-transcription factor signaling pathway controling secretagogin expression. Next, we generated secretagogin knockout$^{(-/-)}$ mice to determine the effects of secretagogin loss on glucose metabolism, integrity and function of pancreatic islets. By using cellular models and acute genetic manipulations combined with global proteomics and histochemical tools, we proposed an unexpectedly distinct mechanism of action for secretagogin.

Results: Secretagogin mRNA expression in human pancreatic islets correlates with their insulin content and the incidence of type 2 diabetes. Single cell RNA-seq reveals retained expression of the TRP family members in β cells from diabetic donors. Amongst these, pharmacological probing identifies Ca^{2+}-permeable transient receptor potential vanilloid type 1 channels (TRPV1) as potent inducers of secretagogin expression through recruitment of Sp1 transcription factors. Accordingly, agonist stimulation of TRPV1s fails to rescue insulin release from pancreatic islets of glucose intolerant secretagogin$^{-/-}$ mice. However, instead of merely impinging on the SNARE machinery, reduced insulin availability in secretagogin$^{-/-}$ mice is due to β cell loss, which is underpinned by the collapse of protein folding, ER stress and deregulation of secretagogin-dependent USP9X deubiquitinase activity.

Conclusion: Therefore, a ubiquitously Ca^{2+}-driven TRPV1-to-secretagogin transcriptional axis is suggested as a signal transduction pathway whose deregulation can curtail the life-long structural integrity and functional competence of pancreatic β cells.

Disclosure: **K. Malenczyk:** None.

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FGF21/analogue suppresses high-fat diet induced islet cell dysfunction and cell apoptosis in mice

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Background and aims: Lipotoxicity in the pancreas is a lipid metabolism disorder, which is due to the excessive accumulation of lipid intermediates in islets, finally leading to islet dysfunction and β-cell apoptosis, as seen in obesity-related type 2 diabetes mellitus (T2DM). Fibroblast growth factor 21 (FGF21) is a potent regulator of lipid homeostasis that has been reported to be beneficial for islet cell health. However, the protective mechanism(s) whereby FGF21 regulates islet fatty acid (FA) metabolism, thereby protecting islet failure under lipotoxic conditions remain elusive. We, therefore, sought to explore the protective effects of FGF21/analogue on islet lipid metabolism in lipototoxicity-induced animal and cell models.

Materials and methods: For in vivo models, C57/BL6J mice and global FGF21 knockout (KO) were fed with a 60% high-fat diet (HFD) and standard diet for 12-20 weeks; the HFD-treated mice were given with a long-acting mimetic of FGF21 (CVX-343/PF-0523102, a gift from Pfizer) for 6 weeks. For in vitro and ex vivo models, rat β-cell line INS-1E and islets isolated from normal C57/BL6J mice were cultured and exposed to palmitic acid (PA normal: 0mM; high: 0.7mM) with/without FGF21 (100nM), mimicking lipotoxic conditions for different time course studies. Real-time quantitative PCR, RNA-seq and Western blot analyses were performed for the expression levels of the genes and proteins of interest.

Results: In animal experiment-treated with the FGF21 analogue (CVX-343), there were significant reductions in the HFD-induced body-weight gain (44.18 ± 1.13 vs 35.13 ± 0.71 g, n=15, $p<0.0001$), the levels of fasting blood glucose (12.77 ± 0.48 vs 9.47 ± 0.48 mM, n=15, $p<0.0001$) and triglyceride accumulation (0.144 ± 0.003 vs 0.080 ± 0.004 ug/mg protein, n=15, $p<0.0001$); meanwhile, the levels of glucose tolerance (2722 ± 134.5 vs 1942 ± 96.4, n=15, $p<0.0001$) and insulin sensitivity (987.3 ± 118.3 vs 575.5 ± 57.1, n=15, $p<0.01$) were markedly improved, in relation to the HFD-fed control mice. Moreover, FGF21 KO mice fed with HFD exhibited decreases in insulin sensitivity (1292 ± 12 vs 1946 ± 137, n=5, $p<0.05$) and glucose tolerance (2984 ± 280 vs 3703 ± 132, n=5, $p<0.05$), in relation to HFD-fed control mice. Exogenous application of FGF21 in isolated islets from wildtype mice displayed higher expression of lipid oxidation-related genes, including carnitine palmitoyltransferase-1 (CPT1, 6.04 ± 0.33 vs 7.58 ± 0.34, n=4, $p<0.05$) and acyl-CoA oxidase (ACO, 2.50 ± 0.06 vs 3.11 ± 0.10, n=4, $p<0.01$), in relation to the PA-treated islets; these observed effects were due, in part, to the activation of the PPARα/RXR signaling, as demonstrated by RNA-seq. In INS-1E cell studies, FGF21 treatment ameliorated the PA-induced FA levels (16.84 ± 0.97 vs 9.66 ± 0.47 mEq/g protein, n=4, $p<0.001$) and triglyceride accumulation (1.32 ± 0.06 vs 0.90 ± 0.04 ug/mg protein, n=4, $p<0.01$); meanwhile, FGF21 reversed high PA-induced cell apoptosis (197.5 ± 10.1 vs 170.3 ± 5.7 %, n=4, $p<0.001$) and improved cell proliferation (62.0 ± 1.2 vs 71.8 ± 2.6 %, n=5, $p<0.05$), as well as enhanced glucose stimulated insulin secretion (1.95 ± 0.16 vs 2.62 ± 0.13, n=4, $p<0.05$).

Conclusion: Our study findings indicate that FGF21 is protective against lipotoxicity-induced islet cell dysfunction and cell demise, probably via the mediation of lipid homeostasis, in HFD-treated mice; these data lend furher support for FGF21 analogue as a therapeutic target for obesity and T2DM.

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Disclosure: **T. Xie:** Grants; Hong Kong Research Grants Council (Ref. # CUHK 14107415).

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Dual Leucine zipper Kinase (DLK) activity is required for beta cell plasticity during gestation, obesity and postnatal development

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Background and aims: Multiple signaling pathways are involved in the physiological adaptation of the functional beta cell mass during gestation, obesity and postnatal development. We performed integrative unbiased

analyses including transcriptomics and kinomics, to identify most potent signaling pathways involved in the rat postnatal islet beta cell maturation.

Materials and methods: mRNA profiling was performed by Arraystar using Agilent Technologies and global serine/threonine kinases (kinome STK) was done by PamGene technology in isolated islets from newborn (p10) and weaned rats (p21 or p31). Islets were isolated from pregnant rats, obese mice fed high fat diet for 15 weeks and ob/ob mice. DLK knock out mice was used for measuring beta cell mass. The role of DLK was investigated in rat or human islets dispersed cells or insulin secreting cells using siRNAs. DLK mRNA and protein levels were quantified by quantitative real-time PCR and western blotting respectively. Proliferation and apoptosis were assessed by Ki67 staining and by counting pycnotic nuclei, respectively.

Results: Microarray analysis revealed a 70 fold increase in the expression of the MAPK activator Dual Leucine Zipper Bearing Kinase (DLK) as the most important modified gene in islets of newborn rat islets cells when compared to weaned rats. Increase of DLK expression was also found in compensatory islets of obese individuals when compared to obese diabetic patients, and in gestational rats and obese mice models including ob/ob and high fat diet, which are characterized by an increase in the beta cell number and function. Furthermore, the kinome analysis revealed that the dramatic increase of DLK expression was associated with a strong JNK activity, particularly JNK3, a kinase target of GLP-1 that is required for beta cell function and survival. We found that DLK positively regulates JNK3 activity, and that this regulation is essential for beta cell survival, proliferation and insulin secretion. DLK KO mice displayed reduced JNK activity and had drastic reduction in the beta cell number, whereas the alpha cells number was unchanged. The role of DLK was confirmed by in vitro experiments. Silencing of DLK in islet beta cells reduced JNK3 activity, beta cell proliferation and increased beta cell apoptosis. Inversely, overexpression of DLK improved beta cell survival and insulin secretion via JNK3.

Conclusion: DLK regulates the beta cell plasticity by controlling JNK3 activity. Activating DLK may have therapeutic implications for enhancing the functional beta cell mass in T2D

Disclosure: **A. Abderrahmani:** None.

OP 31 SGLT2 inhibitors: new opportunities

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Safety and tolerability of empagliflozin in patients with type 2 diabetes and advanced kidney disease: a large pooled analysis of placebo-controlled trials

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Background and aims: Patients with T2D and overt CKD are considered at high risk of AEs. Since current European prescribing information for empagliflozin (EMPA) offers its use in patients with some degree of impaired renal function, we assessed its safety and tolerability in patients with T2D and advanced kidney disease.

Materials and methods: We pooled 12,620 patients randomized (1:1:1) to EMPA 10 mg, 25 mg or placebo (PBO) in 15 PBO-controlled trials and 4 extension studies: this identified 1590, 586 and 32 individuals with CKD Stage 3A, 3B and 4, respectively. Total exposure to PBO, EMPA 10 mg and EMPA 25 mg was 1099, 1183 and 1169 years (CKD Stage 3A); 441, 415 and 422 years (CKD Stage 3B); and 15, 19 and 26 years (CKD Stage 4). Analyses are descriptive and based on investigator-reported AEs.

Results: Rates of SAEs, AEs leading to discontinuation, and AEs of special interest were generally balanced between the 2 EMPA doses and PBO across all 3 CKD subgroups. Consistent with current European prescribing information, AE reporting for genital infections was higher for EMPA, and a numerical increase in UTI with EMPA in CKD Stage 4 was seen. The latter may be interpreted with caution due to the low number of events.

Conclusion: Our comprehensive analysis suggests that safety and tolerability of EMPA in patients at advanced stages of CKD is reassuring. However, careful consideration of the current prescribing information for the use of EMPA in this population is recommended.

	Placebo		Empagliflozin 10 mg		Empagliflozin 25 mg	
	n/N (%)	Rate/100 PY	n/N (%)	Rate/100 PY	n/N (%)	Rate/100 PY
Serious AE	**1150/4203 (27.4)**	**19.21**	**1020/4221 (24.2)**	**15.52**	**1052/4196 (25.1)**	**16.45**
eGFR 45-60	231/529 (43.7)	28.33	196/530 (37.0)	21.16	198/531 (37.3)	22.21
eGFR 30-45	102/197 (51.8)	33.28	90/192 (46.9)	29.05	86/197 (43.7)	26.37
eGFR <30	3/7 (42.9)	29.79	5/9 (55.6)	39.97	9/16 (56.3)	43.22
AEs leading to treatment discontinuation	**540/4203 (12.8)**	**7.57**	**490/4221 (11.6)**	**6.49**	**484/4196 (11.5)**	**6.43**
eGFR 45-60	114/529 (21.6)	10.99	98/530 (18.5)	8.70	92/531 (17.3)	8.16
eGFR 30-45	61/197 (31.0)	14.87	64/192 (33.3)	17.15	51/197 (25.9)	12.72
eGFR <30	4/7 (57.1)	36.40	2/9 (22.2)	10.69	4/16 (25.0)	15.22

Bold rows show overall pool population. AE frequencies n/N = patients with ≥1 event/ all patients who received ≥1 study drug dose; PY = patient-years, defined as time from the first dose to onset of the first event (for patients with an event) or to last dose (for patients without an event). eGFR (mL/min/1.73m^2) calculated using Modification of Diet in Renal Disease equation.

Clinical Trial Registration Number: NCT00885118, NCT00789035, NCT00558571, NCT00749190, NCT01011868, NCT01193218, NCT01210001, NCT01177813, NCT01159600, NCT01289990, NCT01131676, NCT01164501, NCT01370005, NCT01306214, NCT01947855

Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance

Disclosure: **A. Levin:** Grants; AstraZeneca. Lecture/other fees; Janssen.

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Consistent effect of empagliflozin on composite outcomes related to heart failure: results from EMPA-REG OUTCOME

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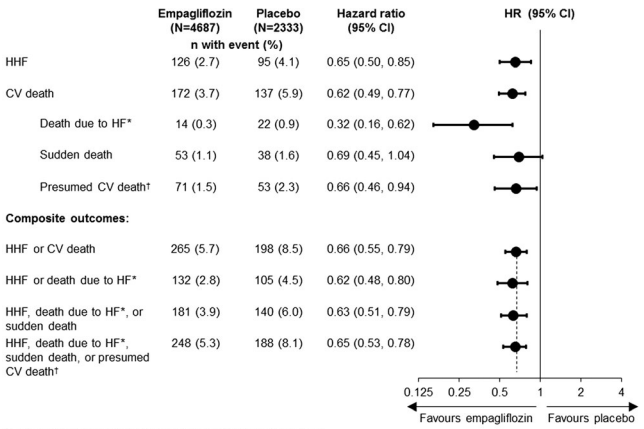
Background and aims: In the EMPA-REG OUTCOME trial, empagliflozin (EMPA) given in addition to standard of care significantly reduced the risk of cardiovascular (CV) death by 38%, hospitalisation for heart failure (HHF) by 35%, and the composite of HHF or CV death by 34% vs placebo in patients with type 2 diabetes (T2DM) and established CV disease. CV outcomes and deaths were prospectively adjudicated by clinical event committees. Post-hoc, we investigated composite outcomes of HHF and modes of CV death commonly observed in patients with heart failure (HF): sudden death, death due to HF (worsening of HF or cardiogenic shock), and presumed CV death (insufficient data for adjudication committees to definitively categorise the cause).

Materials and methods: Patients were randomised to receive EMPA 10 mg, EMPA 25 mg, or placebo. Using a Cox proportional hazards model, we analysed three additional composite outcomes in the pooled EMPA group vs placebo: a) HHF or death due to HF; b) HHF, death due to HF, or sudden death; c) HHF, death due to HF, sudden death, or presumed CV death.

Results: A total of 7020 patients were treated. At baseline, mean age was 63.1 years, body mass index was 30.6 kg/m^2, HbA1c was 8.1%, 71.5% were male and 10.1% had investigator-reported HF. Risk reductions for the composite outcomes studied are shown in the figure.

Conclusion: Risk reductions in composite outcomes of HHF and modes of CV death commonly observed in patients with HF were comparable to the 34% reduction in the pre-specified composite of HHF or CV death in the EMPA-REG OUTCOME trial.



Cox regression analysis in patients treated with ≥1 dose of study drug.
*Death due to worsening of HF or cardiogenic shock.
†Insufficient data for adjudication committees to categorise cause of death.

Clinical Trial Registration Number: NCT01131676
Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance
Disclosure: **J. Schnee:** Employment/Consultancy; Employee of Boehringer Ingelheim.

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Effects of the SGLT2 inhibitor dapagliflozin on tissue specific insulin sensitivity and liver fat content in type 2 diabetes patients: a randomised placebo controlled study

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Background and aims: The sodium-glucose cotransporter 2 inhibitor, dapagliflozin, improves glycaemic control and reduce weight in patients with type 2 diabetes mellitus (T2DM). Some studies also suggest that dapagliflozin ameliorates insulin resistance. The aim of this study was to investigate tissue specific effects of dapagliflozin on insulin sensitivity using positron emission tomography (PET) - imaging in patients with T2DM.

Materials and methods: This randomized, parallel-group, double-blind, placebo-controlled study enrolled 32 patients whose T2DM was inadequately controlled on oral medication. One patient did not complete the study. Groups were similar regard to age (mean 61 y), BMI (32 kg/m^2) and glycemic control (HbA1c 6.9 %). The majority of patients were male (81 %). All patients were on metformin and 16 patients (50 %) were also on sitagliptin. Patients were randomly assigned 1:1 to receive either dapagliflozin 10 mg/day or placebo daily for 8 weeks. Tissue specific insulin sensitivity was measured using PET and [18F]-fluorodeoxyglucose during an euglycemic hyperinsulinemic clamp before and after the intervention. M-values (μmol/kg/min) corrected for steady state (ss) insulin were calculated. Adipose tissue volumes and liver fat percent were assessed using MRI and blood biomarkers were measured.

Results: At week 8, placebo-corrected changes with dapagliflozin were as follows: body weight -2.9 kg ($p<0.001$), HbA1c -0.39% ($p<0.01$), plasma glucose -1.32 mmol/L ($p<0.01$), visceral adipose tissue volume -0.35 L ($p< 0.01$), liver fat% -3.74% ($p< 0.01$) and liver volume -0.10 L ($p<0.05$), interleukin-6 -1.87 pg/ml ($p<0.05$) and pro-BNP -96 ng/L ($p=0.03$). Whole body insulin sensitivity (M-value corrected for ss insulin) was numerically increased by dapagliflozin but the effects were not significantly different between the groups ($p=0.10$). In patients on sitagliptin (placebo n=7, dapagliflozin n=9), dapagliflozin improved ss insulin corrected M-value compared to placebo ($p=0.03$). There was no significant difference between the treatment groups in skeletal muscle, heart, white or brown adipose insulin stimulated glucose uptake. Changes in skeletal muscle insulin stimulated glucose uptake and M-values were significantly correlated in the dapagliflozin group ($r=0.75$, $p<0.01$). Statistical analyses were performed using two-way analysis of covariance (ANCOVA). Correlations were tested using Spearman rank correlation.

Conclusion: In addition to better glycemic control, dapagliflozin reduced visceral fat volume and lowered liver fat in obese insulin resistant T2DM patients. No direct effect on muscle, heart or adipose tissue insulin sensitivity was found suggesting that glucose utilisation was not changed in these tissues.

Clinical Trial Registration Number: NCT02426541
Supported by: AstraZeneca
Disclosure: **A.E. Hyypia:** None.

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Drug-specific or class effects of SGLT2 inhibitors (SGLT2i) in the heart: SGLT2i inhibit NHE, reduce $[Na^+]_c$ and induce vasodilation in isolated mouse cardiomyocytes/hearts

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Background and aims: Empagliflozin (EMPA) reduced cardiovascular diseases in type 2 diabetes patients, yet the cardioprotective mechanism of this kidney-targeted diabetes drug remains unknown. We recently reported that EMPA lowers cytosolic sodium ($[Na^+]_c$) and calcium, while increasing mitochondrial calcium, through inhibition of the myocardial sodium/hydrogen

exchanger (NHE). Cardiac NHE has been shown to be upregulated in, and contribute to, heart failure and diabetes. We therefore investigated 1) whether two other SGLT2i (dapagliflozin (DAPA) and canagliflozin (CANA)) block cardiac NHE and reduce $[Na^+]_c$ as well, and 2) whether SGLT2i (EMPA, DAPA and CANA) affect physiological performance of the isolated-perfused mouse hearts.

Materials and methods: Molecular docking simulations were conducted on a homology model of the NHE-1 protein structure. Furthermore, NHE activity was indirectly measured from recovery of intracellular pH after loading of NH^{4+}-pulse in isolated mice cardiomyocytes. $[Na^+]_c$ was determined in cardiomyocytes using a fluorescent sodium indicator, SBF-1. Finally, Langendorff-perfused mouse hearts, perfused with 7 mM glucose, 1 mM lactate, 0.1 mM pyruvate, 0.5 mM glutamine and 50 mU/L insulin and 0.1% albumin, were subjected to either vehicle, 1 μM EMPA, 1 μM DAPA or 3 μM CANA for 30 minutes.

Results: Molecular docking simulations predicted that all three compounds bind with relatively high binding affinity to the extracellular Na+-binding site of NHE-1 (EMPA: -8.2, DAPA: -7.7 and CANA: -8.9 kJ/mol). Subsequently, all three SGLT2i inhibited NHE activity compared to vehicle (7.09±0.04), with EMPA showing the strongest inhibition of NHE (1 μM EMPA 6.69±0.03, p<0.001; 1 μM DAPA 6.79±0.03, p<0.001; 3 μM CANA 6.87±0.06, p<0.05). Moreover, measurements of [Na+]c showed that all three SGLTi significantly lowered [Na+]c (1 μM EMPA 9.9±0.3, p<0.001; 1 μM DAPA 10.7±0.3, p<0.01; and 3 μM CANA 11.0±0.5, p<0.01; vs. vehicle: 13.0±0.4 mM). Finally, in Langendorff-perfused mouse hearts, both EMPA and CANA induced vasodilation, determined by a 19% and 22% reduction in perfusion pressure respectively (p<0.05) compared to vehicle. In addition, EMPA significantly increased oxygen consumption (DMSO vs. EMPA; from 35.8 ±2.4 to 42.4±1.3 μmol/min/gdw; p<0.05). SGLTi did not alter cardiac mechanical performance.

Conclusion: Here, we show that SGLTi exhibit a class effect by blocking NHE and reducing $[Na^+]_c$ directly in the cardiac cell. CANA and EMPA induce vasodilation of the coronary circulation of the intact heart, and EMPA increases oxygen consumption. These direct cardiac effects may contribute to understanding previously reported cardiac protection by SGLTi.

Disclosure: **L. Uthman:** None.

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24-week efficacy and safety of sotagliflozin, a dual SGLT1 and SGLT2 inhibitor, as adjunct therapy to insulin in type 1 diabetes (inTandem2)

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Background and aims: Sotagliflozin (SOTA) is a dual SGLT1 and SGLT2 inhibitor in Phase 3 development for type 2 diabetes (T2D) and as adjunct therapy to insulin in type 1 diabetes (T1D). SGLT1 inhibition delays and reduces glucose absorption in the proximal intestine, improving postprandial glycemic control. SGLT2 inhibition reduces renal glucose reabsorption.

Materials and methods: In a double-blind, 52-week international Phase 3 trial, 782 adults with T1D treated with MDI or pump therapy, with A1C 7.0-11.0% at Screening, were randomized 1:1:1 to placebo, SOTA 200 or 400 mg after a 6-week insulin optimization period. Baseline characteristics were comparable among groups. The primary outcome was change from Baseline in A1C at 24 weeks.

Results: SOTA 200 and 400 mg were statistically significant vs. placebo in lowering A1C and achieving the prespecified net benefit[a] endpoint. Overall incidences of TEAEs[b] were similar across groups. There were 2 deaths on placebo. There were more genital mycotic infections, diarrhea events, and DKA in the SOTA arms.

Conclusion: The international inTandem2 trial of SOTA as adjunct therapy to insulin met its primary endpoint with a statistically significant and clinically meaningful A1C reduction after 24 weeks, while demonstrating the general safety and tolerability of dual SGLT1 and SGLT2 inhibition in T1D. These results confirm the findings of inTandem1.

Efficacy and Safety Results from Randomization to Week 24 on a Background of Optimized Insulin Therapy

	Placebo n = 257	SOTA 200 mg n = 261	SOTA 400 mg n = 263
Efficacy			
A1C at Screening, %	8.43	8.35	8.38
A1C at Baseline, after 6-week insulin optimization, %	7.80	7.74	7.71
A1C at Week 24, %	7.79	7.36	7.35
A1C at Week 24 LSM Change from Baseline, % (*p*-value)	-0.03 (*p*=0.54)	-0.39 (*p*<0.001)	-0.37 (*p*<0.001)
A1C at Week 24 LSMD vs. placebo[c], % (*p*-value)	N/A[e]	-0.36 (*p*<0.001)	-0.35 (*p*<0.001)
Patients with Safety Event			
Any TEAE, n (%)	132 (51.4)	146 (55.9)	143 (54.4)
AE as primary reason for early discontinuation of Core Treatment Period, n (%)	4 (1.6)	5 (1.9)	8 (3.0)
Serious adverse events, n (%)	9 (3.5)	11 (4.2)	11 (4.2)
Death, n (%)	2 (0.8)	0 (0)	0 (0)
DKA, n (%)	0 (0)	1 (0.4)	3 (1.1)
Severe hypoglycemia, n (%)	7 (2.7)	10 (3.8)	6 (2.3)
Diarrhea[d], n (%)	10 (3.9)	14 (5.4)	19 (7.2)
Genital mycotic infection, n (%)	4 (1.6)	19 (7.3)	22 (8.4)
Efficacy and Safety			
Net benefit [A1C < 7.0% at Week 24 and no SH and no DKA Randomization to Week 24], n (%)	39 (15.2)	83 (31.8)	85 (32.3)
Net benefit difference, % responders vs. placebo[c], n (*p*-value)	N/A[e]	16.6 (*p*<0.001)	17.1 (*p*<0.001)

LSM, least squares mean; LSMD, least squares mean difference; SH, severe hypoglycemia. [a]Net benefit: A1C <7.0% at Week 24 and no SH and no DKA from Randomization to Week 24. [b]TEAE: Treatment-emergent adverse event. [c]Statistical comparisons of each SOTA arm to placebo were preplanned and performed using a generalized linear model with repeated measures statistics. [d]Discontinuation of drug due to diarrhea was: 0.4% placebo, 0.4% SOTA 200 mg, and 0.8% SOTA 400 mg. [e]N/A: Not applicable.

Clinical Trial Registration Number: NCT02421510

Disclosure: **T. Danne:** Honorarium; Bristol-Myers Squibb, Johnson & Johnson, Unomedical. Lecture/other fees; AstraZeneca, Boehringer Ingelheim, DexCom, Eli Lilly, Medtronic, NovoNordis, Roche, Sanofi, Ypsomed.

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Efficacy and safety of sotagliflozin, a dual SGLT1 and SGLT2 inhibitor, as adjunct to insulin in young adults with poorly controlled type 1 diabetes (JDRF Study)

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Background and aims: Sotagliflozin (SOTA) is a dual SGLT1 and SGLT2 inhibitor in Phase 3 development for type 2 diabetes (T2D) and as adjunct to insulin in type 1 diabetes (T1D). SGLT1 inhibition delays and reduces glucose absorption in the proximal intestine, improving postprandial glycemic control. SGLT2 inhibition reduces renal glucose reabsorption.

Materials and methods: In a double-blind Phase 2 trial of young adults (age 18-30 years) with poorly controlled T1D (A1C ≥9.0%), 87 patients were randomized 1:1 to placebo or SOTA 400 mg once daily for 12 weeks. The primary outcome was change from Baseline in A1C at 12 weeks.

Results: SOTA decreased A1C by 0.35% compared with placebo (p=0.10). SOTA treatment resulted in lower postprandial glucose (PPG), higher net benefit[a], lower body weight, and lower A1C in a prespecified subgroup analysis (9.0% ≤ screening A1C ≤ 10.0%) than placebo. Overall incidences of treatment-emergent adverse events (TEAEs) were similar across groups. There were more genital mycotic infections and diarrhea events on SOTA. There was no DKA on SOTA.

Conclusion: In young adults with poorly controlled T1D, SOTA for 12 weeks as adjunct therapy to insulin, was well tolerated with evidence of improvements in glycemic control and weight reduction consistent with dual SGLT1 and SGLT2 inhibition.

Efficacy and Safety Results from Randomization to Week 12		
	Placebo (n = 42)	SOTA 400 mg (n = 43)
Efficacy		
A1C at Screening, % (SD)	10.3 (0.95)	10.6 (1.3)
A1C at Baseline, after 2-week screening + 2-week run-in, % (SD)	9.7 (0.93)	9.9 (1.4)
A1C at Week 12, % (SD)	8.7 (1.0)	8.4 (1.5)
A1C at Week 12, LSM Change from Baseline, % (SE, *p*-value[c])	-0.99 (0.15, *p*<0.001)	-1.33 (0.14, *p*<0.001)
A1C at Week 12, LSMD vs. placebo[b], % (SE, *p*-value[c])	N/A[f]	-0.35 (0.21, *p*=0.10)
A1C at Week 12, LSMD vs. placebo[b] with 9.0% ≤ screening A1C ≤10.0% , % (SE, *p*-value)	N/A[f]	-0.75 (0.26, *p*=0.006)
2-hr PPG LSMD vs. placebo[b], mmol/L (SE, *p*-value[c])	N/A[f]	-3.1 (0.92, *p*=0.001)
Daily CGM time in range 3.9-10.0 mmol/L vs. placebo[c] % (SE, *p*-value[c])	N/A[f]	+7.7 (3.9, *p*=0.057)
Daily CGM time in range 3.9-10.0 mmol/L vs. placebo, hours[g]	N/A[f]	+1.8
Body weight LSMD vs. placebo[b], kg (SE, *p*-value[c])	N/A[f]	-2.4 (0.6, p<0.001)
Patients with Safety Events		
Any TEAE, n (%)	26 (61.9)	25 (58.1)
AE as primary reason for early discontinuation of treatment period, n (%)	2 (4.8)	0 (0)
Serious adverse event, n (%)	3 (7.1)	2 (4.7)
Diabetic ketoacidosis (DKA), n (%)	1 (2.4)	0 (0)
Severe hypoglycemia (SH), n (%)	2 (4.8)	1 (2.3)
Nausea[e], n (%)	3 (7.1)	1 (2.3)
Diarrhea[e], n (%)	0 (0)	2 (4.7)
Genital mycotic infection[e], n (%)	0 (0)	2 (4.7)
Efficacy and Safety		
Net benefit [A1C <7.0% at Week 12 and no SH and no DKA Randomization to Week 12], n (%)	1 (2.4)	7 (16.3)
Net benefit difference, % responders vs. placebo[b], n (*p*-value[c])	N/A[f]	13.9 (*p*=0.026)

CGM, continuous glucose monitoring; LSM, least squares mean; LSMD, least squares mean difference; PPG, postprandial glucose [a]Net benefit: A1C <7.0% at Week 12 and no SH and no DKA from Randomization to Week 12. [b]Statistical comparisons of each SOTA arm to placebo were preplanned and performed using a generalized linear model with repeated measures statistics. [c]Because the primary endpoint was not significant, *p*-values attached to nonprimary/secondary endpoints are descriptive and cannot be used to declare statistical significance. [e]Mild to moderate with no discontinuations. [f]N/A: Not applicable. [g]Estimated by assuming 100% of daily CGM data available for analysis; therefore, 1.0% of daily CGM time = 0.24 hours.

Clinical Trial Registration Number: NCT02383940

Supported by: JDRF

Disclosure: **B. Bode:** Grants; Abbott, DexCom, Eli Lilly, Janssen, Lexicon Pharmaceuticals, Inc., Medtronic, Novo Nordisk, Sanof, Boehringer Ingelheim. Honorarium; Adocia, Astra Zeneca, Insulet, Janssen, Mannkind, Medtronic, Novo Nordisk, Sanofi.

OP 32 Improvement in insulin therapy

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A randomised clinical trial testing for optimal beta cell preserving treatment in Latent Autoimmune Diabetes in Adults (LADA)

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Background and aims: The optimal beta cell preserving treatment of LADA patients is presently uncertain. This is due to a lack of randomized clinical trials in LADA. We aimed to compare the effect on beta cell function during either insulin or sitagliptin, a DDP-4 inhibitor, when added to ongoing treatment with metformin.

Materials and methods: Inclusion criteria included GADA positivity, age 30-75 years, no clinical need for insulin treatment and a diagnosis of diabetes made < 3 years before participation in the study. Further, HbA1C at inclusion was to be less than 60 but more than 5% above upper limit of normal. Patients were stratified by age, BMI and degree of GADA positivity (high/low). Beta cell function was evaluated by C-peptide glucagon tests which were performed after a 48 h temporary withdrawal of study medication.

Results: In a study population of 53 participants the mean age at randomization was 53 years and BMI 27.7 kg/m^2. These parameters and others (male/female participation, HbA1c, fasting C-peptide) were similar between arms of the study. HbA1c did not differ significantly between baseline and after 9 months of intervention (baseline 52.2 ± 1.4 mmol/mol, at 9 months, 50.8 ± 2.1 mmol/mol in the insulin, 50.6 ± 1.8 mmol/mol and 44.9 ± 1.8 mmol/mol in the sitagliptin arm). Stimulated C-peptide after 9 months of intervention was similar in the insulin and sitagliptin arm (incremental C-peptide 0.31 ± 0.04 nmol/l in the insulin, 0.31 ± 0.05 nmol/l in the sitaglitin arm). These findings did not negate a time-dependent negative effect of autoimmunity in the study population (taken as a whole); this was manifested by a decrease in stimulated C-peptide from 0.97 ± 0.09 to 0.82 ± 0.09 nmol/l in those with the highest titer of GADA (p< 0.02 for difference baseline vs 9 months).

Conclusion: This is the first randomized study to compare in LADA patients the effects on beta cell function of early insulin treatment vs. a DDP-4 inhibitor, the latter being a conventional treatment alternative in non insulin dependent diabetes. The results do not give evidence favoring early insulin during the time frame studied. Follow-up of the study population is underway to answer the question whether a longer observation period would detect differences between the arms of treatment.

Clinical Trial Registration Number: NCT01148238

Supported by: NRC Norwegian Research Counsil, SRC Swedish Research Counsil

Disclosure: **I. Hals:** None.

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Systematic analysis of the inflammatory tissue response to CSII catheters: steel versus teflon

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University of Graz, Graz, Austria, [4]Department of Anesthesiology, Thomas Jefferson University, Philadelphia, USA.

Background and aims: Continuous subcutaneous insulin infusion (CSII) catheters are considered the weak link of insulin pump therapy. The choice of CSII catheter material (steel or Teflon) is mostly based on personal experience or preference and wear-time varies between patients. Systematic evaluations of the adipose tissue inflammatory response - to determine advantages of one material over the other - are lacking. This present study evaluated the adipose tissue response to commercially available Teflon and steel CSII catheters over a maximum of 7 days of wear time.

Materials and methods: Steel and Teflon CSII catheters were inserted into the subcutaneous adipose tissue of 10 swine for 1, 4 and 7 days of wear-time. Skin and adipose tissue surrounding catheters were excised and stained with hematoxylin and eosin to determine morphological changes and inflammatory cell infiltration. We performed quantitative real-time PCR (qPCR) to assess changes in gene expression levels of cytokines and macrophages.

Results: While the total area of inflammation around catheters was similar between materials, fibrin deposition and mononuclear cell infiltration were significantly reduced around Teflon catheters compared with steel after 4 days of wear-time. Proinflammatory cytokines were upregulated within 24 hours independent of material but levels were slightly higher around steel over 7 days. Antiinflammatory cytokine gene expression did not resolve over time. The sharp tip of the steel catheter continuously ruptured connective tissue and vasculature throughout wear-time.

Conclusion: The combination of histopathology and qPCR allowed for an adequate trend analysis beyond the clinically recommended wear-time of CSII catheters. Our results suggest a better tolerability of Teflon catheters by the adipose tissue over 4 days of wear-time. These findings may have substantial implications for the choice of material for future CSII catheter development.

Supported by: ConvaTec Inc.

Disclosure: **J.R. Hauzenberger:** None.

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Effect of food intake on the absorption of oral basal insulin

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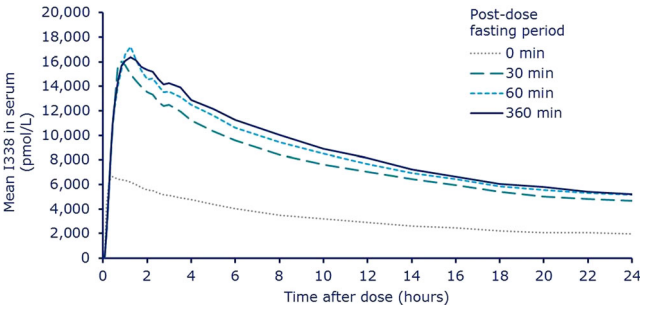
Background and aims: Oral insulin 338 in a GIPET® tablet (OI338GT) is a basal, acylated insulin analogue with a half-life of ~70 hours at steady state. In people with type 2 diabetes OI338GT given 1 hour before breakfast demonstrated safe improvement of glycaemic control to a similar extent as insulin glargine. Since it is well known that oral insulin absorption from the gastrointestinal tract changes with food intake, the aim of this trial was to investigate the effect of timing of food intake on the single dose pharmacokinetics (PK) of OI338GT.

Materials and methods: Healthy men received a single fixed dose of OI338GT in the fasting state at 4 different dosing visits. In a randomised, crossover design, they consumed an identical standardised meal (500 kcal, 57% carbohydrates, 13% fat, 30% protein) 0 min, 30 min, 60 min and 360 min post-dose. After the test meal participants received scheduled standardised meals at the investigational site for 48 hours post-dose after which they resumed their usual dietary habits. PK samples for insulin 338 concentrations were taken at regular time points up until 288 hours post-dose.

Results: Forty-four (44) subjects were enrolled (mean±SD age 35±8 years, BMI 24.2±1.9 kg/m^2) and 42 completed the trial. Mean insulin 338 concentration-time profiles over 24 hours showed the lowest exposure for OI338GT dosing immediately before food intake (0 min) and similar exposure for 30 min, 60 min and 360 min post-dose food intake (figure). Total insulin exposure ($AUC_{0-\infty}$, primary endpoint) and maximum insulin exposure (C_{max}) were lower for OI338GT dosing with food intake (0 min) compared to food intake 360 min post-dose (estimated ratio between geometric means [95% CI] $AUC_{0-\infty}$ 0.36 [0.26; 0.49], C_{max} 0.35 [0.25; 0.49]). There were no statistically significant differences with 360 min post-dose food intake when food was ingested 30 min ($AUC_{0-\infty}$ 0.85 [0.61; 1.21], C_{max} 0.86 [0.59; 1.26]) or 60 min post-dose ($AUC_{0-\infty}$ 0.96 [0.72; 1.28], C_{max} 0.99 [0.75; 1.31]). OI388GT administration was well tolerated with no serious adverse events reported. Hypoglycaemia, including both symptomatic events and asymptomatic events with a blood glucose below 63 mg/dL, was observed across the four post-dose fasting periods with a trend towards fewer hypoglycaemic events for the 0 min post-dose fasting period (13, 26, 25 and 26 events for 0 min vs. 30, 60 and 360 min post-dose periods, respectively). This result is consistent with the lower level of insulin 338 exposure in the 0 min post-dose fasting period.

Conclusion: This trial demonstrates that oral basal insulin 338 absorption in the fasted state is not affected by food intake from 30 min after administration. For convenience and compliance with future treatment this is a highly relevant finding meaning that patients with diabetes should not have to wait more than 30 min to eat their breakfast after a morning oral basal insulin dose.



Clinical Trial Registration Number: NCT02304627

Supported by: Novo Nordisk

Disclosure: **E. Zijlstra:** None.

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The effect of an automated bolus calculator on glucose control, glucose variability and quality of life in patients with type 1 or 2 diabetes treated with insulin pumps

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Background and aims: Automated insulin bolus calculators are widely recommended for patients with type 1 or type 2 diabetes on insulin pumps, but their benefit is still disputed. We investigated whether a bolus calculator reduced glucose variability and improved quality of life in such patients.

Materials and methods: We conducted a 16-week randomized controlled trial, involving 34 patients (mean age 45.9 ± 15.1 years, 35% male, disease duration 26.6 ± 12.8 years, HbA_{1c} 64.2 mmol/mol (8.0%) ± 12.3), who were assigned to treatment with a bolus calculator or to continuation of standard care without a bolus calculator. All patients received extensive education on carbohydrate counting and on insulin therapy. The primary outcome was glucose variability, as assessed by standard deviation of 7-point glucose profiles over 5 days. Secondary outcomes were HbA1c, frequency of hypoglycaemia, low blood glucose index (LBGI) and high blood glucose index (HBGI), and quality of life assessed by different questionnaires.

Results: After 16 weeks of treatment, there were no differences between the two groups with respect to glucose variability (SD, 3.03 ± 0.59 vs. 3.00 ± 0.50 mmol/l, p = 0.096, bolus calculator vs. control group respectively), glucose control (HbA_{1c}, 59.1 ± 19.2 mmol/mol (7.6%) vs. 61.2 ± 9.0 mmol/mol (7.7%), p = 0.932), LBGI (p = 0.528), HBGI (p = 0.338) and frequency of hypoglycaemia (p = 0.534). Quality of life did not change during follow-up in both groups.

Conclusion: Use of a bolus calculator has no benefit with respect to various glycaemic parameters or quality of life in patients with longstanding type 1 or 2 diabetes treated with insulin pumps.

Supported by: EADV

Disclosure: **L.A.G. van Meijel:** Grants; EADV research grant.

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Subcutaneous tissue changes and dermal inflammation at insulin injections sites: a feasibility study using ultrasound to describe characterise and grade lipohypertrophy

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[1]Clinical Research in Diabetes, King's College London, [2]Women's Health - Medical and Vascular Ultrasound, King's College London, [3]Cardiovascular Division, King's College London, London, UK.

Background and aims: Subcutaneous (s/c) tissue changes in patients with type 1 diabetes (T1DM) are common and can affect glycaemic control. There are limited data on the utility of ultrasound to describe changes in s/c tissue with insulin use. This study aimed to assess the feasibility of using ultrasound to describe and characterise tissue changes to inform a future lipohypertrophy (LH) grading study.

Materials and methods: Patients with T1DM thought by diabetes clinicians to have LH or with clinical LH on examination were included. A SonoSite X-Porte ultrasound machine was used with a high frequency linear probe (6-13 MHz). Insulin injections sites and 'normal' non-injected adjacent tissues were scanned by a single operator.

Results: We scanned 15 patients with T1DM to establish a standard operating procedure (SOP). Then 11 additional patients with T1DM were scanned in detail using that SOP; mean age 41 years (range 24-59), mean duration of diabetes 22.7 years (range 4 - 49), and mean HbA_{1c} 7.3% (range 5.8 - 9.6%). Ultrasound images exhibited changes in the s/c tissue at a depth corresponding to approximate needle length (± 2-3mm). This tissue appeared as diffuse reflective areas of differing density in all of the 26 patients. More dense areas formed visible lumps: of the 11 patients studied in detail; 4 (33%) had large palpable lumps, 5 (45%) had small or medium nodules some of which were palpable, and 2 (18%) had large regions of diffuse changed tissue across the injection site. Compiling images from the 26 patients, a potential grading system has been proposed: Grade 0 (no evident nodules or diffuse areas of specific density), Grade 1 (small nodule <10mm), Grade 2 (medium nodule 10-20mm), Grade 3 (large nodule >20mm), Grade 4 (general diffuse area of specific density). Grade 1 to 3 can be single or multiple. Nine (82%) of the 11 participants also showed inflammatory tissue in the dermal layers of injections sites, <3mm thickness in 5 participants, and >3mm thickness in the other 4. Similar inflammation was also seen in a proportion of the first 15 patients, so this may be a common occurrence in this group. The inflammatory tissue has been provisionally graded as: 0 = normal, 1 for <3mm and 2 for >3mm. Normal tissue in all patients showed no increased reflectivity and normal dermal and s/c layers above muscle. See figure 1.

Conclusion: This feasibility study has been the first to report and measure dermal inflammation in people injecting insulin. It demonstrates the potential of ultrasound in characterising s/c tissue changes in people who inject insulin. The observations have informed preliminary grading systems, which will be validated in further studies to assess the clinical implication of these tissue changes in relation to glucose variability and insulin action.

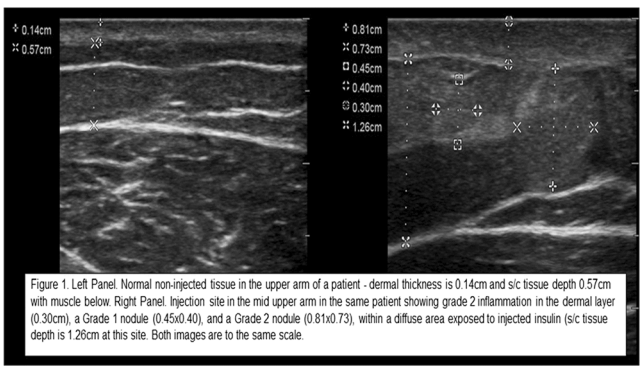

Figure 1. Left Panel. Normal non-injected tissue in the upper arm of a patient - dermal thickness is 0.14cm and s/c tissue depth 0.57cm with muscle below. Right Panel. Injection site in the mid upper arm in the same patient showing grade 2 inflammation in the dermal layer (0.30cm), a Grade 1 nodule (0.45x0.40), and a Grade 2 nodule (0.81x0.73), within a diffuse area exposed to injected insulin (s/c tissue depth is 1.26cm at this site. Both images are to the same scale.

Disclosure: **H. Mulnier:** None.

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A novel formulation of insulin lispro shows significantly faster absorption and improvement in postprandial glucose excursions versus insulin lispro in patients with type 2 diabetes

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[1]Profil, D-41460 Neuss, Germany, [2]Eli Lilly and Company, Indianapolis, USA.

Background and aims: LY900014 (LY) is a novel formulation containing locally-acting excipients citrate and treprostinil to accelerate insulin lispro absorption with the goal of providing an ultra rapid prandial insulin.

Materials and methods: This abstract focuses on the first part of a 2-part, 6-period crossover study evaluating pharmacokinetic and pharmacodynamic differences between LY and insulin lispro (IL). Twenty-nine patients with type 2 diabetes mellitus (T2DM) were randomised to receive individualized s. c. doses of LY or IL administered at the start of a mixed-meal tolerance test. Prior to the test, blood glucose was stabilised at 126 milligrams/decilitre.

Results: Patients treated with LY had accelerated early insulin lispro absorption and faster elimination compared to IL. LY reduced the time to early half-maximal drug concentration by 23% ($p<0.0001$) and increased the insulin lispro area under the concentration curve versus time (AUC) from 0 to 30 minutes by 116% ($p<0.0001$) compared to IL. The 90% CI of the ratio (LY : IL) for insulin lispro AUC(0-∞) was within bioequivalence criteria. The total glucose excursion over the 5-hour test-meal was reduced by 105% ($p<0.0001$) for LY versus IL (Figure 1). No differences were observed between LY and IL for tolerability and incidences or severity of hypoglycaemic events.

Conclusion: These results support continued development of this novel ultra rapid insulin lispro formulation as a next generation prandial insulin for patients with T2DM.

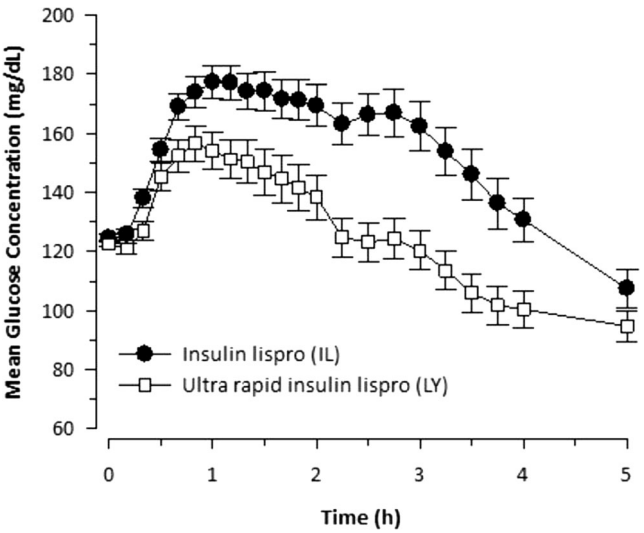

Figure 1. Mean postprandial glucose (±SEM) over time for ultra rapid insulin lispro and insulin lispro

Clinical Trial Registration Number: NCT02703337
Supported by: Eli Lilly and Company
Disclosure: **C. Kapitza:** Other; Research funds from Eli Lilly and Company.

OP 33 Gastro-entero-pancreatic interactions

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Impact of artificial sweeteners on glycaemic control in healthy humans

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[1]Medicine, The University of Adelaide, [2]Nutrition & Metabolism, South Australian Health & Medical Research Institute, [3]Centre of Research Excellence in Translating Nutritional Science to Good Health, [4]Human Physiology, Flinders University, Adelaide, Australia.

Background and aims: Prospective epidemiological studies indicate that a high habitual intake of beverages sweetened with non-caloric artificial sweeteners (NAS) increases the risk of developing type 2 diabetes (T2DM), but the underlying mechanisms are unknown. In animals, acute exposure to NAS activates intestinal sweet taste receptors (STRs) to trigger the release of glucose-dependent insulinotropic polypeptide (GIP) from proximal K-cells, and glucagon-like peptide-1 and 2 (GLP-1, GLP-2) from more distal L-cells, while dietary NAS supplementation increases the function of the sodium-dependent glucose co-transporter-1 (SGLT-1) to augment glucose absorption and increase postprandial glycaemia. It is not known whether NAS alters glucose absorption in humans, and if so, whether this affects postprandial glycaemic control adversely.

Materials and methods: 27 healthy subjects (age 27 ± 2 years, body mass index 24 ± 1 kg/m^2, 14 male) were randomised, in double-blind fashion, to dietary supplementation with a NAS combination (92 mg sucralose plus 52 mg acesulfame-K, equivalent to ~1.5L of diet beverage/day, N=14) or placebo (N=13), taken in capsules three times daily before meals over 2 weeks. Subjects then attended the laboratory after an overnight fast and underwent non-sedated endoscopy incorporating a 30 min intraduodenal glucose infusion (30g/150ml, 3 kcal/min, including 3g of the glucose analogue 3-O-methyl glucose, 3-OMG) and biopsy collection, before and immediately after the intervention. Glucose absorption (serum 3-OMG), plasma glucose, insulin and gut peptides (total GLP-1, GLP-2 and GIP) were measured, and the incremental areas under the curve (iAUC, over 120 min) compared by 2-way ANOVA.

Results: NAS supplementation augmented the iAUC for glucose absorption (23%, $P \leq 0.05$) and blood glucose (27%, $P \leq 0.05$), and attenuated the iAUC for GLP-1 (35%, $P \leq 0.05$) compared to baseline, while none of these measures were altered with placebo. The GLP-2, GIP, and insulin responses to enteral glucose were similar between NAS and placebo groups, although GLP-2 and insulin were lower at 40 and 60 min, respectively, in the NAS group (37% for both vs. baseline, $P \leq 0.05$).

Conclusion: In healthy humans, 2 weeks of dietary NAS supplementation (i) enhances glucose absorption, (ii) augments blood glucose responses to enteral glucose, and (iii) attenuates GLP-1 release, the latter possibly reflecting reduced glucose exposure to more distally located L-cells. This study supports the concept that NAS have a deleterious impact on acute glycaemic control, and highlights the potential for exaggerated postprandial glycaemic excursions in high habitual NAS consumers, which could predispose to T2DM.

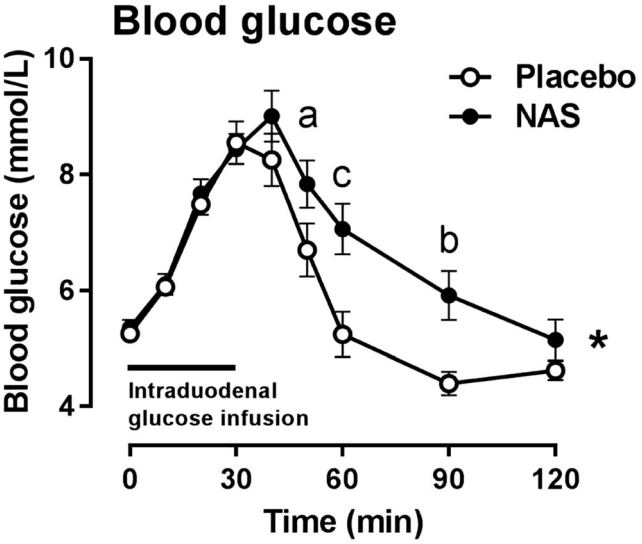

Figure: Blood glucose responses to enteral glucose in placebo (N=13) or NAS-supplemented humans (N=14). NAS vs Placebo: ^{a,b,c} $P \leq 0.05$, 0.01, 0.001; *$P \leq 0.05$ iAUC.

Clinical Trial Registration Number: ACTRN12615000866505
Supported by: NHMRC
Disclosure: **R.L. Young:** None.

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Differential gene profile of human enteroendocrine cells in metabolic diseases

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Background and aims: Enteroendocrine cells (EEC), despite their small proportion (1%) among intestinal epithelial cells represent the largest population of endocrine cells in the body. In adult intestine, EEC precursors are engaged toward terminal differentiation under the control of a complex network of transcription factors such as FOXA1/2, PDX1, PAX4/6, ARX, which define cell lineages, each EEC type secreting one of the 15 gut hormones. Among them, GLP (glucagon-like peptide)-1, contributes to glucose homeostasis and control of food intake. The continuous renewal and differentiation of EEC lineages allow a permanent capacity of cell adaptation to nutritional environment. Our hypothesis is that metabolic diseases, obesity and/or type 2 diabetes (T2D) are associated with different EEC commitment and gene expression patterns. We aim to characterize the differential transcriptional profile of EEC lineages between obese and diabetic obese subjects that could lead to modulation of local and systemic enterohormone levels.

Materials and methods: Jejunum samples were obtained of obese subjects (n=58), normoglycemic, glucose intolerant or diabetic, during gastric bypass procedure and of normal weight individuals (n=22). Expression of the transcription factor network involved in the EEC differentiation was studied by RT-qPCR using Taqman® Arrays Cards on isolated jejunal epithelial cells. A novel cell sorting strategy was developed to select EEC. RNA were purified and the EEC transcriptome was further analysed by RNA-Seq.

Results: The expression of the transcription factor network responsible for the commitment of EEC is altered in metabolic diseases. Multivariate analysis corrected for age and sex, reveals that FOXA1 and PDX1 gene expression is significantly increased in obese subjects when compare to lean subjects whereas ARX and proglucagon (encoding GLP-1) gene expression, is decreased. The diabetes severity (normoglycemia, glucose intolerance and T2D) in obese subjects is associated with an increase of FOXA1 gene expression and a decreased of ARX one. Because EEC are scattered throughout the intestinal epithelium, EEC gene expression profiling requires a cell-enrichment process. We developed a new cell sorting strategy starting from total epithelial cells isolated from fresh gut tissue that gave a 50-fold enrichment in EEC. We confirmed that sorted EEC expressed specific markers such as proglucagon (GLP-1), and chromogranin A at mRNA and protein levels. We obtained EEC fractions from non diabetic and diabetic obese subjects and the RNA seq analysis (Illumina technology) is undergoing.

Conclusion: Our study highlights differences on EEC gene expression profile according to the metabolic status of individuals. The development of this novel cell-sorting strategy to obtain EEC from huma jejunum will be key in the future to identify candidate targets potentially boosting endogenous secretion of enterohormones.

Supported by: INSERM, UPMC, APHP, ICAN Foundation

Disclosure: **A. Ribeiro:** None.

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Endogenous glucagon-like peptide-1 mediates the lowering of glycaemia during small intestinal glucose infusion by bile acids in type 2 diabetes

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Background and aims: Bile acids are recognised to play an important role in glucose homeostasis. We have reported that small intestinal administration of taurocholic acid (TCA) reduces the glycaemic response to intrajejunal (IJ) glucose infusion markedly in healthy humans, associated with an increase in plasma glucagon-like peptide-1 (GLP-1). We have now evaluated the effect of TCA, with and without the GLP-1 receptor antagonist, exendin(9-39), on the glycaemic response to an IJ glucose infusion in patients with type 2 diabetes (T2DM).

Materials and methods: 10 T2DM patients (age 69.5 ± 2.9 years; 8 male; BMI 26.6 ± 0.9 kg/m^2; HbA1c 6.6 ± 2.2% (48.9 ± 2.3 mmol/mol); duration of known diabetes 13.6 ± 3.4 years), managed by diet or metformin alone, were each studied on four study days, separated by at least 7 days, in a double-blind, randomised fashion. On each day, an IJ catheter was positioned and a balloon inflated 30 cm beyond the pylorus to allow proximal aspiration of endogenous bile. An intravenous (IV) infusion of exendin(9-39) (600 pmol/kg/min) or 0.9% saline was commenced and maintained during t = -60-120 min. TCA (2 g in 0.9% saline), or saline, was given via IJ infusion during t = -30-0 min, followed by 2 g TCA or saline, together with 60 g glucose, during t = 0-120 min. Blood glucose and plasma insulin, C-peptide and glucagon were measured at regular intervals. The insulin secretion rate (ISR)/glucose ratio was calculated, based on C-peptide and blood glucose. Incremental areas under the curves (iAUCs) for these measures during t = -60-120 min were compared using two-factor repeated measures ANOVA, with TCA and exendin(9-39) as factors. Subgroup comparisons between TCA and control, with and without exendin(9-39), were also performed.

Results: TCA reduced blood glucose (P = 0.022, treatment effect), and increased plasma insulin (P = 0.007) and the ISR/glucose ratio (P = 0.037), without affecting plasma glucagon. In contrast, exendin(9-39) was associated with higher blood glucose (P = 0.003) and plasma glucagon (P = 0.011), and reductions in plasma insulin (P = 0.008) and the ISR/glucose ratio (P < 0.001). In the absence of exendin(9-39), blood glucose was lower (P = 0.010), and plasma insulin (P = 0.025) and the ISR/glucose ratio (P = 0.039) were greater, with TCA vs. control, without any difference in plasma glucagon. In the presence of exendin(9-39), plasma insulin was greater with TCA vs. control (P = 0.020), without any difference in blood glucose, the ISR/glucose ratio, or plasma glucagon.

Conclusion: In well controlled T2DM, small intestinal administration of TCA reduces the glycaemic response to IJ glucose, associated with an increase in insulin secretion, and these effects are attenuated by exendin(9-39). These observations support the concept of a "bile acid-GLP-1" axis in the regulation of postprandial glycaemia in T2DM.

	IV saline		IV exendin(9-39)		P-value		
	IJ saline	IJ TCA	IJ saline	IJ TCA	TCA	Exendin(9-39)	Interaction
Glucose iAUC (mmol/L × h)	8.8 ± 0.7	6.9 ± 0.7 *	9.8 ± 0.7	9.8 ± 0.8	0.022	0.003	0.033
Insulin iAUC (mU/L × h)	21.1 ± 2.8	30.3 ± 3.9 *	14.2 ± 3.3	20.5 ± 3.8 *	0.007	0.008	0.446
Glucagon iAUC (pg/mL × h)	3.3 ± 6.3	7.1 ± 8.2	24.4 ± 10.5	46.2 ± 15.3	0.125	0.011	0.070
ISR/glucose ratio ((pmol/kg/min)/(mmol/L) × h)	0.4 ± 0.1	0.7 ± 0.2 *	0.2 ± 0.1	0.3 ± 0.1	0.037	<0.001	0.157

Data were analysed using two-factor repeated measures ANOVA, with TCA and exendin(9-39) as factors. Data are means ± SEM.

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GLP-1 receptor agonist impact gut microbiota and intestinal immunity to improve hyperglycaemia and dyslipidaemia in rodents

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Background and aims: Recent evidence demonstrates the role of GLP-1 therapies on immune-metabolism. Since a change in gut microbiota is a feature controlling immune-metabolism, we aimed to demonstrate that GLP-1 receptor agonists liraglutide and exendin-4 (ex-4) control glycemia through their impact on gut microbiota and immune-metabolism. This gain of knowledge would help identify discriminant therapeutic features

Materials and methods: DIO mice were treated i.p. with vehicle, ex-4 10μg/kg BID or liraglutide 100μg/kg QD and drug effects were evaluated after 14 days of treatment. The intestinal immune system was studied on isolated lamina propria cells and the gut microbiota through targeted 16S sequencing. Gut microbiota was used to colonize germ free mice. The immune cell phenotype was identified by flow cytometry.

Results: Compared with vehicle, ex-4 induced a significant body weight loss (-3.6g) after treatment, while liraglutide showed a more pronounced effect (-6.2g). Ex-4 showed better glycemic control over liraglutide after an oral glucose tolerance test, which was due to stronger impact on gastric emptying (as measured with acetaminophen oral administration). However, following a test meal, both ex-4 and liraglutide significantly reduced blood glucose by 40% and triglycerides levels by 25%. Ex-4 significantly reduced intestinal cholesterol absorption by 40% after a ^{14}C-cholesterol labeled olive oil oral gavage, while liraglutide showed a more pronounced effect (50% reduction). Fecal cholesterol mass excretion was significantly increased by 62 and 75% with ex-4 and liraglutide, respectively. Gut microbiota taxonomic profiling further discriminated ex-4 and liraglutide with significant changes in various bacteria taxa, such as bifidobacteriaceae, clostridiaceae or lactobaciliceae. The intestinal immune system was oriented towards a dramatic reduction of the frequency of Th1-inf$^+$ cells (80%), no change in Th17 cells, and an increased frequency of TReg cells (60%). All these effects were abolished by antibiotic treatment suggesting a role of gut microbiota. We then demonstrated in germ free colonized mice that the architecture of the microbiota from liraglutide treated mice was responsible for the glycemic control

Conclusion: ex-4 and liraglutide differentially alter gut microbiota and intestinal adaptive immune system. The changes in gut microbiota improves intestinal adaptive immune system and explain, at least in part, the antidiabetic effect of the peptide. The DIO mouse model, combined with our in vivo phenotyping knowhow in microbiota sequencing, and intestinal immune system expertise represent useful tools to differentiate anti-diabetics, and demonstrate their benefits beyond glucose control.

Disclosure: **R. Burcelin:** None.

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Effect of acute elevations of plasma NEFA on incretin-stimulated insulin secretion

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Background and aims: Plasma nonesterified fatty acids (NEFA) are an important source of energy during both fasting and postprandial periods but their excess could have an inhibitory effect on insulin secretion. Information on the *in vivo* effects of NEFA on incretin-stimulated insulin secretion is lacking. We aimed to assess the effects of acute hyperlipidaemia on incretin-stimulated insulin secretion in healthy subjects.

Materials and methods: Ten healthy subjects aged 34 ± 3 years (mean ±SD), BMI 24 ± 2 kg/m^2 were studied in two sets of experiments. First, each subject received a 75-g oral glucose tolerance test (CT-OGTT) and, a week later, the glycaemic profile from CT-OGTT was matched with an isoglycaemic intravenous glucose infusion (CT-IIGT). Both tests were then repeated during a 20% Intralipid infusion (60 mL/hour) plus a primed-(200 U)-continuous (0.4 U·kg^{-1}·min^{-1}) heparin infusion started 2 hours before either test (L-OGTT and L-IIGT, respectively). The dynamics of insulin secretion were assessed by mathematical modelling of plasma glucose and C-peptide responses. The main parameters are: insulin secretion rate (ISR); glucose sensitivity (ßGS), *i.e.,* the mean slope of the insulin secretion/plasma glucose dose-response curve; glucose-induced potentiation (P_{GLU}), which represents a time-dependent modulation of the dose-response during IIGI; and incretin-induced potentiation (P_{INCR}), which quantifies the time-course of the incretin effect. The incretin effect (IE) was also indexed as the oral-to-IV ratio of insulin secretion.

Results: Plasma NEFA rose from 0.19 ± 0.03 to 3.55 ± 0.53 mmol/L on the L-OGTT, and from 0.23 ± 0.03 to 3.77 ± 0.45 mmol/L on the L-IIGT (p<0.005 for both). Plasma triglycerides increased from 0.7 ± 0.1 to 3.2 ± 0.6 mmol/L during L-OGTT and from 0.6 ± 0.1 to 3.2 ± 0.5 mmol/l during the L-IIGT (p<0.005 for both). While lipid infusion raised plasma glucose levels only marginally (p=ns), it increased total ISR from 61 [26] to 78 [31] nmol·m^{-2}, median [IQR] on the OGTT (p=0.005), and from 29 [26] to 57 [30] nmol·m^{-2} on the IIGT (p=0.02). While neither ßGS nor P_{GLU} was significantly affected (p=ns), P_{INCR} decreased from 1.6 [1.1] to 1.3 [0.2] dimensionless, p=0.05. Moreover, during Intralipid infusion IE was significantly decreased between 0-90 min (p=0.001). The time-courses of GLP-1 and GIP did not change significantly, while the plasma ghrelin response to the OGTT declined from 12.3 ± 2.6 to 8.2 ± 1.2 ng/mL, p=0.005. Lipid infusion also induced mild insulin resistance during the OGTT (as the oral insulin sensitivity index [OGIS] decreased from 402 ± 22 to 355 ± 28 mL·min^{-1}·m^{-2} p=0.05).

Conclusion: An acute increment in plasma lipids stimulates total insulin secretion but selectively impairs incretin-stimulated insulin secretion.

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GIP(3-30)NH$_2$ is an efficacious GIP receptor antagonist in humans

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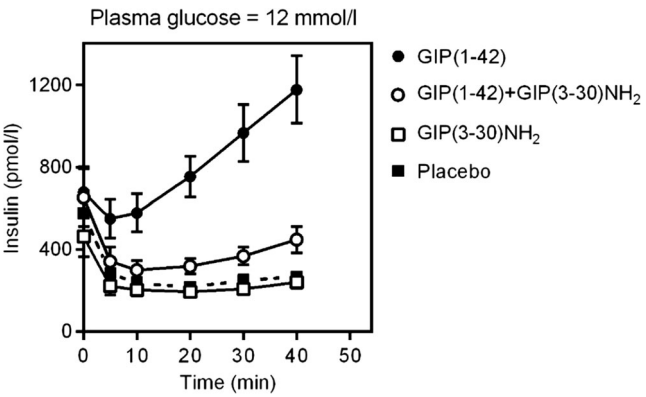
Background and aims: Glucose-dependent insulinotropic polypeptide (GIP) is an incretin hormone secreted from enteroendocrine K cells in the postprandial state. GIP stimulates insulin and glucagon secretion when blood glucose is high and low, respectively. In addition, GIP may inhibit bone resorption and stimulate fat deposition. Despite these therapeutically interesting targets, GIP physiology in humans remains relatively poorly characterised, mainly because until now, a GIP receptor (GIPR) antagonist (GIPRAn) has not been available for use in humans. We previously showed that a naturally occurring GIP fragment (GIP(3-30)NH$_2$) acts as a human GIPRAn *in vitro*. We aimed to establish GIP(3-30)NH$_2$ as a GIPRAn in humans.

Materials and methods: Insulin secretion was evaluated during four separate days in 10 healthy men (mean±SD: age 21.6±1.7 years, BMI 22.7±1.3 kg/m^2). They received a 45-minute infusion of the GIPRAn (800 pmol/kg/min), GIP (1.5 pmol/kg/min) and/or placebo during a hyperglycaemic clamp in a randomised, crossover design (day A: GIP; day B: GIPRAn; day C: GIP+GIPRAn; day D: placebo).

Results: To clamp plasma glucose levels on 12 mmol/l, we used significantly larger amounts of glucose (mean±SD: 54±14 g) on day A (GIP) compared to the three other days (B: 31±7.1 g, C: 37±5.1 g, and D: 39±7.3 g). Steady-state plasma levels of GIPRAn and GIP amounted to ~50 nmol/l and ~90 pmol/l, respectively. Elimination plasma half-life of GIP(3-30)NH$_2$ was 7.6±1.4 min (mean±SD). GIP-induced insulin secretion (day A, incremental $AUC_{0\text{-}40\ min}$ (mean±SEM): 29±3.4 nmol/l × min) was significantly (p<0.0001) lower on day C (GIP+GIPRAn) (12±1.7 nmol/l × min) corresponding to an inhibition of GIP-induced insulin secretion by the GIPRAn by 81±14% (mean±SD). The GIPRAn had no effect on insulin alone compared to placebo (day B: 6.6±1.1 nmol/l × min, day D: 8.1±0.9 nmol/l × min). There were no significant effects of GIP and/or GIP(3-30)NH$_2$ on glucagon, somatostatin, glucagon-like peptide-1 (GLP-1), triglycerides, cholesterol, glycerol or free fatty acids.

Conclusion: Supraphysiological levels of the naturally occurring GIP fragment, GIP(3-30)NH$_2$ robustly inhibits GIP-induced insulin release. Thus, GIP(3-30)NH$_2$ is efficacious GIPRAn in humans that can be used to investigate the physiological effects of GIP.



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OP 34 Nephropathy: from DNA damage to fibrosis

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Short leukocyte telomere length is associated with increased risk for albuminuria progression in type 2 diabetes in Asians

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Background and aims: Progression of albuminuria in type 2 diabetes (T2D) patients is associated with increased cardio-renal complications. Telomere length, a marker for biological aging is implicated with T2D. However, little is known on the association between leukocyte telomere length (LTL) and progression of albuminuria among T2D with preserved renal filtration function. The aim of this study was to investigate whether shorter LTL is associated with albuminuria progression in T2D with preserved renal filtration function (eGFR > 60mL/min/1.73m^2) in Asians.

Materials and methods: This 3-year prospective study included 568 nonprogressors and 123 progressor from participants in the SMART2D cohort. Baseline LTL was measured by real time polymerase chain reaction and expressed as the telomere repeat length (T) / copy number of a single-copy gene (S) ratio (T/S). Albuminuria progression was defined as a change in albuminuria category to a higher level.

Results: Progressors had significantly shorter median LTL compared to nonprogressors (0.58 [0.38-0.79] vs 0.62 [0.45-0.88], p=0.039). Compared to subjects with longer LTL (fourth quartile), subjects with shorter LTL (first quartile) had 1.93 (1.04-3.60, p=0.038) folds increased risk for albuminuria progression after adjustment for traditional risk factors. 1-SD decrement in the natural log transformed LTL was associated with a 25% (OR=1.25. 95% CI 1.01-1.53, p=0.037) higher adjusted odds for albuminuria progression. Sensitivity analysis showed that association of LTL with microalbuminuria to macroalbuminuria progression is stronger than its association with normoalbuminuria to microalbuminuria. Addition of LTL significantly improved the predictive performance of albuminuria as reflected by an increased category-free NRI of 0.428 (95% CI 0.243-0.613, p=1e-5) and IDI of 0.063 (95% 0.63-0.124, p=0.000).

Conclusion: Our results provide evidence that shorter LTL may predispose T2D patients to increased risk for early albuminuria progression.

Table 1. Combined and sub-group univariate and multivariate association between baseline LTL and progression of albuminuria

	Univariate OR (95% CI)	*P value*	Multivariate OR (95% CI)*	*P value*
Combined analysis (1-SD decrement)	**1.21 (1.02-1.43)**	**0.032**	**1.25 (1.01-1.53)**	**0.037**
Sub-group analysis				
normal to microalbuminuria LTL (1-SD decrement)	1.13 (0.91-1.40)	0.263	1.17 (0.93-1.47)	0.185
micro to macroalbuminuria LTL (1-SD decrement)	**1.39 (1.03-1.87)**	**0.032**	**1.54 (1.02-2.32)**	**0.042**

Notes: *Multivariate model analysed adjusting for age, gender, ethnicity, HbA1c, diabetes duration, BMI, TG, systolic blood pressure, uACR, eGFR and usage of RAS antagonist and statins. Bold values represents significant data.

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Disclosure: **R.L. Gurung:** Grants; Alexandra Health Enabling Grant (AHEG1622).

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Genetic deletion of the Set7 lysine methyltransferase attenuates renal damage in a mouse model of diabetic nephropathy

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Background and aims: Chronic hyperglycaemia promotes the production of pro-inflammatory and pro-fibrotic mediators that lead to the development of chronic kidney disease. Increasing evidence implicates epigenetic modifications, particularly histone methylation, in this process. Set7, a lysine methyltransferase that mono-methylates lysine 4 on histone H3 as well as and non-histone proteins, has been associated with increased expression of pro-fibrotic genes in various models of kidney disease. The aim of this study was to further define the role of Set7 in the development of diabetic nephropathy and evaluate it as a target for developing reno-protective therapies in diabetes.

Materials and methods: Diabetes-induced structural and functional kidney damage was studied in *ApoE*$^{-/-}$ and *Set7*$^{-/-}$*ApoE*$^{-/-}$ male mice compared to non-diabetic controls. Mice were euthanised after 10 weeks of streptozotocin-induced diabetes and kidneys were harvested for histological and molecular analyses including transcriptome sequencing (RNA-seq). Renal cell culture models were used for studying the effects of pharmacological inhibition of Set7.

Results: Set7 deletion conferred renal protection as evidenced by attenuated albuminuria, mesangial expansion and glomerular deposition of collagen I and IV in diabetic *Set7*$^{-/-}$*ApoE*$^{-/-}$ when compared to diabetic *ApoE*$^{-/-}$ mice. Global renal cortical gene expression by RNA-seq revealed that diabetes deregulates the expression of almost 5,000 genes in the kidney (adjusted p value<0.05) and that deletion of Set7 largely attenuates these changes in gene expression. Gene Set Enrichment Analysis (GSEA) showed that deregulated genes are associated with pathways related to inflammation, fibrosis and energy metabolism. This approach allowed us to identify novel targets for Set7 regulation in the diabetic kidney such as *Ncf1*, which encodes a NADPH oxidase subunit, and *Bmper*, a regulator of the bone morphogenic protein pathway. The changes in the expression of these genes, as well as known Set7 targets (*Ccl2/Mcp1*, *Fn1*, *Col4a1*, *Icam1*), were validated by qRT-PCR. *In vitro* experiments were used to investigate the effect of pharmacological inhibition of Set7 in specific renal cell populations. Exposure of cultured human podocytes and primary mesangial cells to high glucose and/or TGFβ1 increased the expression of pro-inflammatory (*CCL2/MCP1*, *RELA*) and pro-fibrotic genes (*ACTA2/SMA*, *VEGF*, *COL4A1*, *CTGF*), this was significantly attenuated in the presence of (R)-PFI-2, a potent and selective inhibitor of Set7.

Conclusion: Collectively, our results suggest that Set7 may represent a target for developing therapies aimed at reducing the burden of diabetic nephropathy.

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Disclosure: **H. Rodriguez:** None.

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The effect of transforming growth factor beta1 on cell-cell coupling and cell-to-cell communication in tubular epithelial cells of the diabetic kidney

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Background and aims: Efficient tubular function within the proximal nephron relies on inter-cellular communication between epithelial cells via connexin-mediated gap-junctions. In early diabetic nephropathy, a loss of epithelial (E)-cadherin mediated cell-adhesion instigates a series of events that culminate in disrupted cell-cell coupling, cell-to-cell communication and dissociation of the epithelia. In the current study, we utilized model epithelial cells from human renal proximal tubules, to demonstrate a role for the glucose associated beta1 isoform of the pro-fibrotic cytokine transforming growth factor (TGFβ1) on connexin expression, gap-junction intercellular communication and hemi-channel activity.

Materials and methods: Connexin-26 (Cx26) and connexin-43 (Cx43) expression were assessed by immunoblot analysis in human kidney (HK2) tubular epithelial cells treated with low glucose (5mmol/L) +/- TGFβ1 (10ng/mL) for 48hr or 7days. Whole cell paired-patch electrophysiology assessed junctional conductance between TGFβ1 treated HK2 cells, whilst

carboxyfluorescein (200μM) uptake determined hemi-channel opening at 48hr and 7day, with ATP bio-sensing measuring real-time nucleotide release.

Results: Immunoblot suggests a biphasic change in connexin expression following acute (48hr) and chronic (7day) exposure with TGFβ1 (10ng/mL). At 48hr, Cx26 was down regulated to 58.3±5.7% and Cx43 to 48.1±3.8% of control. Conversely, 7day exposure to the pro-fibrotic cytokine, increased Cx26 and Cx43 expression to 203.9±7.5% and 151.1 ±7.1% respectively (n = 4; P<0.001). Despite time-dependent differences in connexin expression, gap junctional intercellular communication (GJIC) decreased from 4.5±1.3nS (n=5) between control cells to 1.15±0.9nS (P<0.05, n=5) and 0.42±0.2nS (P<0.05, n=5) in cells treated with TGFβ1 at either 48hr or 7day. Removing extracellular calcium opens connexin-mediated hemi-channels and allows uptake of the membrane-impermeant dye carboxyfluorescein. Dye uptake in to TGFβ1-treated cells increased to 346±33% compared of control at 48hr and 430±18% at day 7. These effects were negated by pre-incubation with the hemi-channel blocker carbenoxolone (200μM, 30mins; 110±5.4% of control at 48hr and 64±2.6% at 7day; P<0.001, n=3). ATP bio-sensing confirmed that the TGFβ1 increased ATP hemi-channel release (1.99±0.47μM compared to 0.29± 0.06μM, P<0.01, n=3).

Conclusion: The current study suggests that despite biphasic changes in connexin expression following acute (48hr) and chronic (7day) exposure to the glucose-dependant pro-fibrotic cytokine TGFβ1, hemi-channel mediated ATP release increases at the expense of GJIC as cells attempt to maintain cell-to-cell communication. Linked to fibrosis in the diabetic kidney, a switch favouring local increases in purinergic signalling, may actually exacerbate disease progression in diabetic nephropathy.

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Disclosure: **P.E. Squires:** None.

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A role for connexin mediated cell communication in fibrosis of tubular epithelial cells in the diabetic kidney

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Background and aims: In diabetic nephropathy, pivotal to early tubular injury is loss of expression of cell adhesion protein; epithelial (E)-cadherin. Co-localized with E-cadherin at sites of cell-cell contact, connexins (Cx) oligomerise into hexameric hemi-channels that can form gap-junctions. Intercellular adhesion is a pre-requisite for gap junction formation and in the absence of neighbouring cells, uncoupled Cx-hemi-channels permit local paracrine release of adenosine triphosphate (ATP). Recent studies link elevated ATP levels to increased tissue fibrosis in disease and altered Cx expression &/or function is linked to the development and progression of secondary complications of diabetes. The current study examines if glucose-evoked changes in Cx-mediated ATP release drive extracellular matrix (ECM) remodelling and increased fibrosis.

Materials and methods: Biopsy material was isolated from patients with diabetic nephropathy and stained for Cx-26 and Cx-43. Changes in expression of key candidate proteins were examined by immunoblotting in model epithelial cells from human renal proximal tubules (HK2) cultured in either low glucose (5mmol/L) +/- TGF-β1 (10ng/mL) or high glucose (25mmol/L) for 7days. Lastly to assess the effects of ATP, cells were cultured in non-hydrolysable ATPγS (1-100μM) for 48hrs. ELISA and cytokine arrays measured protein secretion (TGF-β1, Interleukin-6 (IL-6) and Beta Nerve Growth Factor (β-NGF)). Lastly, Carboxyfluorescein uptake assays determined hemichannel activity in control vs TGF-β1 treated (10ng/mL) HK2 cells.

Results: Cx-26 expression was significantly up regulated in biopsy material from patients with diabetic nephropathy, compared to normal control (102700 ±6226 versus 21030±4727; n=10, P<0.01). Similarly, Cx-43 expression increased to 116300±5908 as compared to control 21460±10920 (n=10, P<0.01). In response to high glucose (25mmol/L) treatment for 7days, HK2 cells increased TGFβ1 secretion to 994.4±43.6pg/ml compared to 5mmol/L glucose (334±14.9pg/ml; n=3; P < 0.01). Immunoblot analysis confirmed that TGFβ1 (10ng/mL) up-regulates expression of Cx-26 and Cx-43 to 203.9 ±7.5% and 151.1±7.1% respectively compared to control (n = 4; P<0.001). Dye uptake in TGFβ1-treated cells increased to 430±18% at day 7. Immunoblotting confirmed that ATPγs up-regulated Collagen I to 177±12%, 182±21%, and 187±21% (n=3 P<0.01) and Collagen IV to 233±26%, 344 ±18.5%, and 390±10% compared to control (n=3 P<0.001) at 1, 10 and 100 μM. In addition, ATPγs significantly increased expression of Fibronectin to 274±47%, 350±23%, and 433±81% of control (n=3 P<0.01) at 1, 10 and 100 μM. Array analysis of supernatant from ATPγs treated cells confirmed a significant increase in secretion of both IL-6 and β-NGF to 261±24 and 194 ±44 respectively compared to control (n=3 P<0.01).

Conclusion: Expression of Cx-26 and Cx-43 increased in biopsy material isolated from patients with diabetic nephropathy, changes corroborated in HK2 cells treated chronically with TGF-β1. This increased expression was linked to increased hemi-channel mediated ATP release. ATP increased whole cell expression and secretion of key of ECM components and pro-fibrotic markers, suggesting that increased hemichannel-mediated ATP release may exacerbate fibrosis in the diabetic kidney.

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Disclosure: **C.E. Hills:** None.

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Exposure to high glucose levels or starvation induces cellular tolerance towards harmful levels of the reactive dicarbonyl methylglyoxal in murine kidney cells

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Background and aims: Toxic metabolites, such as reactive carbonyl species (RCS) and reactive oxygen species (ROS), are inevitably produced during energy production and contribute to ageing and degenerative diseases. Whilst ultimately detrimental to a cell, these metabolites can also have the potential to be beneficial. Methylglyoxal (MG) is formed at an early stage in energy metabolism as compared to ROS, which are mainly produced from the respiratory chain at the end of energy production. MG accumulates with increasing glycolytic flux, which can be sensed by a cell, as a first indicator for rising metabolic stress. In yeast cells, we have previously shown that high glucose or low MG concentrations can initiate a complex defense response that protects against toxic levels of reactive metabolites. In this study we focus on whether a similar mechanism exists in mammalian cells and if it can also be activated by short-term starvation, a treatment shown to be effective in type 2 diabetes.

Materials and methods: Murine mesangial and tubular epithelial cells were studied, cultured in low glucose (5mmol/l) conditions. Cell viability in response to MG, following either glucose starvation (1.5mmol/l) or pre-stimulation with high glucose (HG, 35mmol/l) was assessed by MTT assay. MG-induced protein aggregation was quantified by flow cytometry and fluorescence microscopy. Quantitative PCR and Western Blotting were used to analyze induction and expression of mediators and effectors of either the HG pre-treatment or starvation.

Results: Treatment with HG or starvation over 12 hours increased tolerance against MG in both cell lines. Furthermore, cellular MG-H1 concentrations that can ultimately lead to MG-induced protein-aggregation, were decreased in cells that were either starved or pre-treated with HG. This increased tolerance was associated by an up-regulation of the antioxidant stress response (Nrf2 & SOD2), as well as of the protein quality control system (Hsp70).

Conclusion: Short-term elevation of glucose levels as well as starvation induces cellular defense mechanisms that enable cells to handle reactive metabolites released during increased metabolic stress. Further studies are needed to identify the underlying signaling mechanisms. As RCS and ROS are involved in the development of diabetic late complications, this might lead to new therapeutic options for diabetic nephropathy.

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Disclosure: **J. Zemva:** None.

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Prediction of progression to microalbuminuria based on baseline metabolomic profile in patients with type 1 diabetes

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Background and aims: Biomarkers are important measures in explaining onset and progression of complex diseases. In previous studies, metabolomic profile panels have been shown to predict progression of diseases better than single biomarkers. In order to detect the onset and progression of diabetic nephropathy (DN), albumin excretion rate (AER) and estimated glomerular filtration rate (eGFR) are the most widely used measures. Here we performed an untargeted metabolomic study to assess baseline differences between patients with type 1 diabetes (T1D) who either progressed to microalbuminuria or who remained normoalbuminuric during the follow-up.

Materials and methods: Altogether 200 subjects were recruited from the Finnish Diabetic Nephropathy Study (FinnDiane) cohort. All patients had T1D and normal AER at baseline. Of these patients 102 progressed to microalbuminuria during follow-up, whereas 98 remained normoalbuminuric. The prospective metabolomic study was performed using the Metabolon platform. The metabolite concentrations were expressed as relative intensities and scaled to the median of 1. Machine learning based random forest (RF) analysis was performed with normalized metabolite concentrations. Odds ratios (OR) are presented as per standard deviation of the index.

Results: Metabolomic screening identified 1,242 peaks, of which 770 metabolites had known chemical composition. Progressors developed microalbuminuria during a median of 3.2 years. Baseline HbA_{1c} (OR 2.46 [1.65-3.82]), AER (2.29 [1.51-3.65]) and age of diabetes onset (2.03 [1.40-3.02]) were higher in progressors than in non-progressors. ROC_{AUC} for these clinical variables was 0.797. Of 30 metabolites selected by RF, 17 were named ones. An index score formed from these named metabolites resulted in ROC_{AUC} of 0.841, and an OR of 6.85 [4.06-12.60]. When we formed an index by selecting only 5 of the top 30 metabolites from the biochemical groups of polyols (erythritol), amino acid- and oligopeptide intermediates (γ-glutamylglutamate, cis-Cyclo[L-ala-L-Pro] and 3-phenylpropionate), and cortisone together with C-glycosyltryptophan (previously associated with DN but not within in our top 30 metabolites), the ROC_{AUC} for the progression index was 0.821 and the OR 4.57 [2.93-7.64].

Conclusion: The progression index based on the chosen panel of metabolites was able to predict the progression to microalbuminuria significantly better than age of diabetes onset, baseline HbA_{1c} or AER.

Disclosure: **J. Haukka:** None.

OP 35 Inflammation in obesity and type 2 diabetes: studies in animals and humans

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Plasma WISP1 is a marker of systemic and adipose tissue inflammation in subjects with type 2 diabetes

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Background and aims: WISP1 is a novel adipokine participating in adipose tissue (AT) dysfunction; so far, no data on WISP1 in diabetes are available. Aim of this study was to evaluate plasma WISP1 in subjects with type 2 diabetes for detecting its correlates linked to AT inflammation.

Materials and methods: For this cross-sectional study, 97 consecutive dysmetabolic subjects were recruited in our Diabetes outpatient clinics; among them, 71 had type 2 diabetes, with (n=35) or without (n=36) obesity, and 26 were obese non-diabetic subjects. Twenty-one normal weight non-diabetic individuals were enrolled as a control group. Study participants underwent clinical workup and blood sampling for metabolic/inflammatory characterization. Moreover, magnetic resonance imaging (MRI) data on subcutaneous (SAT) and visceral (VAT) AT area, hepatic fat content and VAT homogeneity were available for the majority of diabetic patients.

Results: Results: Plasma WISP1 significantly increased throughout classes of obesity and correlated with greater VAT area, IL-8, IL-6, TNFa and lower adiponectin levels, without differing between diabetic and non-diabetic subjects. Higher IL-8 was the main determinant of increased WISP1 (r^2= 0.58, p<0.001). MRI sequences highly suggestive of VAT inflammation were detected in 13.4% diabetic subjects and associated with higher WISP1, IL-8 and CRP independent of obesity. High WISP1 predicted VAT inhomogeneity with AUROC= 0.87 (p<0.001).

Conclusion: WISP1 levels are increased in obese subjects and directly related to adiposity, independently from glycemic status or insulin resistance; moreover, they tightly associate with increased plasma IL-8 and signal abnormalities of VAT. The overall data add new insights to the mechanisms underlying metabolic alterations and may open a novel scenario for innovative therapeutic approaches for diabetes prevention and care.

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Senescent $CD8^+$ T cells contribute to development of diabetes via enhancing hepatic inflammation and glucose production

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Background and aims: Chronic inflammation has an essential role in the pathogenesis of insulin resistance and diabetes. The aging of the immune system contributes to the development of chronic inflammation and has an important effect on metabolic disease. However, the role of immunosenescence of T cells in patients with prediabetes is not fully understood.

Materials and methods: The participants who visited the hospital for routine health check-up were divided into the following two groups: normal controls and prediabetes. Microarray was conducted to evaluate gene expression profiling in peripheral blood mononuclear cells (PBMCs) from normal controls and drug-naïve patients with diabetes. We have also analyzed the functional characteristics of senescent T cells by fluorescence-activated cell sorting (FACS) in patients with prediabetes and normoglycemia. Furthermore, we co-cultured senescent T cells with hepatocytes or islets to investigate the effect of senescent T cells on hepatic gluconeogenesis and glucose-stimulated insulin secretion, respectively. Finally, we transferred splenic $CD8^+CD28^{null}$ T cells ($1x10^6$) into the mice via

tail vein 3 times per week for 1 week for studying the role of senescent T cells on hepatic inflammation.

Results: Gene expression profiling of the PBMCs revealed elevated levels of the molecules such as CD44, CD69, CD83 and CTLA-4, which link to T cell activation. Gene annotation enrichment analysis based on DAVID algorithm showed that most of the upregulated genes in diabetes might be functionally responsible for T cell activation, phagocytosis, and inflammatory response. Patients with prediabetes also exhibit increased expression of pro-inflammatory cytokines in PBMCs. FACS analysis showed that there are more immunosenescent $CD8^+$ T cells in patients with prediabetes compared to subjects with normoglycemia. Pro-inflammatory cytokines and cytotoxic molecules are highly expressed in senescent $CD8^+$ T cells from patients with prediabetes. In ex vivo co-culture model system, the expression of G6pase and Pepck in hepatocytes was significantly increased by co-culturing with senescent $CD8^+$ T cells. Moreover, glucose-stimulated insulin secretion was reduced in co-cultured islets compared to controls. The mice injected with $CD8^+CD28^{null}$ T cells showed significantly enhanced composition of infiltrating monocytes ($Ly6C^{high}CD11b^+F4/80^{int}$) in the liver, but the population of hepatic resident macrophages ($CD11b^+F4/80^{high}$) was reduced. Populations of $CD4^+CD44^+CD62L^-$ and $CD8^+CD44^+CD62L^-$ (memory/effetor) T cells were not changed in the liver between the mice injected with $CD8^+CD28^{null}$ T cells and vehicles. However, $CD8^+CD44^-CD62L^+$ (naive) T cells were markedly decreased in the liver from the mice injected with $CD8^+CD28^{null}$ T cells.

Conclusion: Senescent $CD8^+$ T cells are more frequently observed in patients with prediabetes compared to normal subjects. Senescent $CD8^+$ T cells are also important for the development of hepatic inflammation and gluconeogenesis in mice.

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Disclosure: **H. Yi:** None.

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Diabetes, obesity and inflammation: persistence of elevated Il-1b after bariatric surgery

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Background and aims: A growing body of evidence suggests that interleukin-1β (IL-1β) is involved in obesity-associated inflammation and the pathogenesis of type 2 diabetes mellitus (T2DM). The aim of the present study was to correlate serum IL-1β levels with weight loss as well as changes in glucose metabolism and insulin resistance after bariatric surgery.

Materials and methods: Thirty-seven morbidly obese subjects with T2D (O-T2D) and 33 morbidly obese subjects without T2D (O), who had undergone Roux-en-Y gastric bypass (RYGB), received an oral glucose tolerance test (OGTT) before and 1 year after surgery. Eleven healthy individuals (C), age-matched to the Mo group, served as control group. Insulin sensitivity was assessed by oral glucose insulin sensitivity (OGIS) method. Fasting plasma IL-1β levels were measured at the time of OGTTs.

Results: At baseline, BMI was similar in O and O-T2D (46.5±5.6 *vs* 43.2±6.3 kg/m^2) groups, while being in the normal range in the C group (22.2±4.0 kg/m^2). Fasting plasma glucose and HbA_{1c} were higher in O-T2D, as compared to O and C ($p<0.05$ for both variables). Insulin sensitivity was progressively lower from C to O to O-T2D ($p<0.0001$). Fasting plasma IL-1β levels were significantly lower in C as compared to O and O-T2DM (2.4±0.6 *vs* 4.7±2.8 *vs* 4.9±1.9 pg/ml, p<0.004), and were positively related to BMI ($r=0.34$, $p=0.03$), mean glucose during OGTT ($r=0.34$, $p<0.02$), and HbA_{1c} ($r=0.69$, $p=0.0001$) and inversely related to insulin sensitivity ($r=0.37$, $p<0.02$). After surgery, BMI decreased to similar levels (32.5±5.9 and 31.1±5.6 kg/m^2, $p<0.0001$), and insulin sensitivity improved to a similar extent ($p<0.0001$), in O and O-T2D. In contrast, plasma IL-1β levels did not change after surgery (4.6±3.5 and 5.2±2.2 pg/ml, in O and O-T2D, respectively) and were not different from pre-surgery values. Based on fasting glycaemia and HbA_{1c}, at 1 year post-surgery T2D resolved in 20 of 37 patients. Plasma IL-1β concentrations did not differentiate regressors from non-regressors.

Conclusion: Plasma IL-1β concentrations are higher in very obese subjects regardless of the presence of diabetes, and are inversely related to insulin sensitivity. However, following surgery-induced major weight loss and improved insulin sensitivity, plasma IL-1β levels do not change, suggesting the persistence of a systemic inflammatory condition.

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Adipocyte specific deletion of integrin-linked kinase regulates lipid and glucose homeostasis in high fat diet-fed C57BL/6 mice

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Background and aims: ILK (integrin-linked kinase) as an adaptor protein, is a key component of the IPP (ILK-Parvin-PINCH) complex which associates with the β-subunit of integrin, linking the extracellular matrix (ECM) with intracellular signalling. Deletion of ILK in muscle or liver, respectively decreases insulin resistance in high fat diet (HFD)-fed mice. HFD-induced insulin resistance and obesity is tightly associated with adipose tissue (AT) remodelling and inflammation. We hypothesize that adipocyte-specific deletion of ILK diminishes insulin resistance in HFD-fed mice via regulating lipid and glucose homeostasis and inflammation.

Materials and methods: ILK was knocked out (KO) in AT using Cre transgenic mice under an adiponectin promoter. Both wildtype (WT) and KO mice were fed with 60% HFD for 16 weeks.

Results: Body weight, water and food intake, energy expenditure, respiratory exchange ratio and physical activity did not differ between WT and KO mice. The KO mice had decreased percent fat mass (21.6 ±1.6% vs. 27.3±1.4%; p=0.01) and increased percent lean mass (73.4 ±1.7% vs. 67.8±1.6%; p=0.02) than WT mice. Glucose tolerance was improved in the KO mice (2412±142 Area under the curve (AUC)) than WT mice (2921±70 AUC; p=0.003). In AT, the KO mice displayed a trend of decrease in expression of both anti- and pro-inflammatory genes including Arg-1, TNFα, IL-1β, IL-6, and collagen 24α with a significant decrease in IL-10 (p=0.02), whilst a trend of increase in adipogenic genes including PPARγ and CEBPα with a significant increase in fatty acid synthase (p=0.006) than WT mice.

Conclusion: In summary, we show that AT-specific ILK deletion in HFD-fed mice increases lean mass, decreases fat mass and improves glucose tolerance possibly via regulating inflammation-associated and adipogenic genes. These results further suggest a pivotal role of the ECM-integrin-ILK pathway in AT in adipogenesis and inflammation, which could contribute to obesity-associated insulin resistance and may represent as a therapeutic target.

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The TNF family member 4-1BBL is a key player in the pathogenesis of B cells driving obesity-induced insulin resistance by inducing cytotoxic $CD8^+$T cells in the peritoneal cavity

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Background and aims: The prevalence of obesity is increasing the risk of chronic inflammation, type 2 diabetes and fatty liver disease. In these conditions, resident B lymphocytes (B cells) in insulin-sensitive organs, such as adipose tissue (AT) and liver, are altered due to excess of glucose and fatty acids resulting in an accumulation of cytotoxic $CD8^+$T lymphocytes (CTL) and recruitment of M1 macrophages. This scenario favors sustained secretion of pro-inflammatory cytokines, specifically TNFα, which directly promotes insulin resistance (IR). The TNF

'superfamily' member 41BBL (alias TNFSF9) found in immune cells, liver and AT, plays a key role in sustaining TNFα secretion by interacting with its receptor, 4-1BB (TNFRSF9) a costimulatory molecule found in activated CTL, besides transducing signals on its own. Within the peritoneal cavity (PC), B1-a cells ($CD19^+CD5^+$) are known to be the most abundant B cells. In a tumor environment, B-1a cells expressing 4-1BBL (4BL cells) highly accumulate in the PC of old mice as inducers of CTL. Since IR is a key feature associated with obesity-induced low-grade inflammation, we hypothesize that 4BL cells play a key role in triggering the recruitment and activation of CTL. In this study, we aimed to investigate the role of PC infiltrating 4BL cells, as a source of TNFα, in driving IR and regulating glucose homeostasis in obese mice

Materials and methods: Eight week-old *C57BL/6* WT and *Tnfsf9*$^{-/-}$ mice on a *C57BL/6* background (4-1BBL KO) were placed on HFD for 24 weeks and compare to age-matched WT mice on SD. PC B cells ($8x10^6$) from different donors were adoptively transferred (i.p.) into recipient mice lacking mature B cells, J_HT (BKO), fed a HFD for 12 weeks. After 7 days, intraperitoneal glucose tolerance test and PC lymphocytes immune profiling were analyzed in recipient mice

Results: 4-1BBL KO-HFD mice showed improved insulin sensitivity ($p^*<0.001$), with a 1.5-fold reduction in liver size, when compared to their WT counterparts. Immune profiling of PC infiltrating immune cells indicated a 3-fold increase in 4BL cells secreting TNFα in WT-HFD when compare to WT-SD mice. Moreover, the number of B-1a cells secreting TNFα in obese 4-1BBL KO mice were reduced to half when compare to WT-SD and WT-HFD mice. Also, the number of CTL secreting TNFα were increased 2.3-fold in WT-HFD mice when compare to WT-SD mice, but only a 1.6-fold increase was observed in 4-1BBL KO-HFD mice. Likewise, CTL secreting IFNγ were augmented 2-fold in HFD, but only 1.6-fold in 4-1BBL KO mice. HFD caused a 10-fold increase in the PC infiltrating B cells ($CD19^+$) in both strains, when compared to SD. Interestingly, the concentration of the pro-inflammatory adipokine leptin, which upregulates B cell cytokine production and T cell responsiveness, increased more than 2-folds in the PC of WT-HFD mice when compared to SD, while only a 1.6-fold increase was observed in 4-1BBL KO-HFD mice. Recipient mice adoptively transferred with WT-HFD B cells had worse glucose tolerance and a 2-fold increase in the number of infiltrating CTL secreting TNFα, when compared to mice receiving WT-SD B cells or saline. In contrast, mice receiving B cells from 4-1BBL KO-HFD, had similar glucose tolerance as saline-injected mice

Conclusion: These findings point to the blockade of 4-1BBL as a promising therapeutic treatment against the deleterious effects of obesity, bringing new insights in the pathogenesis of PC 4BL cells driving IR

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Disclosure: **S. Santa Cruz Calvo:** None.

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Invalidation of the small GTPase Rab4b in T cells leads to adipose tissue inflammation and dysfunction, and insulin resistance

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Background and aims: Changes in adipose immune cells in obesity is involved in the inflammation of adipose tissue (AT) and in its impaired expandability leading to the development of insulin resistance (IR) and metabolic disease. However, the underlying mechanisms involved in this change remain not completely understood. A fine-tune control of intracellular trafficking is crucial for the function and fate of the immune cells. The Rab GTPases govern intracellular trafficking and we previously found that the expression of Rab4b, a key protein of the early/recycling endosomes, was decreased in the AT of obese mice and humans. We recently found a down-regulation of Rab4b in the adipose T lymphocytes, both in obese mice and humans. We thus aimed at determining the consequences of the down-modulation of Rab4b in T cells on AT function and glucose and lipid homeostasis.

Materials and methods: We generated mice with a specific invalidation of Rab4b in T cells ($Rab4b^{Tcell\ KO}$) by crossing $Rab4b^{flox/fox}$ mice with Lck-Cre mice. We analysed the metabolic phenotype of these mice fed a normal chow diet (NCD) or a high-fat diet (HFD) and characterized the cellular events underlying the phenotypic changes.

Results: On NCD, 35-week-old $Rab4b^{Tcell\ KO}$ mice have the same body and AT weight but exhibited a defect in AT expandability with hypertrophic adipocytes and an increase in pro-inflammatory macrophages compared to wild-type mice. Consequently, $Rab4b^{Tcell\ KO}$ mice exhibit ectopic lipid deposition in liver and muscles and are glucose intolerant and IR at this age. Those mice when fed a HFD, became as obese as wild-type but exhibited an exacerbation of IR. Young 10-week-old $Rab4b^{Tcell\ KO}$ mice have no metabolic disturbance but their AT contains more adipose mesenchymal stem cells and the secretome produced by this AT inhibits adipocyte differentiation. This secretome contains inflammatory cytokines, especially IL-17 and IL-6. Moreover, the lack of Rab4b in T cells induced a decrease in Treg and an increase in Th17 in the AT of 10-week-old $Rab4b^{Tcell\ KO}$ mice. This early inflammation of AT can explain the perturbation of AT expandability and the development of IR observed in 35-week-old $Rab4b^{Tcell\ KO}$ mice. The change in the balance between Treg and Th17 found in AT of 10-week-old $Rab4b^{Tcell\ KO}$ mice is a cell autonomous effect driven by the lack of Rab4b in T cells because ex vivo differentiation of Th0 from $Rab4b^{Tcell\ KO}$ mice is skewed towards Th17 whereas Treg differentiation is impaired.

Conclusion: This study uncovers a control of T cell fate by Rab4b and reveals that the loss of Rab4b expression in T cell during obesity contributes to the insulin resistance by promoting disturbance in adipose T cell homeostasis leading to inflammation and impaired expandability of AT.

Supported by: SFD

Disclosure: **M. Cormont:** None.

OP 36 Biomarkers

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IGFBP-3 is a marker of impaired adipose tissue function and a novel causal mediator of type 2 diabetes risk: a report from the ORIGIN study

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Background and aims: Obesity is a strong risk factor for type 2 diabetes (T2D), yet only 15% of obese individuals develop diabetes. There is currently no satisfactory explanation for this paradox and the mediators linking adiposity to T2D are incompletely understood. We hypothesized that genetically determined body mass index (BMI) can distinguish metabolically deleterious from neutral and beneficial BMI, and can help identify blood biomarkers mediating adiposity's metabolic effects.

Materials and methods: We partitioned 1.9 million genetic variants associated with BMI in the GIANT consortium into three categories according to genetic covariance with diabetes (DIAGRAM consortium): BMI-increasing variants linked to low T2D prevalence (20,255 SNPS) were assigned to the "beneficial" BMI genetic risk score (GRS); those linked to high T2D prevalence to the "deleterious" GRS (190,372 SNPs); with the remaining ones allocated to the "neutral" GRS (1,691,424 SNPs). We then assessed the association of each GRS with BMI itself, T2D and 237 cardiometabolic biomarkers in 4,147 participants from the ORIGIN trial with genetic material.

Results: All 3 GRSs were positively associated with BMI. However, the beneficial GRS was inversely associated with T2D prevalence (OR=0.50 [0.29-0.85] per 1 kg/m^2 increase, p=0.011) and the deleterious GRS was positively associated with T2D prevalence (OR=1.26 [1.07-1.50] per 1 kg/m^2 increase, p=0.006). No relationship was observed between the neutral GRS and prevalent T2D. Of the 237 biomarkers tested, only the adipokine Insulin-like Growth Factor-Binding Protein 3 (IGFBP-3) discriminated between genetically beneficial and deleterious BMI: IGFBP-3 levels were inversely associated with both the beneficial GRS (β= -0.27±0.07 SD per kg/m^2, p=0.0001) and the measured BMI (β= -0.01±0.002 SD per kg/m^2, p=4.9x10^{-07}). Moreover, after adjustment for clinical covariates, higher IGFBP-3 levels predicted a higher prevalence of T2D (OR=1.11 [1.03-1.20] per SD IGFBP-3 level, p=0.005); and a higher incidence of major adverse cardiovascular events (MACE) (HR=1.11 [1.05-1.18] per SD IGFBP-3 level, p=0.0005). Finally, when applying a Mendelian randomization approach to the DIAGRAM consortium, genetic determinants of higher IGFBP-3 levels were also linked to a higher prevalence of T2D (OR=1.26 [1.11-1.43] per SD IGFBP-3 level, p=0.0004).

Conclusion: Our data suggest that high IGFPB-3 concentrations reflect low "beneficial" BMI and may be a causal mediator of T2D. These results are consistent with *IGFBP3* transgenic mice, which exhibit fasting hyperglycemia and impaired glucose tolerance. Together, these data position IGFPB-3 as a marker of impaired adipose tissue function, as well as a potential therapeutic target.

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Disclosure: **M. Pigeyre:** None.

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Blood metabolites associate with glycaemic control in type 2 diabetes independent of glucose lowering medication

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Background and aims: To facilitate personalized therapeutics in type 2 diabetes patients, there is a growing interest to discover factors associated with poor treatment response. In a search of better markers we have used a targeted metabolomics approach to study the association of metabolites in blood with treatment and treatment response.

Materials and methods: Blood metabolites (n=164) were measured using a targeted approach (Nightingale Health, FI) in independent samples from four type 2 diabetes patient cohorts from the Netherlands (Hoorn DCS cohort, CODAM, NEO and Maastricht study, n=2653). We applied linear and logistic regression analysis with adjustment for age, sex, BMI, lipid lowering medication, glucose lowering medication (if applicable) and diabetes duration as covariates. We first analysed the effect of different drugs on metabolite levels. Next we examined associations of metabolite levels with HbA1c levels and with the ability to reach the clinical target of an HbA1c below 53 mmol/mol. In addition we used stratified analyses to examine the effect of different glucose lowering drugs on the association of metabolite levels and HbA1c. A Bonferroni adjusted threshold p-value was used to account for multiple hypothesis testing after meta-analysing the results of the four independent studies in a fixed effects model (164 tests, P<3.1x10^{-4}).

Results: Our results show that a substantial number of metabolites were affected differently by the various types of glucose lowering medication. Especially insulin treatment affected the metabolome (n=46 altered, all p≤3.1x10^{-4}). In addition a large number of metabolites were associated with HbA1c levels (n=80) or the ability to reach the treatment target (HbA1c <53 mmol/mol, n=63, all p≤3.1x10^{-4}). Most significantly associated metabolites were amino acids (Gln, Leu, Ile) and measures of lipoprotein subclasses (mainly VLDL). Interestingly the associations of metabolites with HbA1c were hardly affected by the different types of drugs these patients are treated with.

Conclusion: Blood metabolites are associated with treatment response in type 2 diabetes patients. These associations are independent of glucose lowering treatment regimen and other covariates.

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Disclosure: **L.M. 't Hart:** None.

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Metabolic phenotyping of first degree relatives of patients with type 1 diabetes

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Background and aims: Incidence rates of type 1 diabetes (T1D) are rising by more than 3% / year worldwide. New cases of T1D are rising by even 70% in children <15 years while the frequency of high risk genotypes is decreasing in newly diagnosed T1D cases. The risk for children to develop T1D is ~8% when they have a T1D affected sibling; it increases up to 20% if two first-degree relatives (FDR) have T1D. Recent studies suggest a profound influence of "diabetogenic" environmental factors in the pathogenesis of T1D, making early prediction of T1D development a topic of major interest. Metabolomics is a new tool to identify patterns of metabolites to detect early changes in people developing T1D. The advantage of metabolomics is, that metabolites fall downstream of genetic, proteomic and environmental variation, thus providing the most integrated phenotype measure. But since T1D is seen as a primarily immune mediated disease, metabolomics studies of T1D are scarce. This study aimed to identify metabolite patterns indicative of a high risk of developing T1D and consequently identify candidate biomarkers for early prediction of T1D. FDR with a 10-fold higher risk to develop T1D should

have characteristic metabolite patterns compared to matched groups of healthy and T1D patients.

Materials and methods: Three subgroups of the Austrian Diabetes Prevention Program cohort were selected: 19 newly diagnosed T1D patients (diabetes duration <3 months), 17 healthy FDR and 19 unrelated healthy controls (age, gender and BMI matched). The metabolomics plasma sample analysis was performed using HILIC-HRMS (sample extraction with cold 80% MeOH) for targeted and untargeted data analysis.

Results: The preliminary targeted analysis yielded 186 known metabolites covering all major classes with 156 metabolites showing the highest possible analytical quality (median technical variability 7.4%). Principal Component Analysis (PCA) of these 156 metabolites showed a distinct separation of patients with T1D from healthy controls. FDR clustered between T1D and healthy controls (Fig. 1, left). The PCA loadings plot showed differences in 14 metabolites among the three groups ($p<0.01$, Fig. 1, right).

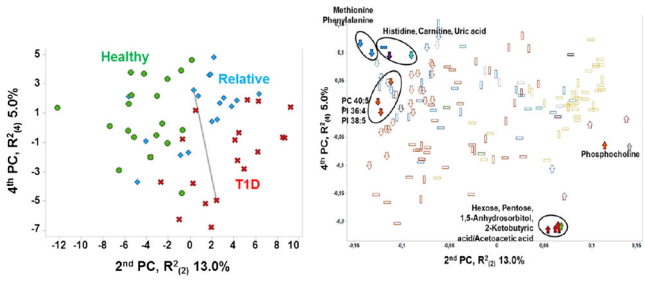

Figure 1: Selected PCA of 156 known metabolites, performed on logarithmic data (scaled to unit variance, centered). Left: PCA scores plot. The line indicates one direct relationship between a T1D patient and a first-degree relatives. Right: PCA loadings plot. Filled arrows: p-value <0.01; hollow arrows: p-value <0.05; up/down-arrows: higher/lower metabolite levels in T1D patients relative to healthy contro

Conclusion: In this study we were able to show that healthy FDR of patients with T1D showed distinct metabolic patterns correlating with the higher risk of developing T1D. We hypothesize that this metabolic pattern may be a biomarker for early diagnosis or prediction of T1D.

Disclosure: **E.E. Zügner:** None.

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Liver and plasma metabolomics on two mouse models with progressive diabetes development uncovers shared biomarkers for beta cell dysfunction in pre-diabetes

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Background and aims: Early diagnosis of beta-cell failure remains unsuccessful despite its crucial importance in the diabetes prevention. Identification of patients with beta-cell dysfunction in the pre-diabetic stage is therefore essential. In recent years, metabolomics emerged as a powerful tool in providing read-outs of early perturbations in diseases. We aimed to identify novel biomarkers for beta-cell dysfunction in the pre-diabetic stage of two distinct mouse models displaying progressive diabetes development independently of drug or diet treatments, i.e. db/db mice and beta-cell specific prohibitin-2 knockout mice (beta-Phb2$^{-/-}$).

Materials and methods: Liver and plasma were collected from 18 male C57BLKS/J db/db mice aged 4, 6 and 8 weeks, and 18 age-matched heterozygous db/+ controls. Same collection was also done from a total of 21 male beta-Phb2$^{-/-}$ mice aged 4, 5 and 6 weeks and 24 age-matched control mice. Total pancreas was fixed for immunohistochemistry quantification of beta-cell mass and islets were isolated for glucose-stimulated insulin secretion (GSIS). Metabolite profiling was performed by non-targeted flow injection-time-of-flight mass spectrometry (MS), complemented by targeted liquid chromatography (LC)-MS and gas chromatography (GC)-MS.

Results: At the age of 8 weeks and 6 weeks, respectively, db/db mice and beta-Phb2$^{-/-}$ mice began to display hyperglycaemia. Before the appearance of diabetes, both db/db mice and beta-Phb2$^{-/-}$ mice had a transient expansion of beta-cell mass, along with decreased GSIS from isolated islets. This was followed by the progressive loss of beta-cells and development of hyperglycaemia a few weeks later. However, unlike db/db mice with marked body weight gain during the pre-diabetic stage, beta-Phb2$^{-/-}$ mice maintained the same body weights as their age-matched controls. In the liver, cortisol increased in db/db mice during pre-diabetes, not in beta-Phb2$^{-/-}$ mice. Branched-chain amino acids (BCAA) increased in the liver of db/db mice, while they slightly decreased in beta-Phb2$^{-/-}$ mice, showing the association of BCAA with obesity and insulin resistance rather than with beta-cell dysfunction. Importantly, a specific group of deoxy sugars decreased in liver and plasma of the two mouse models at the pre-diabetic stage, showing strong correlation with the development of early asymptomatic beta-cell failure before the appearance of hyperglycaemia. These biomarkers continued the same trend after the loss of functional beta-cells in both db/db and beta-Phb2$^{-/-}$ mice.

Conclusion: The present study identified metabolite signatures specifically associated with early beta-cell dysfunction by comparing two diabetes mouse models. We also uncovered metabolic similarities between liver and plasma, providing insights into the systemic effects caused by early changes in beta cells in pre-diabetes.

Supported by: SNF Sinergia, SNF Doc Mobility

Disclosure: **L. Li:** None.

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Skin autofluorescence predicts 4-years risk of incident type 2 diabetes in a large scale general population

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Background and aims: The rising prevalence of type 2 diabetes mellitus (T2DM), accompanied with its cardiovascular complications, is a major problem worldwide. Screening of high-risk individuals might be beneficial as early treatment may delay the development into overt disease and its complications. In the present study, we examined whether the measurement of skin autofluorescence (SAF), a non-invasive biomarker of advanced glycation end products (AGEs) accumulation, is able to predict 4-years risk of T2DM in a large-scale, general population.

Materials and methods: For this prospective analysis, we included 80,253 participants of the Dutch Lifelines Cohort Study, who underwent baseline investigations between 2007-2013 and had a SAF measurement available. Individuals were diagnosed with incident T2DM in 2014-2016 by self-report or by a fasting blood glucose >6.9 mmol/l or HbA1c ≥6.5% at 4-years of follow-up. SAF was measured non-invasively at baseline using the AGE Reader. Subjects were divided into quartiles of SAF (quartile 1=low; quartile 4=high). Odds ratio (OR) for pre-specified risk factors were calculated.

Results: After 4 years of follow-up (range 1-9 years), 995 subjects had developed T2DM (1.2%). Baseline SAF was 2.19 ± 0.45 arbitrary units (AU) in T2DM and 1.91 ± 0.43 AU in subjects without diabetes ($p<0.01$). Univariate factors predicting incidence of T2DM were SAF, age, gender, BMI, waist circumference, fasting glucose, HbA1c, current smoking and coffee consumption. After adjusting for these common risk factors, SAF remained associated with a higher risk of T2DM (OR 1.27 per AU; 95% CI 1.06-1.52, $p<0.01$), but not after further adjustment for HbA1c. Individuals with a high SAF level at baseline had an increased risk of T2DM compared to subjects with a low SAF level (OR 1.37 per AU; 95% CI 1.03-1.81, $p<0.05$). For individuals in the third

and second SAF quartile, SAF was not associated with incident T2DM (p=0.27 and p=0.91).

Conclusion: SAF is elevated in subjects with incident T2DM compared to individuals without diabetes. Individuals with a high SAF level have an increased risk of T2DM after 4 years of follow-up compared to subjects with a low SAF level, even after adjusting for other conventional risk factors.

Disclosure: **R.P. van Waateringe:** None.

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Plasma N-glycans and C-reactive protein as biomarkers of HNF1A-MODY in young adults

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Background and aims: HNF1A-MODY is frequently misdiagnosed due to lack of accepted diagnostic protocols and limited availability of genetic testing. Benefits of making the diagnosis include first-line treatment with sulphonylureas and screening of relatives. While absence of islet antibodies and preserved insulin secretion can differentiate MODY from type 1 diabetes, non-genetic biomarkers are not routinely available to differentiate from type 2 diabetes in adults, and this remains challenging. We previously showed that defects in key processes regulated by HNF1A result in a distinctive plasma N-glycan profile and lowered CRP in HNF1A-MODY. We aimed to evaluate these agents in identifying those at high risk of HNF1A-MODY in an unselected population of young adults with antibody negative, C-peptide positive diabetes.

Materials and methods: Individuals (n=1032) with diabetes onset below 45 years were recruited in the UK and Croatia. N-glycans were analysed using Hydrophilic Interaction Liquid Chromatography-UPLC and profiles were divided into 38 glycan groups (GP1-GP38) with glycan structures assigned. HsCRP was measured by immunoassay. Sanger sequencing of HNF1A was performed and rare HNF1A variants (MAF&lt1%) underwent a systematic assessment of their functional effect.

Results: Twenty-five rare HNF1A variants were found in 29 probands. Fourteen variants were previously reported as disease causing, of which 12 had familial co-segregation of the variant allele with diabetes. Seven variants were protein truncating and assumed to be functionally damaging, while 18 were missense. Eight of the missense variants were predicted by three bioinformatics tools as likely damaging. In vitro functional studies reported before, or performed in this study, in 14 variants supported disease causality of the variant allele in 6 cases. Thirteen out of 25 variant alleles were present in the ExAC database with minor allele frequency (MAF) of 0.001-0.1%. After the above assessment, we assigned 13 variant alleles, found in 18 individuals, as HNF1A-MODY-causing. The glycan groups GP30, GP36 and GP38, all containing fucosylated glycans, showed good discriminative power between HNF1A-MODY and those without variants (p&lt5x10^{-8}, C-statistics of 0.86-0.90). Glycan group GP30 had the best performance, with sensitivity of 89% and specificity of 76%. Using cut-off of 0.73, GP30 detected 16 out of 18 patients with damaging HNF1A alleles. Median hsCRP of those with damaging HNF1A variants was 0.30 mg/L (IQR 0.86) versus 2.20 mg/L (IQR 4.78) in the remainder (p=2.4x10^{-5}). HsCRP had a C-statistic of 0.82 in classification performance, and using a cut-off of 0.9 mg/L, showed sensitivity of 78% and specificity of 73% and identified 14/18 probands with damaging HNF1A variant alleles.

Conclusion: Fucosylated glycans and hsCRP are good discriminative markers for HNF1A-MODY among young adults with non-type 1 diabetes. Use of these markers in diagnostic protocols could assist in identifying adults at high risk of HNF1A-MODY.

Supported by: EFSD/Novo Nordisk; Diabetes UK; Oxford NIHR BRC

Disclosure: **O. Gornik:** None.

OP 37 Insulin secretion in vivo

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Kisspeptin: a novel regulator of glucose homeostasis during pregnancy

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Background and aims: During pregnancy, the maternal pancreatic islets of Langerhans undergo adaptive changes in order to compensate for the normal progression of insulin resistance that occurs during gestation. Recently placentally-derived kisspeptin (KP) and its cognate receptor, G protein-coupled receptor-54 (GPR54), have been suggested to play a role in the regulation of these adaptive changes and maintaining glucose tolerance during pregnancy. Although global GPR54 knockdown mice have previously been established, they are unsuitable for studying the role of KP during pregnancy because they do not progress through puberty and lack adult reproductive function due to effects of KP within the hypothalamus. Consequently, we have generated a novel, beta cell-specific GPR54 knockout (KO) mouse model in order to investigate the effects of KP on glucose homeostasis during pregnancy.

Materials and methods: Transgenic targeting of GPR54 specifically within the islet beta cells was achieved by crossing MIP-Cre/ERT^{1Lphi} mice with LoxP-flanked GPR54 animals and beta cell-specific deletion of GPR54 was induced by injection of i. p. tamoxifen (TMX, 75 mg/kg body weight). Islet GPR54 knockdown was confirmed by quantitative PCR and immunohistochemistry, and normal reproductive function was confirmed by analysis of vaginal smears. Following GPR54 knockdown and TMX washout, female mice were mated and i. p. glucose (30% glucose/kg body weight) and insulin tolerance tests (0.75 IU/kg body weight) were performed at gestational days 16 and 18 respectively. Mice of identical genotype but without TMX administration (Cre controls) and Cre negative mice administered tamoxifen (TMX controls) were used as controls. Characterization of insulin secretion from islets isolated from beta cell-specific GPR54 knockdown and control animals exposed to stimulatory glucose (20 mmol/l) together with exogenous KP (1 μmol/l) were assessed by dynamic perifusion.

Results: Tamoxifen administration induced a 60% knockdown in beta cell *GPR54* mRNA expression (beta cell GPR54 KO: 0.69±0.17 x10^{-3}, Cre control; 1.79±0.41 x10^{-3} relative to *beta-actin*, n=3). Both male and female mice demonstrated no adverse phenotype and normal reproductive function. In pregnant mice, GPR54 knockdown resulted in significantly impaired glucose tolerance (AUC glucose excursion in beta cell GPR54 KO vs Cre controls; 482.8±54.0 vs 306.8±44.5; n=4-7, p=0.027) but no change in insulin tolerance (AUC glucose excursion in beta cell GPR54 KO vs Cre controls; 405.7±10.7 vs 400.9±28.9; n=4-7), when compared to controls. Similar effects were seen when compared to TMX controls, confirming that this effect was due to GPR54 knockdown. Islets isolated from beta cell-specific GPR54 knockdown mice demonstrated normal glucose-stimulated insulin secretion but an impaired incremental response to the addition of exogenous KP, when compared to control islets (AUC insulin excursion in response to KP, beta cell GPR54 KO: 49.9±8.2 pg/islet/20min, Cre controls: 80.3±34.0, TMX controls 98.7 ±14.3; n=4, p<0.05 beta cell GPR54 KO vs TMX controls).

Conclusion: These data validate the hypothesis that KP could represent a novel and important placentally-derived signal that plays a physiological role in the islet adaptation to pregnancy and helps to maintain normal maternal glucose homeostasis by acting through the beta cell GPR54 receptor.

Supported by: Wellcome Trust

Disclosure: **T.G. Hill:** None.

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Reduced production of isoprostanes by the peripancreatic adipose tissue from Zucker fa/fa rats as a new mechanism for beta cell compensation in insulin resistance and obesity

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Background and aims: In type 2 diabetes, ectopic fat deposits develop in parallel with visceral adipose tissue and participate to the onset of metabolic disorders. Among them, peripancreatic adipose tissue (PPAT) is increased in obesity and infiltrates the pancreas to establish direct contact with pancreatic islets. Interestingly, we have shown that factors secreted by the PPAT can modulate both function and proliferation of β-cells, what is in favor of a local biocommunication between PPAT and pancreatic islets. Among them, F_2-isoprostanes (F_2-IsoPs) are oxygenated metabolites derived from the non-enzymatic peroxidation of arachidonic acid, mainly present in phospholipids of cellular membranes. Our aim was to investigate whether IsoPs released by the PPAT could be modulators of β-cell function and proliferation, to influence its ability to compensate for insulin resistance.

Materials and methods: We used the Zucker fa/fa rat model, which displays hyperinsulinemia, insulin resistance and obesity, as a consequence of hyperphagia. Secretome from the PPAT was obtained after 24h culture in RPMI medium. Pure IsoPs were obtained by total synthesis, and were quantified by liquid chromatography-mass spectrometry after an initial solid phase extraction. Secretome or IsoPs were then incubated with INS-1 cells or isolated islets from Wistar rats and insulin secretion or proliferation were measured.

Results: When INS-1 cells were cultured 24h with the PPAT secretome from fa/fa rats, we observed an increase in glucose-induced insulin secretion *versus* lean (NS) and Wistar rats (P<0.05), along with an enhanced proliferation of 40 % (P<0.05). We then asked whether changes in local production of IsoPs by PPAT could be responsible for the observed effects. In PPAT secretome from fa/fa rats, the concentration of 5-F_{2t}-IsoP and 15-F_{2t}-IsoP reached respectively 18.41 ± 2.42 and 4.23 ± 0.61 ng/ml *versus* 135.28 ± 42.16 and 53.89 ± 19.1 ng/ml in lean *fa/+* rats (NS), and 129.6 ± 18.26 and 49.27 ± 8,24 ng/ml in Wistar rats (P<0.001). At the opposite, we observed an increased concentration of 5-F_{2t}-IsoP in plasma from fa/fa rats (10.11 ± 1.25 ng/ml) *versus* lean (3.86 ± 0.32 ng/ml; P<0.001) and Wistar rats (5.28 ± 0.29 ng/ml; P<0.01), as well as that of 15-F_{2t}-IsoP (1.44 ± 0.23 in fa/fa rats *versus* 0.57 ± 0.044 in lean (P<0.01) and 0.83 ± 0.072 ng/ml in Wistar rats (P<0.05)). Finally, we evaluated the effects of synthetic IsoPs on insulin secretion in isolated islets from Wistar rats. After a 24h-incubation period, 15-F2t-IsoP and its 15 epimer decreased glucose-induced insulin at the concentration of 10 μM by respectively 42.8 ± 5.3% (P<0.001) and 30.7 ± 6.5% (P<0.01), but not at lower concentrations.

Conclusion: PPAT secretome obtained from obese Zucker rats stimulated both insulin secretion and proliferation of β-cells. Part of this effect could be related to a decreased production of 5-F_{2t}-IsoP and 15-F_{2t}-IsoP by the PPAT, inhibiting insulin secretion at high concentrations. These data possibly highlight a new mechanism for β-cell compensation in insulin resistance and obesity.

Disclosure: **J. Laget:** None.

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Impaired beta cell function predicts the islet functional capacity following partial pancreatectomy

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Background and aims: Several lines of evidence suggest that β-cell functional defects temporally anticipate (and eventually cause) the subsequent development of hyperglycemia. Still, a significant reduction (~50%) of beta-cell mass is observed at diagnosis of type 2 diabetes.

Materials and methods: To further investigate the relative role of beta-cell mass reduction/dysfunctionin the development of diabetes we performed oral glucose tolerance tests (OGTT) and hyperglycemic clamps (HC), followed by arginine stimulation, in 22 patients undergoing pancreatoduodenectomy (PD), pre- and post-surgery. Based on post-surgery OGTT, subjects were divided into 3 groups depending on glucose tolerance: normal (NGT, n=6), impaired (IGT, n=6) or diabetic (DM, n=10) (12 F/10 M, 51±15 yrs.). To evaluate β-cell function, β-βcell glucose sensitivity (GS) during HC was calculated as the ratio of insulin secretion and glucose increments.

Results: Before surgery, Arginine-stimulated Insulin Secretion rates (AIS) were similar across groups, whereas GS and incremental 1st phase insulin secretion were significantly lower in IGT and DM as compared with NGT subjects (p=0.02 and p<0.01). Following PD, AIS decreased in all patients (p<0.05 for all groups) but the reduction was greater in DM compared to IGT and NGT patients (p<0.001). A similarly scaled reduction was observed in 1st and 2nd phase of insulin secretion (p=0.03 and p<0.01) and in GS (p<0.01). Compared to baseline secretion, only DM group experienced a significant reduction in 2nd phase insulin secretion (348±118 vs. 105±40.3 pmol·min^{-1}·m^{-2}, p=0.05) and in GS (34.4±11.3 vs.13.1±6.13 pmol·min^{-1}·m^{-2}mM^{-1}, p=0.01).

Conclusion: In this study the acute beta-cell mass reduction had a different impact on insulin secretory capacity, despite comparable functional mass at baseline, according to pre-surgery insulin secretion characteristics. This suggests that underlying beta-cell dysfunction anticipates the decline of beta-cell responses, being the pivotal mechanism for the development of hyperglycemia.

Clinical Trial Registration Number: NCT02175459

Disclosure: **C.M.A. Cefalo:** None.

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Overexpression of c-Kit in aged mice stimulates beta cell proliferation, but leads to impaired beta cell function

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Background and aims: Activation of the c-Kit receptor on β-cells regulates intracellular pathways that enhance insulin secretion, up-regulation of the insulin receptor (IR), β-cell proliferation and survival, and islet vascularization. c-Kit overexpression in β-cells is able to improve glucose levels in mice up to 28 weeks of age and on a short-term high fat diet. However, the prolonged effect of c-Kit activity on sustained insulin release from β-cells or on overall β-cell function has not been previously examined. This study determines if the long-term overexpression of c-Kit on murine β-cells maintains glucose homeostasis through increased insulin release.

Materials and methods: We generated mice with a β-cell specific c-Kit overexpression (*c-KitβTg*) and monitored aged males to 60 weeks compared with wild-type (WT) littermates. Metabolic tests (IPGTT, IPITT, *in vivo* and *ex vivo* GSIS) were performed to measure glucose tolerance and insulin release. Islet histology and isolated islet protein were analyzed to examine intracellular signalling, proliferation, and expression of transcription factors and proteins that maintain β-cell function. To examine the effects of c-Kit-induced insulin resistance during aging, a β-cell specific, tamoxifen inducible IR knockout mouse line (*βIRKO*) was generated and crossed with *c-KitβTg* mice (*c-KitβTg:βIRKO*). IR loss was induced at 40 weeks of age and glucose metabolic tests were performed at 60 weeks.

Results: Aged *c-KitβTg* mice progressively developed high fasting blood glucose and demonstrated significant glucose intolerance (p<0.05) compared to age matched WT mice. The survival rate of *c-KitβTg* mice declined with aging over a two-year period. *In vivo* and *ex vivo* GSIS results revealed a reduction of insulin secretion from islets of *c-KitβTg* mice (p<0.05 vs WT). Histological analyses of *c-KitβTg* pancreatic islets found enlarged β-cell mass and high proliferation (p<0.05 vs WT), indicating that high glucose levels in *c-KitβTg* mice are not due to a loss of the β-cell population. Protein analyses from isolated islets showed an increase in MAPK/Erk/cyclin D1 signalling pathway in *c-KitβTg* mice (p<0.05 vs WT), with no change through Akt signalling between WT and *c-KitβTg* mice. Reduced syntaxin 1a and Munc18-1 levels were detected in aged *c-KitβTg* islets (p<0.05 vs WT), suggesting possible dysfunctions in insulin granule exocytosis. Preliminary studies have found that loss of β-cell IR in aged *c-KitβTg:βIRKO* mice resulted in normal glucose tolerance when compared to aged *c-KitβTg* mice.

Conclusion: This study shows that c-Kit activation maintains increased β-cell mass and proliferation in *c-KitβTg* mice over 60 weeks of age, yet chronic overexpression of c-Kit results in reduced insulin release and glucose intolerance as associated with the reduction of exocytotic proteins. The results from this study demonstrate that regulation of c-Kit signalling (time- and dose-dependent) is important for optimal β-cell function and insulin secretion.

Supported by: CIHR

Disclosure: **A.M. Oakie:** None.

OP 38 Lifestyle factors and diabetes

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The effects of ethnicity on the development of prediabetes among immigrant populations living in low vs high walkability areas: a population-based cohort study

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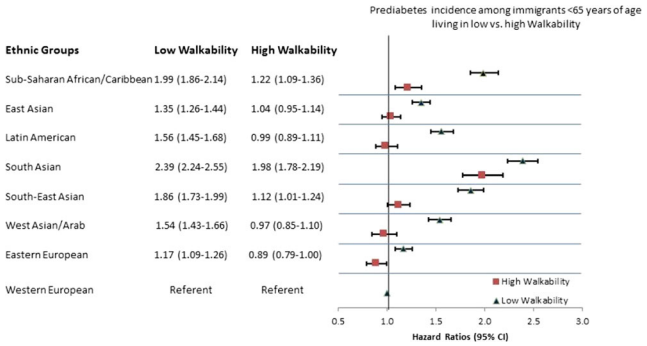
Background and aims: Evidence on urban environments have shown that immigrants living in less activity-friendly neighbourhoods are at a higher risk of developing diabetes compared to long-term residents. However, the association between neighbourhood walkability and prediabetes has not been studied. We conducted a population-based cohort study to examine whether walkability modifies the relationship between ethnicity and prediabetes among immigrant populations living in Canada.

Materials and methods: We used linked health, laboratory and immigration data for the province of Ontario, Canada to identify adults aged 20-85 who immigrated to Canada and were living in urban areas within Southern Ontario between Jan 1, 2002 and Dec 31, 2013. We used Cox proportional hazards modeling to examine the incidence of prediabetes across ethnic groups, stratifying by high and low walkability. Walkability was assigned to individuals using their postal code of residence at cohort entry. We previously found a threshold effect between walkability and diabetes; therefore, we divided walkability into two categories: high, based on the top quintile (Q5) and low, based on the bottom 4 quintiles (Q1-Q4).

Results: Our cohort consisted of 193,899 immigrants (mean age 40 years) of different ethnic origins including Sub-Saharan African and Caribbean (N=20,324), South Asia (N=38,441), South-East Asia (N=18,541) and Western European (N=14,227). The incidence of prediabetes was higher among all non-Western European populations after adjusting for age, sex and area income. However, these effects were more marked in low walkability neighbourhoods and reduced or eliminated in high walkability neighbourhoods (Figure 1). For example, the incidence of prediabetes among Sub-Saharan and African Caribbeans was twofold higher among those living in low walkability areas (HR: 1.99, 95%CI: 1.86-2.14, p<0.001) but only 1.2 times higher in high walkability areas (HR: 1.22, 95%CI: 1.09-1.36, p<0.001). Similarly, West Asian/Arab populations living in low walkability areas had a 1.5-fold higher incidence compared with Western Europeans (HR: 1.54, 95%CI: 1.43-1.66, p<0.001) but this association was not significant in high walkability settings. The interaction between ethnicity and walkability was less among South Asians (HR:2.39, 95%CI: 2.24-2.55, p<0.001 in low vs. HR:1.98 95%CI: 1.78-2.19, p<0.001 in high walkability areas). The effects of ethnicity on prediabetes development were less pronounced among adults aged 65+ living in both high and low walkability areas.

Conclusion: Neighbourhood designs may amplify the risk of prediabetes development among immigrant populations. Further research on interactions between socioeconomic, environmental and immigration factors are necessary to guide the design of population interventions and policies targeting prediabetes among high-risk populations.



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Disclosure: **G.S. Fazli:** None.

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Sodium intake and the risk of type 2 diabetes and Latent Autoimmune Diabetes in Adults (LADA)

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Background and aims: It has been suggested that salt (sodium chloride) may increase the risk of T2D, hypothetically through an effect on insulin resistance and/or by way of promoting hypertension and weight gain. Whether sodium intake is related to onset of autoimmune diabetes has not been investigated. However, experimental studies have shown that excessive sodium intake may initiate an autoimmune reaction by enhancing the production of T_H17 cells which are highly proinflammatory. We aimed to study, for the first time, whether sodium intake is associated with an increased risk of LADA.

Materials and methods: We used data from ESTRID (epidemiological study of risk factors for LADA and T2D), a Swedish population-based study which includes incident cases of LADA (n=355) and T2D (n=1136) from ANDIS/ANDiU, together with population-based matched controls (n=1379). Dietary intake reported in a food frequency questionnaire was used to calculate daily energy and nutritional intake, using standard values cited in the Swedish National Food Agency Database and taking into account age-specific portion sizes. Sodium intake was energy adjusted using nutrition density method. HLA genotype was dichotomized as high risk or other. Odds ratios (OR) with 95% confidence intervals (CI) were calculated and adjusted for age, sex, BMI, education, smoking, physical activity, family history of diabetes, total energy, alcohol and potassium intake.

Results: Sodium intake was associated with an increased risk of LADA (OR per gr/day; 1.73, 95% CI; 1.23-2.43); comparing the highest to lowest tertile of sodium intake indicated an OR of 2.19 (95% CI; 1.33-3.61) (Table 1). The risk was even more pronounced for LADA patients with high risk HLA genotypes; an almost four-fold (OR 3.87, 95% CI 1.87-8.01) increased risk was seen in the high consumers. We could also confirm that sodium intake was associated with an increased risk of T2D (OR per gr/day; 1.43, 95% CI; 1.09-1.88).

Conclusion: Our findings suggest that high sodium intake may be a risk factor for LADA, especially in carriers of high risk HLA genotypes. We could also confirm an association between sodium intake and T2D. If confirmed in other populations, these findings may have important implications in the primary prevention of diabetes with adult onset.

Table 1. OR of LADA and type 2 diabetes in relation to sodium consumption, ESTRID study, 2010-2016

	Type 2 diabetes			LADA		LADA With other HLA genotype [a]		LADA With high risk HLA genotype [a]	
	No. controls	No. cases	OR (95% CI) [b c]	No. cases	OR (95% CI) [b c]	No. Cases [d]	OR (95% CI) [b c]	No. Cases [d]	OR (95% CI) [b c]
Energy adjusted sodium intake (mg/day); tertiles									
Low (0.001–2412)	607	319	Reference	110	Reference	32	Reference	42	Reference
Medium (2413-3150)	554	549	1.31 (0.99-1.73)	173	1.64 (1.18-2.30)	48	1.37 (0.78-2.42)	77	2.40 (1.47-3.93)
High (=3151)	218	268	1.58 (1.06-2.35)	72	2.19 (1.33-3.61)	22	1.88 (0.82-4.35)	33	3.87 (1.87-8.01)
Sodium intake (per gr/day)	*1379*	*1136*	*1.43 (1.09-1.88)*	*355*	*1.73 (1.23-2.43)*	*102*	*1.87 (1.02-3.42)*	*152*	*2.43 (1.47-4.01)*

[a] High risk HLA genotype: Individuals with *DR4-DQ8, DR3/4, DR3/3, DR4/4, DR4/3-DQ8,* and *DQA1*0501-DQB1*0201*; Other HLA genotypes: individuals with *DR3/X, DR4/X, DR4-DQ7,* and *DR/X/X*
[b] Adjusted for age, sex, BMI, education, smoking, physical activity, alcohol intake, family history of diabetes, total energy intake, and potassium intake
[c] Additional adjustment for other dietary factors including protein, saturated fat, magnesium and calcium did not change the results
[d] Genetic information was available for 71% of LADA patients

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Protein intake in relation to development of type 2 diabetes and the role of obesity

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Background and aims: Type 2 diabetes (T2D) is one of the most common chronic diseases with increasing prevalence over the world. Diet is considered an important component of a healthy lifestyle in the prevention of T2D. Investigations into the effect of dietary factors on risk of T2D have become very important. One of the dietary factors of interest is protein intake. Overall, total and animal protein intake seems to be associated with higher risk of T2D, but the association between plant protein intake and risk of T2D is still uncertain, appears to be either inverse or null. Besides, the evidence for protein intake in relation to earlier stages in the development of T2D including insulin resistance and prediabetes is scarce. Therefore, we aimed to examine the associations between habitual protein intake with insulin resistance, risk of prediabetes and risk of T2D in a large population-based cohort study.

Materials and methods: Our current study was embedded within three cohorts of the Rotterdam Study (RS), a population-based cohort study including people aged ≥45 years living in the Ommoord District of Rotterdam, the Netherlands. The study has been approved by the Medical Ethics Committee of Erasmus University Medical Center and all participants gave written informed consent. We followed 2976 subjects in Rotterdam Study I (1990-2012), 1410 subjects in Rotterdam Study II (2000-2012), and 2418 in Rotterdam Study III (2006-2012). All 6814 subjects in our current study were free of diabetes at baseline; of whom 5795 were without prediabetes, and 3932 had repeated assessments of glucose and insulin, from which we calculated the homeostatic model assessment for insulin resistance (HOMA-IR). Protein intake was assessed at baseline with the use of validated food-frequency questionnaires. We used multivariable cox proportional hazard regression models to analyze the associations between protein intake and risk of prediabetes and T2D. We used multivariable linear mixed models to analyze the associations between protein intake and insulin resistance, and we used joint models to examine the role of longitudinal obesity data in the associations of protein intake with insulin resistance and risk of prediabetes and T2D.

Results: We documented 643 cases of T2D during a median 7.2 years of follow up; and 931 cases of prediabetes during a median 5.7 years of follow up. In pooled multivariable models, higher intake of total protein was positively associated with HOMA-IR (β=0.10 (95%CI 0.07-0.12) per 5 energy percent (E%)), risk of prediabetes (HR=1.35 (95%CI 1.20-1.51) per 5E%), and risk of T2D (HR= 1.38 (95%CI 1.20-1.60) per 5E%). Additional adjustment for repeatedly measured BMI or waist circumference attenuated these associations, but the associations of protein intake with higher insulin resistance and prediabetes risk remained significant. Results were mainly explained by protein from animal food sources. Results were irrespective of whether protein substituted total fat or carbohydrates, independent of protein intake at follow-up, and independent of other cardio metabolic risk factors.

Conclusion: Total protein intake is positively associated with insulin resistance, risk of prediabetes and T2D, which is mainly driven by animal protein. It appears likely that obesity partly mediates the associations.

Disclosure: **Z. Chen:** None.

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Physical activity, fatty liver and glucose metabolism over the life course: the LifeLines cohort

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Background and aims: Fatty liver is strongly associated with type 2 diabetes mellitus (T2DM), and may be influenced by lifestyle factors. We aimed to examine whether there is an association of habitual physical activity with biochemical markers of Non-alcoholic fatty liver disease (NAFLD) and whether this association is stronger with age and degree of impaired glucose metabolism.

Materials and methods: We included 44,491 participants from the Dutch LifeLines Cohort Study, aged 18-80 years (39.2% males, 44±12 years of age, BMI 26±4.3 kg/m^2 and Waist Circumference (WC) 90.3±12.3 cm). Moderate-to-vigorous physical activity (MVPA) was assessed by the SQUASH, a self-reported questionnaire. Glucose status was defined as (1) normal glucose tolerance (NGT) based on fasting plasma glucose (FPG) <6.1 mmol/L, (2) impaired glucose metabolism (IGM) - FPG 6.1 - 6.9 mmol/L or HbA1C 5.7 - 6.4%, and (3) diabetes - FPG ≥7.0 mmol/L or HbA1C ≥6.5% or self-report of diagnosis by a physician, or use of glucose-lowering agents. NAFLD was defined as a fatty liver index (FLI) >60, which is based on BMI, WC, triglycerides and gamma-glutamyltransferase (GGT). Furthermore, we assessed alkaline phosphatase (AP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Exclusion criteria were previously diagnosed hepatitis or cirrhosis and excessive alcohol use. All analysis were adjusted for age, gender and education level.

Results: Participants spent a median of 420 (165-1200, 25th-75th %) minutes per week in MVPA. NAFLD was found in 21.4% of the participants. Higher MVPA reduced the risk for NAFLD in a dose-dependent manner. Compared to "no MVPA" odds ratios (OR) and 95% confidence intervals (CI) for quintiles of MVPA (all participants with MVPA >0 minutes/day) were 0.73 (0.66; 0.80), 0.57 (0.51; 0.63), 0.53 (0.48; 0.59), 0.49 (0.45; 0.55) and 0.50 (0.45; 0.55) for the highest level of MVPA. Regarding glucose status, the highest level of MVPA, compared to "no MVPA", showed an OR of 0.56 (0.48; 0.64) for NGT, 0.48 (0.41; 0.56) for IGM and 0.38 (0.21; 0.67) for T2DM. Furthermore, the association between MVPA and NAFLD was dependent on age. The OR was 0.75 (0.57; 0.99) in young people (<35 years) and 0.15 (0.05; 0.51) in older people (> 65 years) when comparing the highest level of MVPA with "no MVPA". Furthermore, the association was dependent on gender. The OR was 0.44 (0.39; 0.50) for men and 0.58 (0.49; 0.65) for women. In linear regression, MVPA was inversely associated with Log GGT (p<0.01), Log ALT (<0.01) but not Log AP (p=0.248) and positively associated with Log AST (p<0.01).

Conclusion: A higher level of MVPA is associated with a lower level of NAFLD risk as well as the individual liver enzymes such as GGT and ALT. This association is stronger in diabetic and older subjects.

Disclosure: **O. Byambasukh:** None.

OP 39 Improving outcomes by individualising health care delivery

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Diabetes care and mortality in England and Wales 2006-2014

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Background and aims: There is substantial excess mortality among people with diabetes. We investigated whether completion of NICE recommended standards of diabetes care (annual care processes and treatment targets) and levels of HbA1c and BP achieved are related to subsequent mortality.

Materials and methods: The National Diabetes Audit for England and Wales (NDA) extracts data annually from primary care and specialist electronic records. Cohorts of people were selected from the 2006-07 NDA with Type 1 and Type 2 diabetes (also includes secondary diabetes, MODY etc) whose data completeness fulfilled the following criteria: age at least 20 years and still alive on 31st March 2013. This comprised 113,748 Type 1 and 863,850 Type 2 records i.e. 80% of the people in the 2006-07 NDA. The cohort was split: (A) into groups according to completion of three annual care processes (measurement of HbA1c, BP, cholesterol) over the seven year period (Good = all three checks each year between 2006/07 and 2012/13. Poor = not more than 12 out of a possible 21 checks over the seven year period); (B) according to measured levels of HbA1c, BP and cholesterol (the boundaries were dictated by trying to get an even spread of records across the groups, rather than by any clinical significance). Mortality rates for "good" and "poor" care process completion groups were determined, as were rates for the highest and lowest levels of HbA1c, BP and cholesterol at the start and end of the 6 year period.

Results: For both Type 1 and Type 2 diabetes there were age related relationships between mortality (between 1st April 2013 and 31st March 2015) and care process completion and treatment target achievement over the previous 7 years. For each age group and for both people with Type 1 diabetes or Type 2 diabetes, mortality rates were halved for those with a good record of care process completion compared to those with a poor record. For people with Type 2 diabetes, mortality rates were about one third lower for those with consistently low HbA1c recorded compared those with consistently high HbA1c whereas the pattern for Type 1 diabetes was more complex. For baseline systolic blood pressure those in the highest groups as compared to the lowest groups mortality rates were 100% greater in Type 1 and 20% greater in Type 2

Conclusion: Successful completion of NICE recommended annual care processes are associated with lower mortality in all types of diabetes in all age groups. A more complex picture is observed for achieved levels of HbA1c and BP, but higher levels of HbA1c and blood pressure are associated with higher mortality rates.

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Factors associated with poor glycaemic control in adults with type 1 diabetes in Norway

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Background and aims: Randomized studies in patients with type 1 diabetes have shown that improved blood glucose control significantly reduces the risk of developing microvascular complications. Observational studies have demonstrated a substantial increase in the risk of death with higher HbA_{1c} levels. A registry based study from Norway has shown that 22% of adult patients with type 1 diabetes had an $HbA_{1c} \geq 9\%$ in 2012. The aim of this study is to assess factors associated with poor glycaemic control using variables registered in the Norwegian Diabetes Registry for Adults (NDV) between 2013 - 2015, together with socioeconomic data obtained from Statistics Norway in 2015.

Materials and methods: 7602 participants over 18 years of age with a diabetes duration of more than two years who had an HbA_{1c} recorded in the NDV between 1st January 2013 and 31st December 2015 were included in the study. The primary outcome of the study was poor glycaemic control defined as $HbA_{1c} \geq 9\%$. Variables potentially associated with glycaemic control were investigated using univariable and multivariable regression analyses adjusted for clustering of observations within hospital diabetes clinics. Odds ratio for $HbA_{1c} \geq 9\%$ and 95% confidence intervals are reported. In view of the large sample size and the number of variables evaluated $p < 0.01$ was chosen as the level of statistical significance. Statistical analyses were performed in Stata 14.0.

Results: The average age of the 7602 participants was 43 years, average duration of diabetes was 22 years and 46.8% were female. The overall mean HbA_{1c} level was 8.0% and 18.7% had an $HbA_{1c} \geq 9\%$. Multivariable regression analysis showed that age between 18 - 25 years and current smoking were significantly associated with an increased risk of having an $HbA_{1c} \geq 9\%$, whereas higher level of education, increasing numbers of glucose measurements and exercise more than three days a week were significantly associated with attenuated risk. In addition several variables from the univariable analysis (not included in the multivariable regression analysis due to feedback issues) were significantly associated with poor glycaemic control. For example insulin pump therapy and higher daily insulin dose per kg were associated with increased risk of $HbA_{1c} \geq 9\%$, whereas increasing numbers of hypoglycaemic episodes per month was associated with attenuated risk of $HbA_{1c} \geq 9\%$.

Conclusion: This study has provided representative national data that demonstrate a strong association between blood glucose control and several patient and socioeconomic factors. A better awareness and understanding of these factors could lead to more individualized management strategies, better glycemic control and a lower risk of diabetes complications.

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Predictors of transitioning from adherence to non-adherence among elderly patients with diabetes

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Background and aims: Good medication adherence to evidence-based drug therapies to control blood sugar levels is a significant factor in reducing cardiovascular disease risk in patients with diabetes. There is very little research that examines the predictors of good medication adherence in older diabetes patients, or on barriers and facilitators to maintaining good adherence over time. The purpose of this study is to determine factors that predict transitioning from good medication adherence to non-adherence for oral diabetes agents in patients with diabetes age 65 and older.

Materials and methods: Kaiser Permanente (KP) is an integrated delivery system that provides comprehensive health care to its members in 8 regions of the United States. This retrospective cohort study included patients aged $\geq$65 from 3 KP regions who received oral diabetes agents in 2010 for whom the Proportion of Days Covered (PDC) could be calculated ($\geq$2 dispensings per year). The study sample was limited to the 83% of the population that were considered adherent using a definition of PDC$\geq$0.8 in 2010. Patients were followed through 2014 or until they disenrolled from the health plan or had less than 2 dispensings of oral diabetes agents per year. To assess predictors of transitioning from adherent to non-adherent we employed the standard generalized estimating equation approach for longitudinal data.

Results: The cohort included 46,406 patients. Characteristics associated with transitioning from adherent in 2010 to non-adherent in 2011-2014 included being female (OR=1.15; 95%CI 1.10-1.10), age ≥75 (OR=1.27; 95% CI 1.20-1.33) compared to age 65-69, African American (OR=1.24; 1.14-1.34) or American Indian/Alaska Native (OR=1.43; 95%CI 1.05-1.94) compared to White, and ≥2 comorbidities (OR=1.38; 95%CI 1.29-1.48) compared to no comorbidities. Patients were less likely to become non-adherent if the mean days' supply dispensed was >90 days (OR=0.56; 95%CI 0.53-0.60) or they received oral diabetes agents via mail order >50% of the time (OR=0.69; 95%CI 0.66-0.72).

Conclusion: Patient characteristics can increase the likelihood of transitioning from adherent to non-adherent to oral diabetes medications. However, health care system-level factors including days' supply and mail order pharmacy use can decrease the likelihood of becoming non-adherent over time. These findings can help policy-makers, clinicians, and health plans to develop interventions that target establishing and maintaining long-term adherence to oral diabetes medications.

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Overtreatment of older patients with type 2 diabetes

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Background and aims: According to EASD Guidelines, in older people with type 2 diabetes, especially those with macrovascular complications and other comorbidity and with a long duration of diabetes, less stringent HbA1c levels are requested. In the Netherlands the National Diabetes Guidelines on type 2 diabetes include an HbA1c algorithm with HbA1c targets above 7% (53 mmol/mol) for people ≥ 70 years, who are using more than metformin alone. If they are known < 10 years with diabetes, the HbA1c target is ≤ 7.5% (58 mmol/mol), when the diabetes duration ≥ 10 years the target is ≤ 8% (64 mmol/mol). In this study we investigated, three years after the publication of the Dutch guidelines, whether these personalised HbA1c targets are implemented or whether overtreatment is common.

Materials and methods: Observational study using routine care date of 1002 patients with type 2 diabetes treated in Health Centers. Data were retrieved from the electronic patient files. Frailty was assessed using the Frailty Index. In overtreated persons we scrutinised the files for records of hypoglycaemia, fall accidents and visits to the emergency room of the hospital. Patients with an HbA1c target > 7% were compared with those with an HbA1c target ≤ 7% using the Pearson Chi square test. Characteristics of the persons on target were compared with those who were either undertreated or overtreated using the Pearson Chi square or Kruskal-Wallis test.

Results: Among all 1002 people with type 2 diabetes and treated in the health centers, 31.8% (n=319) were 70 years or older. All people < 70 years had an HbA1c target < 7%. The same held true for 154 people ≥ 70 years who were treated with only a lifestyle advice or with metformin monotherapy. For the remaining people (n=165, 16.5%) a target ≥ 7% should be defined; 53 persons had an HbA1c target ≤ 7.5% and 112 persons ≤ 8%. These 165 persons had more micro (54.0% vs. 35.2%) - and macrovascular (33.3% vs. 17.7%) complications, used more often ≥ 5 medicines (87.3% vs 53.2%) and were more often frail (44.2% vs 13.9%) compared to those with a target < 7%. Of these 165 persons 47 (28.4%) were above their respective HbA1c targets, whereas 64 (38.8%) had an HbA1c below their target and even below 7%. Especially in the 'severely' overtreated group of persons with an average age of 76 years, many were frail, on insulin and complaining of hypos according to their medical files. In this group, almost 3 out of 10 persons reported fall accidents (Table).

Conclusion: Of the 165 persons ≥ 70 years with an HbA1c target > 53 mmol/l 64 persons were overtreated, representing 20% of all 319 people with type 2 diabetes of 70 years or older. Almost half of the overtreated persons were frail. Many experienced hypoglycaemic events. Evidence based, HbA1c targets > 53 mmol/ l in older persons with type 2 diabetes are not well practiced in daily diabetes care and cause iatrogenic damage.

Table: Characteristics of overtreated patients with HbA1c < 7%.
Numbers and percentages, unless otherwise stated

	Target HbA1c ≤ 7.5% (58 mmol/mol) (n=23)	Target HbA1c ≤ 8% (64 mmol/mol) (n=41)
Age (years, median, IQR)	72 (9)	76 (10)
Diabetes duration (years, median, IQR)	5.0 (6)	14 (8)
HbA1c (mmol/mol, median, IQR)	6.5% (6)	6.4% (7)
eGFR <45	5 (21.7)	3 (7.3)
Medication		
- Metformin	18 (78.3)	34 (82.9)
- Insulin	2 (9.1)	13 (31.7)
- Sulfonylureas	20 (87.0)	29 (70.7)
- Other	3 (13.6)	1 (2.4)
Frailty index >0.2	8 (34.8)	21 (51.2)
Living alone	8 (34.8)	13 (31.7)
Hypoglycemia	4 (17.4)	9 (22.0)
Hypoglycemia related ER visit	1 (4.3)	1 (2.4)
Polypharmacy (≥ 5 medications)	21 (90.3)	33 (80.5)
Comorbidities ≥1	13 (56.5)	18 (43.9)
Fall accidents	4 (17.4)	12 (29.2)

Disclosure: **H.E. Hart:** None.

OP 40 Weight regulation: mechanisms and interventions

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Obesity caused by melanocortin-4 receptor (MC4R) defects can be treated with a glucagon-like peptide 1 (GLP-1) receptor agonist

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Background and aims: The most common monogenic form of obesity is caused by loss of function mutations in the hypothalamic expressed melanocortin-4 receptor (MC4R) gene, but treatment options are limited. The gut hormone glucagon-like-peptide 1 (GLP-1) reduces food intake. The central mechanisms underlying this effect may either occur independent of the MC4R by inhibiting orexigenic neurons or dependent of the MC4R by stimulating anorectic neurons. We therefore tested if the GLP-1 receptor agonist (GLP-1 RA) liraglutide could induce weight loss in adult obese MC4R mutation carriers.

Materials and methods: Fourteen adult individuals with loss of function MC4R obesity were identified as the biological parent to a child diagnosed with MC4R mutation, and 28 BMI, age and gender-matched adults without MC4R mutation served as control participants. All participants were treated with liraglutide 3.0 mg for 4 months. Bodyweight, waist circumference, and magnetic resonance (MR)-assessed liver and muscle fat were measured before and after liraglutide treatment.

Results: Baseline BMI was 37.1±1.7kg/m^2 in MC4R carriers and 36.9±0.8kg/m^2 in control subjects (p=0.8 between groups). After 4 months of liraglutide treatment, the MC4R carriers lost -6.8±1.8kg, p=0.003 (equivalent to -5.7 ±1.4% bodyweight) and the obese control subjects lost -6.1±1.2kg, p<0.0001 (equivalent to -5.4±1.1% bodyweight) (p=0.8 between groups). Waist circumference decreased by -5.4±1.4cm, p<0.0001 in MC4R carriers and by -6.4±1.6cm, p<0.0001 in control subjects (p=0.1 between groups). Total body fat percentage decreased by -2.5±0.8%, p=0.009 and by -2.6 ±0.5%, p<0.0001 (between groups, p=0.9), liver fat decreased by -1.2±1.7%, (p=0.5) and by -3.0±1.5%, p=0.06 (p=0.5 between groups) and muscle fat decreased by -0.13±10.7%, p=0.9 and by -0.4±0.8%, p=0.8 (p=0.5 between groups), respectively, in MC4R carriers and control subjects.

Conclusion: We show that liraglutide can treat MC4R-induced obesity. The GLP-1 RA liraglutide caused similar, clinically significant weight loss in obese MC4R carriers and matched controls participants, indicating that the appetite reducing effects of GLP-1 is independent of the MC4R pathway.

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Chronic effects of repetitive transcranial magnetic stimulation on satiety and body weight control

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Background and aims: Repetitive Transcranial Magnetic Stimulation (rTMS) is a non invasive technique inducing long lasting and reversible changes in neural excitability and dopamine release. Hence, rTMS has been developed as a treatment for neuropsychiatric disorders associated with abnormal dopamine release. Consistently with its modulatory effect on the reward system, we hypothesized a potential role of rTMS in inducing satiety and body weight loss, through modulation of food craving circuitries ("food reward system").

Materials and methods: Twenty-eight obese patients (8 M, 20 F; age: 46.3±9.4; BMI: 36.6±4.9 kg/m^2) were randomized to receive 15 daily sessions of high frequency (18 Hz, promoting cortical excitability), low frequency (1 Hz, inhibiting cortical excitability) or sham stimulation (3 per week for 5 weeks). Prior to stimulation, obese subjects were either shown a series of palatable food images (cue) or not (no cue). The coil version used in this study (H-coil) was designed to stimulate deeper brain regions (3 cm *vs* 1.5 cm from the skull) and targeted to the Pre-frontal Cortex and Insula, bilaterally. Body weight, metabolic, neuro-endocrine parameters and food craving were evaluated at baseline, at the end of 15 rTMS sessions, after 1 and 6 months of follow-up.

Results: After 5 weeks, a significant weight loss was found in the 18 Hz group (-4.1±2.5% *vs* basal, p<0.001) which continued up to 1 month (-5.5±3.2% *vs* basal, p<0.001) and 6 months of follow-up (-4.8±3.5% *vs* basal, p=0.014). 18 Hz group obese patients experienced also a decrease in food craving (-42.3±15.2% *vs* basal, p<0.0001) which continued even after 1 month (-38.5 ±14.5% *vs* basal, p=0.0001) and 6 months of follow-up (-41.5±8.8% *vs* basal, p<0.0001; p<0.01 *vs* sham). In the 18 Hz (cue) sub-group, a significant decrease of glucose (-5.3±4.8% *vs* basal, p=0.042) and a trend to decrease of cholesterol (-8.2±8.1% *vs* basal, p=0.086) were observed after 5 weeks of treatment. Concerning pituitary hormones, in the 18 Hz group, significant reductions of ACTH (-36.6±17.1% *vs* basal, p=0.002), prolactin (-43.1 ±11.7% *vs* basal, p<0.0001), and TSH (-20.6±21.7% *vs* basal, p=0.019) were found at the end of the 15 rTMS sessions; TSH decrease persisted up to 1 month of follow-up (-19.2±25.9% *vs* basal, p=0.009). In the 18 Hz group, a trend to reduction in the norepinephrine levels (-10.8±32.9% *vs* basal, p=0.078) was observed after 15 rTMS sessions. Conversely, in the 1 Hz group, neither significant weight change, or the above described hormone and metabolite variations were shown, whilst a significant decrease in Beta-endorphin levels was found (-10.6±7.6% *vs* basal, p=0.05).

Conclusion: In summary, high frequency rTMS has proven to be effective in reducing food craving leading to a significant decrease of body weight up to 6 months since the end of treatment, via modulation of the HPA axis and sympathetic activity. These findings support the role of rTMS as a novel promising treatment for obesity.

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Voluntary exercise can modify the preference for palatable food through the modulation of central reward circuit by peripheral ghrelin signal

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Background and aims: We previously reported that voluntary exercise contributed to an amelioration of abnormal feeding behavior with a concomitant restoration of ghrelin production in high fat diet (HFD)-induced obese model (Peptides, 2015). In obese human subjects, hedonic eating is often present because palatable foods may promote addiction like drugs. Because it's known that hedonic eating is related to the activation of central reward circuit via ghrelin receptor in ventral tegmental area (VTA), we investigated whether voluntary exercise could modify the food preference in relation to peripheral ghrelin and central dopamine systems in rats.

Materials and methods: Male Sprague-Dawley (SD) rats at 4 weeks old were either housed as sedentary group (Se) in ordinary cages throughout the experiment or exercise group (Ex) in ordinary cages from Monday to Thursday and in special acrylic chambers equipped with a running wheel from Friday to Sunday. All rats were allowed to freely access to either control chow diet (CD;10 kcal%fat) or HFD (60 kcal%fat) according to their preference. Food preference was measured on Monday morning up to 10 weeks old. Subsequently, dopaminergic activity in nucleus accumbens (NAc) in either Ex or Se was measured using microdialysis method. Furthermore, synthesized rat ghrelin (3 nmol/2mL) was administered intravenously to Se at 10 weeks old, therafter microdialysis procedures were performed as well.

Results: Body weight at 10 weeks old was higher in Se than in Ex (459±12 vs. 412±16 g, $P<0.05$). In Se, most of consumed food was HFD throughout experimental periods. In contrast, the preference for HFD was attenuated by the induction of voluntary exercise, and around 40% of food consumption was substituted for CD after 5 weeks old to the end of the experimental period (Figure). In Se, dopamine (DA) level in NAc was increased in response to either CD or HFD fed for 20 minutes by 50% of basal levels. Voluntary exercise abolished HFD-induced DA surge in spite of no effect on CD-induced one. Systemic ghrelin administration to Se abolished HFD-induced DA surge with no effect on CD-induced one in the same manner as voluntary exercise did.

Conclusion: It was reported that systemic ghrelin administration brought about the inhibition of DA surge associated with HFD through the activation of dynorphin A/kappa opioid receptor pathway of DA neurons in VTA (Neuropharmacology, 2013). It is thus plausible that voluntary exercise could attenuate the food preference for palatable HFD through the modulation of central dopamine system by peripheral ghrelin signal, taking the amplification of ghrelin production by regular exercise into account.

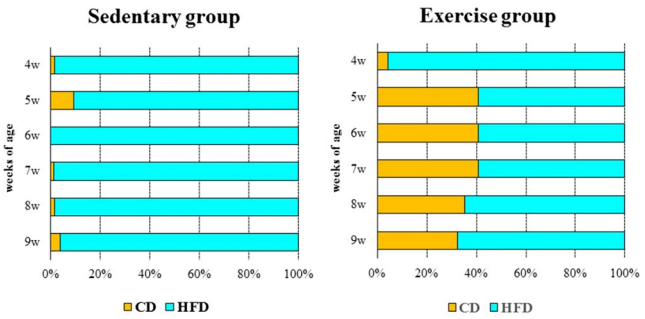

Figure. Effects of voluntary exercise on food preference in SD rats

Disclosure: **Y. Tajiri:** None.

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Hypothalamic ATP up-regulation is the mechanism for the amelioration of leptin resistance by celastrol and withaferin A

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Background and aims: Leptin is an adipocyte-derived hormone involved in the regulation of food intake and energy expenditure. Obese subjects generally have hyperleptinemia and leptin resistance. Leptin sensitizers rather than leptin itself are expected as anti-obesity drugs. Although the precise mechanism underlying leptin resistance is still unclear, it has been reported that endoplasmic reticulum (ER) stress in the hypothalamus plays a key role. Recently, two natural compounds, celastrol and withaferin A, has been identified as leptin sensitizers (Cell 161: 999, 2015, Nat Med 22: 1023, 2016). Withaferin A has a similar gene expression signature to that of celastrol. Both of them alleviate hypothalamic ER stress and restore the sensitivity of the hypothalamus to leptin. Thus, celastrol and withaferin A are promising agents for the treatment of obesity and its complications including diabetes. However, the molecular mechanism by which celastrol and withaferin A alleviate ER stress and restore leptin sensitivity remains completely unknown. Under ER stress, protein folding in the ER is impaired leading to the accumulation of misfolded proteins. The accumulation of misfolded proteins is harmful to cells and thus the ER has evolved mechanisms designed to detect misfolded proteins and either refold them or target them for degradation. These responses against ER stress are called unfolded protein response (UPR). UPR requires appreciable amounts of ATP. Indeed, ATP-deficient cells are vulnerable to ER stress and treatment of ATP protects cells against ER stress. For these reasons, we investigated the role of ATP in the development of hypothalamic ER stress and leptin resistance and their improving effect of celastrol and withaferin A. It is well known that high fat diet induces ER stress and leptin resistance in the hypothalamus. Thus, we measured ATP concentrations in the hypothalamus of mice with or without celastrol and withaferin A treatment.

Materials and methods: 8 weeks old male C57B/6J wild type and ob/ob mice were used. For high fat diet loading, mice were fed an ad libitum high-fat pellet diet containing 20% weight for weight (wt/wt) protein, 20% wt/wt carbohydrate, and 60% wt/wt fat. Celastrol (100 ng/gBW) and withaferin A (2 mg/gBW) were intraperitoneally administered once a day. Hypothalamic ATP concentrations were measured by luciferase activities.

Results: A month of high fat diet feeding decreased hypothalamic ATP concentrations in C57B/6J mice. Celastrol and withaferin A suppressed body weight gain and food intake and increased hypothalamic ATP concentrations in C57B/6J mice fed high fat diet. Celastrol and withaferin A had no effect not only on body weight and food intake but also on hypothalamic ATP concentrations in C57B/6J mice fed standard diet, indicating that celastrol and withaferin A do not increase hypothalamic ATP concentrations but inhibit high fat diet-induced ATP reduction. Celastrol and withaferin A increased hypothalamic ATP concentrations but showed only minimal effect on body weight and food intake in ob/ob mice, suggesting that increase of hypothalamic ATP concentrations by celastrol and withaferin A is not the secondary effect of metabolic improvement.

Conclusion: Taken together, it is suggested that celastrol and withaferin A maintain leptin sensitivity by up-regulating hypothalamic ATP concentrations under high fat diet.

Disclosure: **C. Ebihara:** None.

OP 41 Islet and pancreas transplantation

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The SGLT2 inhibitor dapagliflozin preserves human beta cell function in diabetic mice

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Background and aims: Dapagliflozin is a sodium-glucose cotransporter 2 (SGLT2) inhibitor used for the treatment of type 2 diabetes. Dapagliflozin decreases plasma glucose levels by inhibiting the renal reabsorption of glucose, leading to an increased urinary glucose excretion. Our aim was to examine the effect of dapagliflozin treatment on human β cell function in vivo using a human islet transplanted diabetic mouse model.

Materials and methods: Human pancreatic islet equivalents from three different donors (Prodo Labs, USA) were transplanted into the subrenal capsular space in the left kidney (1,500 islets per mouse) of normoglycemic immunodeficient male NUDE (NMRI-Foxn1nu; Charles River) mice (n=59, 9 weeks of age). After two weeks, diabetes was induced (alloxan monohydrate, 72.5 mg/kg) and five days later, the mice were randomized into two groups per donor based on non-fasting glucose levels. Dapagliflozin (3 mg/kg/day) was provided in the drinking water for 53 days. Non-fasting glucose and human C-peptide plasma levels were monitored during the study and an intravenous arginine/glucose tolerance test was performed. At termination, the grafted human islets were stained for insulin, glucagon and the proliferation marker Ki67.

Results: Blood glucose before treatment start was 15.5±1.5 mM. Dapagliflozin treated mice showed reduced plasma glucose levels compared to control mice over the 53 day study period ($p<0.0001$, 2-way ANOVA). Human C-peptide levels were similar between the two groups at start (veh: 319±32 pM vs. Dapa: 382 ±33 pM, not significant (NS)). After 4 weeks, the levels started to decline in both groups (veh: 120±27 pM vs. Dapa: 221±24 pM, NS). However at termination, hC-peptide levels were significantly elevated in Dapagliflozin treated mice (322 ±77 pM vs. 101±58 pM in controls, $p<0.05$). Dapagliflozin also improved the human β cell acute insulin response to the iv glucose/arginine test compared to controls (0.38±0.05 ng/ml vs. 0.20±0.06 ng/ml in controls, $p<0.05$). At the end of the study, dapagliflozin treated mice had increased number of insulin positive cells in the grafted human islets (56.4±2.1 % vs. 47.4±3.4 % in controls, $p=0.031$), while the number of glucagon positive cells were decreased (39.4±1.8 % vs. 50.8±3.5 % in controls, $p=0.006$). Proliferation, measured as insulin or glucagon co-staining with Ki67 was, however, increased in both β cells (0.9±0.1 % vs 0.4±0.1 % in controls, $p=0.007$) and α cells (3.3±0.7 % vs 1.4±1.7 % in controls, $p=0.018$), following dapagliflozin treatment.

Conclusion: The SGLT2 inhibitor dapagliflozin maintained long-term glycemic control by preserving human β cell number and in vivo function in a humanized diabetic mouse model.

Disclosure: **D. Karlsson:** Employment/Consultancy; All authors are employed by AstraZeneca. Stock/Shareholding; All authors are shareholders of AstraZeneca.

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Impact of primary graft function long-term (10 years) outcome of islet allotransplantation in type 1 diabetes

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Background and aims: Islet transplantation (IT) is a treatment of type 1 diabetes in case of brittleness, hypoglycaemia unawareness or after kidney transplantation. The procedure is demanding for the patient requiring 2 to 3 islets injections under an immunosuppressive regimen. Short-term results are continuously improving but long-term results have not been studied. The **aim** of this work was to assess long-term results and the predictive factors of success of IT.

Materials and methods: This prospective observational single-arm study combines the analysis of 33 consecutive patients having received since 2003 either an islet transplantation alone (ITA, n=19) or an islet-after-kidney transplantation (IAK, n=14), under an Edmonton immunosuppressive regimen. Each patient received 2 to 3 intraportal islet injections through surgical or radiological route over a 6-month period. Metabolic results and graft survival assessed by a fasting C-peptide level greater than 0.3 ng/mL) and insulin-independency with normal A1c were prospectively assessed during more than 10 years. The β-score calculated 1 month after the last islet injection was classified as optimal (β-score greater or equal to 7) or sub-optimal (β-score below 7) to define the primary graft function (PGF).

Results: The median islet-transplanted mass was 13.9[11.1-15.8] x 10^3 islet-equivalent/kg of body weight. 32 patients (97%) reached insulin-independence. At last news (September 2016), the graft was functional in 25 patients (14 ITA, 11 IAK) among whom 11 patients still insulin-independent (4 ITA, 7 IAK) with a median follow-up duration of 3723[2141-4028] days. In the whole group, the percentage of graft survival (Kaplan-Meier, KM) was respectively 85% at 5 and 76% at 10 years. The percentage of insulin-independence (KM) was 46% at 5 and 28 % at 10 years. The graft type (IAK or ITA) had no significant influence on the results. In contrast, graft survival and insulin-independence rates were significantly higher in patients with optimal PGF (n=21) *vs.* sub-optimal PGF (n=12), with respectively at 5 years 100 *vs.* 64 % and at 10 years 93 *vs.* 53% of graft survival ($p=0.0008$; log-rank test) The insulin-independence rate was at 5 years 66 *vs.* 18 % and at 10 years 42 *vs.* 0 % ($p=0.0002$) respectively in the optimal *vs.* suboptimal graft function.

Conclusion: This study shows the long-term persistence of graft function and insulin-independence with the Edmonton protocol, both in ITA and IAK. The primary graft function is essential for long-term good results.

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Disclosure: **M. Vantyghem:** None.

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Mcl-1 is a key anti-apoptotic protein in human and rodent pancreatic beta cells

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Background and aims: Induction of endoplasmic reticulum (ER) stress and activation of the intrinsic apoptotic pathway contribute to β-cell death in type 1 diabetes (T1D). MCL-1 is an anti-apoptotic member of the BCL-2 protein family, whose depletion causes apoptosis in rodent β-cells in vitro. Importantly, decreased MCL-1 expression was observed by histology in islets from T1D patients. Given the important role of MCL-1 in the survival of β-cells, the aim of this study was to clarify the role of MCL-1 both in human β-cells and in an in vivo murine model of T1D and to determine the mechanisms involved in the post-transcriptional regulation of the MCL-1 protein in β-cells.

Materials and methods: The in vitro studies were performed in the human β-cell line EndoCβH1, the rat insulinoma cell line INS-1E and human islet cells exposed or not to IL-1β or TNF +, IFNγ. Knockdown of the proteins of interest were performed by the use of specific small interfering RNAs while overexpression was achieved using adenoviral vectors. To evaluate the role of Mcl-1 in vivo, a β-cell specific Mcl-1

knockout (βMcl-1 KO) mouse was generated. Both βMcl-1 KO and wild type mice were subjected to multiple low-dose streptozotocin (MLDS) treatment. Non-fasting blood glucose levels were measured every 7 days for 10 weeks. Pancreases were then collected for histological analysis and measurement of insulin content.

Results: Exposure of EndoC-βH1 cells to IL-1β+IFN-γ or TNF+IFN-γ for 24 hours decreased MCL-1 protein expression by 42 and 50%, respectively ($p<0.05$). Silencing MCl-1 sensitised EndoC-βH1 cells to apoptosis induced by the different cytokine combinations, (36-48% increase in cell death; $p<0.01$). In mirror experiments, overexpression of MCl-1 protected EndoC-βH1 cells and dispersed human islet cells against cytokine-induced apoptosis (36 and 52% protection, respectively; $p<0.05$). βMcl-1 KO mice showed normal development and preserved islet function/morphology under basal condition. Islets from βMcl-1 KO mice, however, were more susceptible to IL-1β + IFN-γ- and TNF+IFN-γ-induced β-cell apoptosis (50%-81% increase respectively, $p<0.05$ vs WT). Moreover, βMcl-1 KO mice displayed higher hyperglycaemia (50% increase AUC, $p<0.05$ vs WT) and lower pancreatic insulin content (54% decrease, $p<0.05$ vs WT) after MLDS treatment. Mechanistic studies in INS-1E cells identified the kinase GSK3β, the E3 ligases MULE and βTrCP and the deubiquitinase USP9x as the key regulators of cytokine-mediated MCL-1 protein turnover in β-cells.

Conclusion: The present findings identify MCL-1 as a critical protein for preventing β-cell death in T1D and unveil the role for E3 ligases and ubiquitination in its down-regulation by pro-inflammatory cytokines. Development of strategies to prevent MCL-1 loss in the early stages of T1D may enhance β-cell survival and thereby serve as a relevant adjuvant therapy to delay or prevent disease progression.

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Disclosure: **N. Violato:** None.

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Beta cell-derived microparticles as early biomarkers of rejection in islets transplanted patients: Toward the identification of the cause of the graft loss?

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Background and aims: Microparticles (MPs) are vesicles shed from the plasma membrane in response to stress and circulating surrogate markers of the initial cell damage. High levels of circulating MVs are associated with transplant rejection of renal, cardiac or mesenchymal stem cell grafts. We previously reported a peak of procoagulant MPs preceding the rise in insulin needs in islets transplanted patients thereby suggesting early graft dysfunction. In the present pilot study, plasma variations of procoagulant MPs released from insulin cell (IC) and bearing the characteristic PSA-NCAM were monitored in islet transplanted patients.

Materials and methods: Venous sampling of 19 T1D transplanted pancreatic islet patients, (Man,Women ratio:10/9. Age (years): 56 (29-74). Diabetes duration (years): 38 (14-44). HbA1c (%) : 7,5 (5,9-9.6). Daily insulin requirements (UI/kg): 0,6 (0,32-1,05). Islet after kidney: 5 patients (26,3%), was prospectively performed before each transplant and 3, 6, 9, 12, 24 and 36 months post-last islet injection. MPs-PSA-NCAM+ (PSA-MPs) were measured after capture on an anti-PSA-NCAM antibody using an original and highly sensitive prothrombinase activity assay. Values of the international graduation β-score, that combines four indicators of transplantation efficacy (HbA1c, C-peptide, daily insulin dose, fasting glucose) were also calculated. Transplanted patients were allocated to 3 groups: functional (FG, n = 8) or partially functional (PFG, n = 8) graft, with respective β-score values ($6\leq FG \leq 8$ and $3 < PFG < 6$), or to Graft failure (GrF β-score ≤ 3, n = 3). Control groups were T1D (n = 4) and T2D (n = 7) patients and healthy subjects (HV, n = 3).

Results: Mean plasma MPs concentrations in FG and HV were similar (0.26 ± 0.05 nM vs. 0.3 ± 0.0 nM) indicating minimal cell damage. A peak of PSA-MPs (x 2-3) was observed before each β-score drop and the rise of insulin needs, suggesting early alteration of ICs. In GrF or PFG, the mean value of PSA-MPs levels is higher than in FG (0.51 ± 0.42 nM 0.44 ± 0.26 nM, vs. 0.26 ± 0.05 nM, $p < 0.0001$) and close to values measured in T2D (0.62 ± 0.17 nM), suggesting that resident dysfunctional β cells still shed MPs. In GrF, the PSA-MP concentrations measured at distance of the peak did not statistically differ from those in DT1 patients (0.40 ± 0.08 nM vs. 0.28 ± 0.04 nM, p= 0,297), in agreement with the massive loss of β cells. Analysis of the circulating PSA-MPs values obtained in the transplanted patients using a survival model (function or non-function of the graft) with time-dependent variables of PSA-MPs showed that a longitudinal study in 40 patients would be suitable for statistical validation of an eventual prognosis value of PSA-MPs.

Conclusion: Our data suggest that PSA-MPs might prove valuable for the early and non-invasive detection of pancreatic islet transplant dysfunction. Combination with values of circulating hepatocyte-derived MPs as a signature of the altered host tissue of the islet graft could increase sensitivity. Finally, measurement of circulating MPs of leukocyte origin could add value to this putative monitoring tool by facilitating the identification of the activated immune cells contributing to graft failure or dysfunction.

Disclosure: **L. Amoura:** None.

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Tissue sodium content is increased in type 2 diabetes

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Background and aims: The measurement of tissue sodium content by 23Na magnetic resonance imaging (23Na-MRI) has been validated in experimental and human studies. Increased tissue sodium content has been reported in patients with treatment resistant hypertension (TRH), hyperaldosteronism and chronic renal failure. We analyzed whether tissue sodium content is increased in patients with type-2 diabetes.

Materials and methods: In patients, most of them treated for hypertension, with cardiovascular risk, we compared patients with type-2 diabetes to those with primary arterial hypertension without type-2 diabetes. Baseline clinical characteristics were determined with same methods: fasting glucose, office blood pressure, body weight, and medical history. Patients with type 2 diabetes were off any antidiabetic therapy (antihypertensives were kept constant) and hypertensive patients off any antihypertensive or antidiabetic therapy for at least 4 weeks. At baseline tissue sodium content was assessed non-invasively with a 3.0 T clinical MRI system (Magnetom Verio, Siemens Health Care, Erlangen, Germany) in each patient. Subject placed their lower legs in the center of a 23Na knee coil and sodium content in skin and muscle (musculus triceps surae) were measured. Coefficient of variation of the same images was 2.1 % for skin sodium and 0.5 % for muscle sodium content (inter-reader variability).

Results: In patients with type 2 diabetes (N=59) we observed significantly greater muscle sodium content (diabetes: 20.6 ± 3.7 vs hypertension: 16.3 ± 2.5 mmol/L, p<0.001) and skin sodium content (diabetes: 24.5 ± 7.2 vs hypertension: 20.8 ± 5.9 mmol/L, p=0.01) than in those with primary hypertension (N=33). Age was similar in the two groups (diabetes: 60.3 ± 7.6 vs hypertension 59.6 ± 10.9 years; p=0.72) and body mass index was not significantly different (diabetes: 29.7 ± 4.4 vs hypertension 28.4 ± 4.0 kg/m², p=0.14). Blood pressure was greater in the hypertensive than in diabetes patients (156 ± 8.3 / 92.8 ± 8.0 vs 130 ± 14 / 79.1 ± 9.4 mmHg, both p<0.001). Estimated glomerular filtration was greater in the diabetic than hypertensive patients (89.9 ± 12 vs 83.8 ± 14 ml/min/1.73 m², p=0.03). When these potential confounders (age, body mass index, gender, systolic and diastolic blood pressure, glomerular filtration rate) were entered in the covariance analysis, skin sodium content (p=0.037) and muscle sodium content (p<0.001) were still clearly elevated.

Conclusion: With the innovative 23Na-MRI technology, we could demonstrate that patients with type 2 diabetes have greater tissue sodium content, irrespective of age, blood pressure and other potential confounders (including renal function). Since tissue sodium content is related to organ damage, therapeutic intervention should aim at reducing tissue sodium content.

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Disclosure: **D. Kannenkeril:** None.

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Hyperkalaemia in patients with diabetes: incidence, risk factors, and clinical outcomes. A Danish population based cohort study

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Background and aims: Elevated potassium levels (hyperkalemia, HK) are frequent in clinical practice and associated with increased mortality. Data are scarce on HK in patients with diabetes (DM). We investigated the incidence of HK in patients with incident type 1 or 2 DM, and examined associated risk factors and clinical outcomes.

Materials and methods: Population-based cohort study based on prospective medical and laboratory databases in Northern Denmark during 2000-2012. We included 76,942 people with a first incident record of DM. The incidence rate of HK among patients with DM was calculated based on a first blood test in primary care or hospital with K+ level >5.0 mmol/L. For each patient with DM who developed HK, a comparison DM patient without HK was matched on age, sex, and time since DM diagnosis. Risk factors were compared for DM patients with HK vs. without HK. Clinical outcomes (including acute hospitalizations, cardiac diagnoses, ICU treatment, and death) were assessed before and after HK in a self-controlled analysis, and were compared with DM patients without HK further matched on comorbidity, diabetes complications, and HbA1c level.

Results: The median age of 76,942 patients with incident DM was 62 years, 47% were female, 18% had concomitant chronic kidney disease, 6% congestive heart failure, and 47% hypertension. At baseline, 22% were treated with ACE-inhibitors, 13% with ARBs, and 6% with K+-sparing diuretics (99% spironolactone). During a mean follow-up of 3.4 years (317,980 person-years), 17.6% (n=13,530) of the DM patients had a first event of HK (incidence rate 42 per 1,000 person-years), increasing with age and comorbidity burden. Median time to first HK event was 2 years; 45% of events were hospital-diagnosed. Patients with DM who developed HK had more chronic kidney disease (odds ratio, OR, 2.4; 95% CI: 2.3-2.5), chronic heart failure (3.0; 95% CI: 2.8-3.2), hypertension (1.4; 95% CI:1.4-1.5), and use of ACE-inhibitors (1.3; 95% CI: 1.3-1.4), K^+ supplements (1.9; 95% CI:1.8-2.0), and spironolactone (3.2; 95% CI: 3.0-3.5), compared with DM patients without HK. In DM patients with HK, 55% experienced any acute hospitalization including and within 6 months after the HK event vs. 32% within 6 months before the HK event [before-after relative risk, RR 1.73 (95% CI 1.68-1.79)]. During this time frame, the risk for any cardiac hospital diagnosis increased from 17% to 28% (1.69, 95% CI 1.61-1.77), ICU admissions from 2% to 15% (6.24, 95% CI 5.56-7.01), and cardiac arrest from 0.1% to 0.7% (11.25, 95% CI 5.46-23.17). Six-month mortality following HK was 22%. Compared with matched DM patients without HK, the prior-event-rate-ratio adjusted hazard ratio was 2.14-fold increased for any acute hospitalization 6 months after HK, while the HR for death was 6.16 (95% CI 5.61-6.75).

Conclusion: One out of six patients with DM develops HK; those with concomitant kidney disease and heart failure are at particularly increased risk. HK was found to be associated with severe clinical outcomes and mortality in a real-world population of patients with DM.

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Hypertriglyceridaemia and osteopenia in type 2 diabetic patients and the effect of TG-rich lipoproteins on RANKL expression in cultured osteoblastic cells

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Background and aims: Skeletal fragility is considered among the complications associated with diabetes. Type 2 diabetic patients have increased fracture risk and lower bone mineral density (BMD). Last year in this meeting we reported that there exists diabetic osteopenia. Thus, association between diabetes mellitus and osteopenia has been clear, but little information is available about the effect of hyperlipidemia on osteopenia. Recently it was reported that midlife women with high fasting plasma triglyceride had an increased risk of incident non-traumatic fracture (SWAN study). In this study we examined whether hypertriglyceridemia (HTG) contributes to lower bone mineral density in type 2 diabetic patients. RANKL produced by osteoblastic cells is the essential factor for osteoclast formation, activation and survival, thus resulting in bone resorption and bone loss. We examined whether TG-rich lipoproteins derived from patients affect RANKL expression in cultured osteoblastic cells to elucidate the association between HTG and osteopenia.

Materials and methods: We evaluated 157 postmenopausal type 2 diabetic patients, ages 50-89 years, with HTG (n=80) and without HTG (n=77). HTG was defined as fasting plasma TG higher than 150 mg/dl. They were divided into 4 groups according to the ages; 50-59 years, 60-69 years, 70-79 years and 80-89 years. Lumbar spine BMD and femoral neck BMD were evaluated with dual energy X-ray absorptiometry (DXA) according to the guideline of Japan Osteoporosis Society 2015. BMD was expressed as the percent ratio to the young adult mean BMD. BMD was compared in each age group with and without HTG. Patients who took drugs affecting bone metabolism or had diabetic nephropathy (renal failure stage) were excluded from this study. Apolipoprotein (apo) E2 is one of the strong genetic factors which cause HTG. Apo E genotypes were determined by isoelectric focusing and immunoblotting method. Human osteoblastic cells from Promo Cell Co. USA were cultured, and then were incubated for 24 h with TG-rich lipoproteins at the concentration of 0 and 100 μg/ml. RT-PCR procedure was performed to evaluate the expression of RANKL m-RNA. TG-rich lipoprotein were isolated from patient's plasma by ultracentrifugation.

Results: In 50s diabetic patients with HTG had significantly ($p<0.001$) lower femoral neck BMD than diabetic patients without HTG (73.9 vs 96.6%). In 60s diabetic patients with HTG had significantly ($p<0.001$) lower femoral neck BMD than those without HTG (79.5 vs 86.6%). In 70s diabetic patients with HTG had significantly ($p<0.001$) lower femoral neck BMD than those without HTG (74.9 vs 86.3%). In 80s diabetic patients with HTG tended to have lower femoral neck BMD. Similarly, in each age group diabetic patients with HTG had significantly ($p<0.001$) lower lumbar spine BMD than diabetic patients without HTG (81.0 vs 94.0% in 50s, 79.7 vs 88.6% in 60s, 78.4 vs 92.4% in 70s and 74.7 vs 84.0% in 80s). The frequency of apo E2 genotype was significantly ($p<0.001$) higher in diabetic patients with HTG than in those without HTG (34% vs 10%). In cultured human osteoblastic cells TG-rich lipoproteins significantly ($p<0.001$) stimulated the expression of RANKL mRNA.

Conclusion: This clinical study showed that HTG contributes to lower BMD in type 2 diabetic patients, and that apo E2 genotype is associated with HTG and diabetic osteopenia. TG-rich lipoproteins stimulated the expression of RANKL. This finding explains one mechanism by which HTG contributes to osteopenia.

Disclosure: **M. Eto:** None.

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A hip fracture risk equation for type 2 diabetes: the Fremantle Diabetes Study

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Background and aims: Type 2 diabetes (T2D) is associated with an increased risk of osteoporotic fractures, resulting in disabilities and increased mortality. Currently available fracture risk calculators do not include diabetes-related variables. Therefore we have developed a hip fracture risk calculator for T2D.

Materials and methods: The Fremantle Diabetes Study is a community-based longitudinal observational study. 1251 participants with T2D and aged 40-89 years at study entry were followed from baseline (1993-6) for 10 years or until they experienced a hip fracture or died. Hip fracture during follow-up was identified from linkage with the Western Australian hospital morbidity data collection and death registry. Cox proportional hazards modelling with backward elimination identified clinically plausible independent predictors of hip fracture within 10 years of study entry. Calibration was assessed using the modified Hosmer and Lemeshow test for survival data. Discrimination was assessed by the area under the receiver-operating characteristic curve (*c*-index), and then adjusted for optimism using 1000 bootstrap re-samples (internal validation). Accuracy was assessed by the Brier score (mean squared error; range 0-1, the lower the better). Positive and negative predictive values (PPV and NPV), sensitivity and specificity were determined for a 10% 10-year hip fracture risk. Sensitivity analyses were performed: Multiple imputation (n=20) generated complete datasets, Cox modelling was applied to each imputed dataset, and the results from these multiple analyses pooled to produce overall regression coefficients, with accompanying 95% confidence intervals (CIs). StataIC 13 and IBM SPSS Statistics 22 were used for statistical analyses.

Results: The 1251 participants were on average 65 years old at study entry, 49% were men, with median diabetes duration 4 years and mean BMI 29.4 kg/m^2. During 10,306 patient-years of follow-up, 50 participants (4.0% (95% CI 3.0%-5.3%); 80% women), had at least one hip fracture. Variables in the final Cox model (n=1162 (44 with incident hip fracture)) comprised age, sex, BMI, any difficulties with activities of daily living (ADL), peripheral sensory neuropathy (PSN), and estimated GFR (eGFR) <45 ml/min/1.73m^2. The proportional hazards assumption was not violated overall or for any of the individual variables ($p>0.19$). The mean 10-year predicted risk of hip fracture was 6.0%. Discrimination of the model was good (*c*-index: 0.84 (95% CI 0.79-0.89), $p<0.001$). The optimism-adjusted *c*-index was 0.83 (0.77-0.88). Accuracy (mean squared error (95% CI): 0.04 (0-0.96)) and goodness-of-fit ($p>0.64$)) were also good. The positive and negative predictive values, sensitivity and specificity for a 10% 10-year hip fracture risk cut-off were 37.5% and 96.4%, 90.9% and 67.5%, respectively. The regression coefficients for the final Cox model were similar to those for the pooled imputed estimates and lay well within the 95% CIs.

Conclusion: Available hip fracture risk calculators developed for the general population do not take important diabetes-related variables into account. We found that community-based women, in particular, aged 40-89 years with T2D and PSN, eGFR<45 ml/min/1.73m^2 or who had any difficulty with ADLs had an increased risk of hip fracture. External validation of this prognostic model is underway.

Supported by: Fremantle Hospital Medical Research Foundation

Disclosure: **W.A. Davis:** Grants; Fremantle Hospital Medical Research Foundation.

OP 43 Metformin: oldie but goodie

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The effect of metformin on a healthy human gut microbiota

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Background and aims: Within recent years it has been shown that patients with type 2 diabetes have an altered bacterial composition of their gut microbiota compared with non-diabetic individuals. However, these alterations may be confounded by medication, notably metformin. We investigated adaptations in gut microbiota composition in response to metformin treatment, independent of the diabetic state.

Materials and methods: Twenty-six young, healthy, lean men were enrolled in the study. They underwent a 6-week run-in period, a 6-week intervention period with metformin, and a 6-week wash-out period. During the intervention participants were given 500 mg metformin daily, increasing with 500 mg weekly to a total dosage of 2000 mg daily. Participants were examined five times in the fasting state, with blood-sampling and recording of gastrointestinal symptoms. Examinations took place before and after the run-in period, halfway through and immediately after the intervention and after the wash-out period. Stools were collected at nine, evenly distributed time-points, and bacterial DNA was extracted and subjected to 16S-rRNA-sequencing in order to evaluate gut microbiota composition.

Results: Twenty-three men completed the intervention. The mean (SD) age was 25.7 (2.7) years with a mean BMI of 22.9 (2.1) kg/m^2. Plasma vitamin B12 and HbA1c concentrations declined following intervention (p=0.01 and p=0.03 respectively). The relative abundance of 20 operational taxonomic units (OTUs) changed significantly (10% false discovery rate) during the 6-week intervention. Several OTUs of the order *Clostridiales* were depleted, including one assigned to *Intestinibacter Bartlettii and* two *Clostridium* spp. In contrast, *Alistipes finegoldi* of order *Bacteroidales* and an OTU assigned to genus *Escherichia*|*Shigella* were enriched. All OTUs recovered to pre-intervention levels within 3-6 weeks of treatment cessation. Changes in gut microbiota composition were accompanied by an increase in self-reported intestinal discomfort.

Conclusion: Metformin intake changes the gut microbiota composition, independent of the diabetic state. This intervention substantiates and extends our previous cross-sectional findings in type 2 diabetes patients. More studies are needed to examine to which extent metformin exert its glucose-lowering effect, and adverse gastrointestinal effects, partly by modifying the gut microbiota.

Clinical Trial Registration Number: NCT02546050

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Metformin potentiates bile acid-induced GLP-1 secretion in patients with type 2 diabetes

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Background and aims: The glucose-lowering mechanisms of the biguanide metformin remain to be fully clarified despite a position as the first-line pharmacotherapy in type 2 diabetes. An increasing focus on potential gut-derived modes of action, including suppression of bile acid reabsorption and consequent increased glucagon-like peptide 1 (GLP-1) secretion, has surfaced during recent years. The aim of the present study was to investigate the effects of cholecystokinin (CCK)-induced gallbladder emptying and concomitant single-dose metformin on GLP-1 secretion and glucose metabolism in patients with type 2 diabetes.

Materials and methods: This was a randomized, placebo-controlled and double-blinded cross-over study including 15 metformin-treated patients with type 2 diabetes. Plasma GLP-1 excursion as measured by incremental AUC (iAUC) was the primary endpoint. Each patient was submitted to four experimental study days with administration of either single-dose metformin 1,500 mg or placebo in combination with intravenous infusion of CCK (0.4 pmol/kg/min) or saline. Patients were instructed to pause ongoing treatment with metformin seven days prior to each of the study days. One-way repeated measures ANOVA with Fisher's LSD post hoc test was used to test for differences between the individual study days.

Results: A significant increase in iAUC for GLP-1 was evident following CCK-induced gallbladder emptying ($P < 0.05$), whereas isolated administration of metformin did not meet statistical significance in terms of GLP-1 iAUC compared to the day with placebo and saline ($P = 0.15$). Interestingly, the combination of metformin and CCK elicited a significant potentiation of bile-acid induced GLP-1 secretion with significantly higher iAUC for GLP-1 compared to the day with CCK alone ($P < 0.05$). Single-dose administration of metformin 1,500 in the applied fasting setting was observed to elicit statistically significant placebo-corrected reductions in plasma glucose excursions, both alone ($P < 0.05$) and in combination with CCK-induced gallbladder emptying ($P < 0.05$), whereas no glucose-lowering effect of isolated CCK infusion was observed ($P = 0.73$). No effects of either metformin or CCK infusion were observed with respect to insulin secretion or resting energy expenditure.

Conclusion: In conclusion, we found single-dose administration of metformin to potentiate bile acid-induced GLP-1 secretion in patients with type 2 diabetes, whereas no significant isolated effect of metformin on GLP-1 secretion was observed. The underlying mechanisms remain speculative, but might involve metformin-mediated modulation of bile acid circulation with potential subsequent implications for bile acid receptor activation in the GLP-1-secreting L cells. No clear associations were evident between the observed GLP-1-inducing effects and insulin secretion or plasma glucose excursions, which might be due to an impaired beta cell responsiveness to GLP-1 in patients with type 2 diabetes.

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Delayed-release metformin targeting the lower bowel elicits sustained improvements in HbA_{1c} and fasting glucose with minimal systemic exposure

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Background and aims: Delayed-release metformin (Met DR) is being developed for T2DM patients with chronic kidney disease (CKD Stage 3B or 4). By utilizing an enteric coating to bypass the stomach and upper intestine, Met DR delivers metformin to the lower bowel to retain its gut-based glucose-lowering properties but with greatly reduced absorption and systemic exposure. This

blinded study evaluated 16 weeks of Met DR or Placebo (PBO) in subjects with T2DM; unblinded immediate-release metformin (Met IR) was included as a reference. Due to Met IR restrictions of use/contraindication in CKD Stages 3B and 4, only subjects with CKD Stage 1 or Stage 2 were enrolled.

Materials and methods: 571 subjects with T2DM and CKD Stage 1 or 2 were randomized to 16 weeks of double-blind Met DR (600, 900, 1200, 1500 mg QD) or PBO, or to unblinded 2000 mg Met IR (1000 mg BID; 1000 mg QD for first week). Data are LS mean±SE change from baseline in the modified-ITT (mITT) population (≥1 post-baseline HbA_{1c}).

Results: 542 subjects (56 y; 53% male; 7.9±6.7 y T2DM; BMI 32±5 kg/m^2; HbA_{1c} 8.6±0.9%) were included in the mITT population. Metformin plasma exposure (estimated $AUC_{0\text{-}24h}$) with Met DR was ≤37% of Met IR. Met DR resulted in a significant ($p<0.05$) dose-dependent HbA_{1c} reduction with no plateau (1200 mg: -0.49±0.13%; 1500 mg: -0.62±0.12%; PBO: -0.06 ±0.13%). Met IR 2000 mg elicited a -1.10±0.13% HbA_{1c} improvement. Fasting plasma glucose (FPG) improvement ($C_{average\ Week\ 4\text{-}16}$) was significantly greater with 900-1500 mg Met DR vs PBO, and the effect with 1500 mg Met DR approached that of 2000 mg Met IR (-25.1±4.1 vs -32.6±4.2 mg/dL, respectively; NS). Thus, 1500 mg Met DR exhibited 1.5-fold (HbA_{1c}) and 2.1-fold (FPG) greater relative efficacy than Met IR when adjusting for plasma metformin exposure. Adverse events were primarily gastrointestinal and incidence was lower with Met DR (<16% at all doses) vs Met IR (28%). Nausea incidence was markedly lower with Met DR (1-3% vs 10% Met IR), likely due to Met DR bypassing the stomach.

Conclusion: The improved efficacy relative to plasma exposure with Met DR may result in an improved benefit/risk profile for patients with CKD Stage 3B or 4, in whom minimizing exposure is desirable. Delivery of Met DR at higher doses may result in greater efficacy but at the expense of increased systemic exposure. Importantly, reducing current metformin dosage in an attempt to achieve the low exposure of Met DR would result in a disproportionate loss in efficacy.

Clinical Trial Registration Number: NCT02526524

Disclosure: **M. Fineman:** Employment/Consultancy; Elcelyx Therapeutics. Stock/Shareholding; Elcelyx Therapeutics.

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Metformin exposure with gut-restricted delayed-release metformin in CKD Stage 4 does not exceed that of current metformin used on-label: results from population PK modelling

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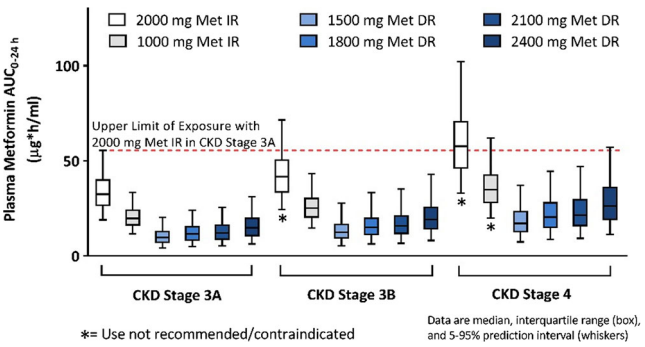
Background and aims: By utilizing an enteric coating to bypass the stomach and upper intestine, delayed-release metformin (Met DR) delivers metformin to the lower bowel and thereby retains its gut-based glucose-lowering properties but with greatly reduced absorption and systemic exposure. The relative bioavailability of Met DR from doses of 1500 mg to 2400 mg is ~40% of that seen with typical clinical doses of immediate-release metformin (Met IR). Thus, a dose of 2400 mg Met DR results in similar systemic exposure to 1000 mg Met IR. In a 16-week study, 1500 mg Met DR provided 56% (HbA_{1c}) and 77% (fasting glucose) of the efficacy of 2000 mg Met IR with only 37% of the exposure. Taken together, 1500 mg Met DR exhibited 1.5-fold (HbA_{1c}) to 2.1-fold (fasting glucose) greater efficacy relative to exposure than 2000 mg Met IR. This greater efficacy/exposure ratio may provide an improved benefit/risk profile in patients for whom minimizing metformin exposure is desirable, such as patients with advanced chronic kidney disease (CKD). Metformin is currently restricted in CKD Stage 3B and contraindicated in CKD Stage 4 due to lactic acidosis risk secondary to increased systemic metformin exposure. Met DR may provide a treatment option for these patients. To describe the range of exposures expected in patients with CKD Stage 3B/4, a population PK model was developed to characterize the absorption and disposition of Met IR, extended-release metformin (Met XR), or Met DR at varying doses in subjects across a range of renal impairment.

Materials and methods: The population PK model was developed using 5,854 plasma and 762 urine observations from 108 subjects who received orally administered single or multiple doses of Met IR, Met XR, or Met DR in subjects with varying degrees of renal impairment. Each simulation comprised 1000 subjects with varying body weight and renal function. eGFR values were assumed to arise from uniform distributions between 45-59.5 mL/min/1.73 m^2 (CKD Stage 3A), 30-44.5 mL/min/1.73 m^2 (CKD Stage 3B), and 15-29.5 mL/min/1.73 m^2 (CKD Stage 4). The simulations also assumed 50% females and 61% Caucasians. Met DR relative bioavailabilities were estimated from noncompartmental analyses of data from two comparative bioavailability studies.

Results: Predicted steady-state exposures ($AUC_{0\text{-}24h}$) with Met DR doses of up to 2100 mg were no higher in CKD Stage 4 than those with on-label use of 2000 mg Met IR in patients with CKD Stage 3A.

Conclusion: We conclude that Met DR at doses up to 2100 mg daily may provide a favorable benefit/risk profile for patients with CKD Stage 3B currently receiving metformin, or a novel option to initiate metformin for patients with CKD Stage 3B/4.



Disclosure: **G. Bakris:** Employment/Consultancy; Merck, Elcelyx. Other; AbbVie, Janssen, Bayer.

OP 44 Novel aspects in the regulation of glucose metabolism

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Fatty liver disease is associated to increased glucogenic amino acids and decreased de novo alanine synthesis

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Background and aims: Amino acids (AA) are the main substrate of muscle proteins and several studies have shown that insulin resistance (IR) is associated to increased protein catabolism with net release of AA. In fasting state, AA, in particular alanine and glutamine, are used as substrate for de novo gluconeogenesis and energy metabolism. During postprandial state, alanine and glutamine are synthesized from glucose and used by the muscle for protein synthesis. The goal of this study was to evaluate 1) if concentrations of glucogenic AA (GNG-AA) were increased in condition of hepatic insulin resistance (Hep-IR) like obesity or NAFLD, 2) if de novo synthesis of alanine/glutamine after a labeled glucose load was associated to severity of NAFLD, explaining the increased risk of sarcopenia present in these subjects.

Materials and methods: We studied 44 non diabetic NAFLD subjects with liver biopsy (29 NAFLD-NO and 15 NAFLD-Ob) and 20 non-obese controls (CT-NO). We measured plasma GNG-AA (alanine, glutamate, branched chain amino acids (BCAA), and aromatic amino acids (AAA)) by GCMS, fasting EGP (by tracer infusions), and calculated Hep-IR (EGPxInsulin), HOMA, Adipo-IR (FFAxInsulin). We also developed a novel protocol to study de-novo AA synthesis by measuring the rate of de-novo alanine, glutamine and glutamate synthesis from U-^{13}C-glucose given orally. We studied 20 subjects with a double tracer OGTT (1.5g U-^{13}C-glucose plus 75g of unlabeled glucose), and ^{13}C-AA production was measured by GCMS

Results: From CT-NO to NAFLD-NO to NAFLD-Ob we observed an increase in total EGP (584±44 to 710±23 to 839±40 umol/min, p<0.0002) and Hep-IR (52±6 to 96±6 to 166±23 umol/kg/min x mU/l, p<0.001). Fasting GNG-AA, in particular alanine and glutamate, were also higher in NAFLD (NAFLD-ob 1588±98, NAFLD-NO 1467±36, CT 1296±55 umol/l, p<0.005). Total GNG-AA positively correlated with both EGP (R=0.47 and p=0.0006) and Hep-IR (R=0.35, p=0.0096). This was associated to an increased protein catabolism as indicated by the higher BCAA concentration in NAFLD compared to controls (NAFLD-ob 355.3±9.8, NAFLD-NO 389.7±23.4, CT 313.1 ±12.9 umol/l, p<0.001). We followed de-novo alanine, glutamine and glutamate synthesis after a 13C-glucose load but we were able to detect reliable levels of 13C-enrichment only in alanine, while the 13C-incorporation into glutamine or glutamate was very low. De-novo alanine production was positively correlated with glucose rate of disappearance (R=0.45, p=0.04) indicating that reduced peripheral insulin sensitivity is playing a major role. The alanine de-novo synthesis tended to be reduced in NAFLD with severe fibrosis independent of obesity.

Conclusion: Subjects with NAFLD exhibit increased GNG-AA and BCAA concentrations and reduced alanine turnover that might explain the increased risk of Hep-IR and sarcopenia.

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Global and tissues-specific Ogt deletion revealed various effects of O-GlcNAcylation in glucose metabolism

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Background and aims: Post-translational modifications to intracellular proteins regulate a variety of cellular functions. Growing evidence indicates that dysfunction of post-translational modifications disrupts cellular homeostasis and is strongly associated with the pathogenesis of numerous diseases. O-GlcNAcylation is one of the post-translational modifications that is characterised by the addition of *N*acetylglucosamine to various proteins by O-GlcNAc transferase (Ogt) and serves as "an intracellular nutrient sensor" by modulating various cellular processes. Although it has been speculated that O-GlcNAcylation is associated with glucose metabolism, its exact role in whole body glucose metabolism has not yet been fully elucidated. Here, we investigated whether loss of O-GlcNAcylation globally and in specific organs affected glucose metabolism in mammals under physiological conditions.

Materials and methods: Tamoxifen-inducible global *Ogt* knockout mice were generated by crossbreeding *Ogt*-flox mice with R26-Cre-ER^{T2} mice. Liver-, skeletal muscle-, adipose tissue- and pancreatic beta cell-specific *Ogt* knockout mice were generated by crossbreeding *Ogt*-flox mice with Alb-Cre, Mlc1f-Cre, Adipoq-Cre and Pdx1^{PB}-CreER^{TM} mice, respectively. Various metabolic phenotypes including glucose metabolism were evaluated in each model.

Results: Tamoxifen-inducible global *Ogt* knockout (*Ogt*-KO) mice exhibited severe weight loss (*Ogt*-flox 29.1±1.4 vs. *Ogt*-KO 9.8±0.46 g, p<0.01), hypoglycaemia (5.65±0.17 vs. 4.87±0.15 mmol/l, p<0.01) and hypoproteinemia (4.3±0.1 vs. 2.9±0.3 g/dL, p<0.01) 3 week after Tamoxifen injection, although food intake was preserved. Moreover, tissue-specific *Ogt* deletion from insulin-sensitive organs, including liver, skeletal muscle and adipose tissues, had little impact on glucose metabolism under physiological conditions. However, pancreatic beta cell-specific *Ogt* knockout (*Ogt*-betaKO) mice displayed transient hypoglycaemia (*Ogt*-flox 5.46±0.41 vs. *Ogt*-betaKO 3.88±0.26 mmol/l, p=0.015) associated with enhanced insulin secretion and accelerated adiposity, followed by subsequent hyperglycemia (*Ogt*-flox 6.34±0.32 vs. *Ogt*-betaKO 26.4±2.37 mmol/l, p<0.01) with insulin depletion accompanied by beta cell apoptosis.

Conclusion: Our findings suggest that O-GlcNAcylation is indispensable for survival also in adult mice. O-GlcNAcylation does not impact glucose metabolism in insulin-sensitive tissues but plays crucial roles in pancreatic beta cell function, and survival under physiological conditions. Our results provide novel insight into O-GlcNAc biology and physiology in glucose metabolism.

Supported by: JSPS

Disclosure: **S. Ida:** None.

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A novel stepwise eu-, hyper- and hypoglycaemic clamp using stable tracer method to compare glucose fluxes and lipolysis in type 1 and type 2 diabetes

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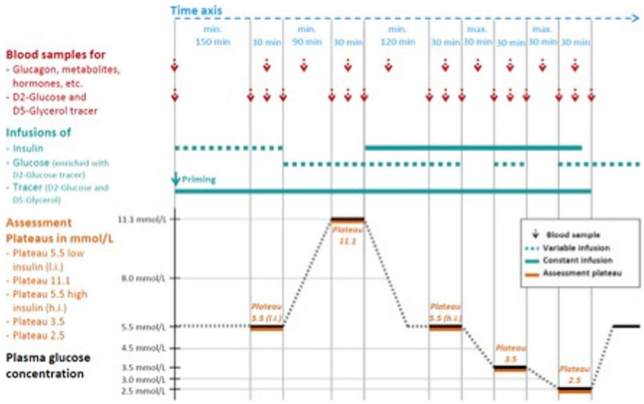
Background and aims: Recently it was proposed that some diabetes medications shift the fuel balance towards lipolysis and ketogenesis. The aim of this study was to establish a novel stable tracer method to assess endogenous glucose production (EGP) and lipolysis (rate of appearance [Ra] glycerol) during eu-, hyper-, and hypoglycaemia in type 1 (T1DM) and type 2 diabetes mellitus (T2DM).

Materials and methods: A stepwise eu- (5.5 mmol/L) and hyperglycaemic (11.1 mmol/L) clamp with ambient insulin levels was followed by an insulin induced (T1DM: 1.5 mU/kg/min; T2DM: 2.5 mU/kg/min) hypoglycaemic

clamp (5.5, 3.5, 2.5 mmol/L). Primed tracer infusion of [6,6-2H2]glucose (prime: 9.6 mg/kg, constant: 0.08 mg/kg/min) and [1,1,2,3,3-2H5]glycerol (prime: 1.5 μmol/kg, constant: 0.1 μmol/kg/min) was used to assess EGP, peripheral glucose uptake (PGU) and Ra glycerol throughout all glycaemic levels (Fig.). Glucagon was measured by specific double sandwich ELISA (Mercodia AB, Sweden), all other hormones by routine assays, isotopic glucose and glycerol by gas-chromatography mass spectrometry. Ten C-peptide negative T1DM (age 42±14 ys, diabetes duration 21±13 ys, BMI 23.2±2.0 kg/m^2, HbA1c 60±11 mmol/mol) were compared to 17 metformin only treated T2DM patients (age 53±6 ys, diabetes duration 6.6±5.1 ys, BMI 28.9±3.3 kg/m^2, HbA1c 61±11 mmol/mol).

Results: Glucagon levels were significantly lower in T1DM compared to T2DM during euglycaemia (2.2±1.1 vs. 9.4±4.1 pmol/L; p<0.001) and during hypoglycaemia (at 2.5 mmol/L: 1.5±1.1 vs. 18.9±14.0; p<0.001). At euglycaemia insulin (17.0±8.0 vs. 28.7±48.3 mU/L, ns), EGP (2.3±0.4 vs. 2.2±0.5 mg/kg/min; ns), Ra glycerol (2.5±0.8 vs. 2.0±0.9 μmol/kg/min; ns) and ketones (0.09±0.06 vs. 0.06±0.04 mmol/L; ns) were comparable in T1DM and T2DM. During hyperglycaemia, EGP (3.5±1.8 vs. 1.8 ±0.4 mg/kg/min, p<0.02), Ra glycerol (4.1±2.0 vs. 2.3±0.9 μmol/kg/min; p<0.02) and ketones (0.85±0.36 vs. 0.14±0.15 mmol/L; p<0.001) were substantially higher in T1DM, but insulin lower (5.3±4.9 vs. 30.8±38.4 mU/L; p<0.02). During induction of hypoglycaemia EGP and ketones were comparable, but T2DM had higher Ra glycerol (1.3±0.4 vs. 0.9 ±0.3 μmol/kg/min; p<0.01) despite higher insulin levels (229±42 vs. 104±38 mU/L; p<0.001). NEFA levels were not different throughout the clamp.

Conclusion: This novel stepwise clamp using a stable tracer method allows assessment of glucose fluxes (EGP and PGU) and lipolysis (Ra glycerol) over a wide range of glycaemic levels in subjects with diabetes. Distinct differences in fuel shifts (e.g.in lipolysis) are not detected by NEFA measurement, but by rate of appearance of glycerol using a stable tracer method.



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Disclosure: **E. Svehlikova:** None.

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Mild physiologic hyperglycaemia induces hepatic insulin resistance in healthy Normal Glucose Tolerant (NGT) subjects

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Background and aims: Chronic hyperglycemia worsens skeletal muscle insulin resistance and causes beta cell dysfunction; i.e. glucotoxicity. However, effect of mild chronic hyperglycemia on hepatic insulin sensitivity never has been examined. The aim of the present study was to evaluate effect of a physiologic increase in plasma glucose concentration on endogenous glucose production (EGP) in healthy NGT individuals.

Materials and methods: 16 NGT subjects: 8 with family history of T2DM (FH+((5M/3F, age = 47± 14 yrs, BMI = 27 ± 1 kg/m^2) and 8 without FH (FH-) (4M/4F, Age = 42±9, BMI = 26±1 kg/m^2) participated in a 3-step hyperinsulinemic (10, 20, 40 mU/m^2·min) euglycemic clamp before and after a 48 hour glucose infusion to increase plasma glucose conc by ~50 mg/dl above baseline. Endogenous (primarily reflects liver) glucose production was measured with [3-3H] glucose and gluconeogenic rate was measured as (EGP) (plasma C5/ C2 glucose ratio) using the deuterated water method.

Results: FPG concentration increased from 97± 4.5 to 138± 5 mg/dl following 48 hours of glucose infusion while basal EGP increased from 2.05±0.09 to 3.06±0.29 mg/kg•min (p=0.003) and hepatic insulin resistance index (EGP X Fasting Plasma Insulin) increased markedly and similarly (20.1±1.8 vs 51.7± 6.6, p<0.005) following glucose infusion in FH+ and FH- subjects. Following chronic glucose infusion, the rate of gluconeogenesis (5.58± 1.8 vs 5.78±2.06 μmol/kg•min) remained unchanged. The rate of glycogenolysis (total EGP minus gluconeogenic rate) increased following chronic glucose infusion (6.55± 3.3 to 8.25±2.4 μmol/kg•min, p<0.10). Thus, both increased glycogenolysis and gluconeogenesis contributed to the increase in basal EGP following chronic glucose infusion. Following 48 hr glucose infusion, total body insulin sensitivity declined from 8.75±2.1 to 6.92± 6 mg.kg/min (p<0.05) during the 40 mU/m^2•min insulin clamp. There was no significant change in plasma glucagon or free fatty acid concentrations.

Conclusion: Chronic (48 hr) physiologic hyperglycemia to levels seen in T2DM increases basal endogenous (hepatic) glucose production and induces hepatic and skeletal muscle insulin resistance in healthy NGT subjects. These results demonstrate, for the first time in man, that glucotoxicity causes hepatic insulin resistance to the suppressive effect of insulin on hepatic glucose production.

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OP 45 Classifying diabetes

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Clustering of diabetes into novel subgroups provides improved prediction of outcome

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Background and aims: The current classification of diabetes into two main forms (T1D and T2D) has been useful in delineating T1D as an insulin-deficient form requiring insulin therapy but less useful for dissecting the heterogeneity of T2D. A refined classification could provide a powerful tool to identify those at greatest risk of complications already at diagnosis and tailor individualized treatment.

Materials and methods: We performed unsupervised clustering (k-means and hierarchical) based on six variables (age, BMI, HbA1c, HOMA2-B, HOMA2-IR and GAD auto-antibodies) in ANDIS, a Swedish cohort of 15,000 newly diagnosed diabetes patients. Disease progression, treatment and development of complications were followed using medical records and national registries. Risk of complications was analyzed using cox regression with the largest cluster as reference. Genetic loci known to be associated with T2D and related traits were analysed using MLE comparing each cluster to a non-diabetic cohort from the same geographical region.

Results: ANDIS patients clustered into one GADA-positive cluster (referred to as SAID, Severe Autoimmune Diabetes) and four GADA-negative clusters. This was replicated in three independent cohorts from Sweden and Finland. Cluster 2 (SIDD, Severe Insulin Deficient Diabetes; 17.5% of patients) was characterized by early onset, insulin deficiency and high HbA1c. During a mean follow-up of 4 years SIDD had higher HbA1c, was more likely to be prescribed insulin and develop diabetic retinopathy compared to other GADA-negative clusters. Cluster 3 (SIRD, Severe Insulin Resistant Diabetes; 15.3%) had the highest risk of kidney disease including CKD stage 3B (HR 3.30[2.67-4.08], $p=3.6\times10^{-28}$) and macroalbuminuria/end-stage renal disease (ESRD; HR 2.40[1.69-3.42], $p=9.8\times10^{-7}$). This was replicated in a cohort with longer follow-up (SDR, mean 11 years), where SIRD had five-fold increased risk of ESRD (HR 5.04[2.76-9.23], $p=1.5\times10^{-7}$). Cluster 4 (MOD, Mild Obese Diabetes; 21.6%) and cluster 5 (MARD, Mild Age-Related Diabetes; 39.1%) showed only modest metabolic derangements. Using $p<0.01$ as cut-off, no genetic variant was associated with all clusters. Strikingly, the T2D-associated variant, rs7903146, in the *TCF7L2* gene was associated with SIDD (OR 1.51[1.33-1.71], $p=2.8\times10^{-10}$), MOD (OR 1.38[1.21-1.56], $p=25.7\times10^{-7}$) and MARD (OR 1.41 [1.28-1.55], $p=1.1\times10^{-12}$), but not with SIRD (OR 1.00[0.87-1.15], p=0.86). A variant in *IGF2BP2* (rs4402960) was associated with SIDD (OR 1.23[1.08-1.40], $p=2.0\times10^{-4}$) and MARD (OR 1.22[1.11-1.33], $p=2.1\times10^{-6}$), but not with SIRD (OR 1.01[0.88-1.16], p=0.53) or MOD (OR 1.04[0.92-1.18], p=0.31).

Conclusion: This study provides a first step towards a more precise, clinically useful, classification of diabetes representing an important step towards precision medicine in diabetes.

Supported by: Swedish Research Council, ERC, Novo Nordisk Foundation, ALF

Disclosure: **E. Ahlqvist:** None.

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Clustering on baseline clinical variables identifies subgroups of type 2 diabetes patients with different rate of progression over 18 months: a DIRECT study

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Background and aims: Type 2 diabetes is characterized by a heterogeneous presentation with varying degrees of insulin resistance and β-cell failure. Taking advantage of the detailed clinical information collected for type 2 diabetes patients included in the DIRECT study, our aim was to characterize inter-individual heterogeneity and identify subgroups with different presentations of diabetic phenotypes at baseline, and investigate the effect on the rate of progression during follow-up. Genotyping data was used to evaluate genetic differences between subgroups.

Materials and methods: 836 newly diagnosed individuals were enrolled in the DIRECT study. The clustering was based on 20 clinical variables from the baseline visit consisting of anthropometric, biochemical and glycaemic modeling variables. We clustered the individuals using an unsupervised, agglomerative clustering method. Diabetes progression was assessed using individual HbA1c slopes obtained from a conditional linear mixed-model using data at 0, 9 and 18 month adjusted for weight, diabetes medication and baseline HbA1c. An extra time-varying covariate defined to be 1 at baseline and zero for all other visits was introduced to account for presence of effect from baseline to subsequent visits. Linear regression models with cluster membership as predictor was used to calculate effect, 95% CI and p-values for differences between subgroups. A genetic risk score (GRS) was calculated from the cumulative number of risk alleles of 65 published GWAS SNPs for type 2 diabetes.

Results: Full clinical data was available for 790 individuals. We identified three major patient subgroups that differed significantly in regards to their baseline characteristics. The three groups could broadly be described as insulin resistant (IR), β-cell deficient (βD), and mixed. The most significant difference was seen in insulin sensitivity (ln(Matsuda), beta (95% CI): Mixed= -0.61 (-0.68- -0.55), $p=9.9*10^{-68}$; IR=-1.3 (-1.3- -1.2), $p=4.9*10^{-203}$) compared to the βD group, but also insulin secretion, C-peptide, BMI, basal glucose, triglycerides and ALT ($p<10^{-16}$). The IR group was treated with significantly more metformin compared to the βD group (% maximum dose: IR= 5.13 (1.79-8.47), p=0.003). Investigating the rate of progression in HbA1c between baseline and 18 months showed that the IR group had the fastest progression compared to the βD group, which had the slowest progression (change in HbA1c (mmol/mol)/year: IR=0.77 (0.32-1.22), p=0.0008). There was no difference for the GRS constructed of 65 GWAS SNPs.

Conclusion: We have demonstrated that the newly diagnosed type 2 diabetes cohort from DIRECT has a heterogeneous presentation of their diabetic phenotype at baseline. Clustering identified three major subgroups, driven by their level of insulin resistance-related traits. The subgroups showed differences in their rate of progression over 18 months with the insulin resistant group showing the fastest progression.

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Disclosure: **C.A. Brorsson:** None.

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Single antibody positivity associates with two distinct phenotypes in people with young-onset diabetes: insights from the MY DIABETES study

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Background and aims: Pancreatic autoantibody status may be used as a biomarker to differentiate diabetes subtype. Single antibody positivity in individuals presenting at older age has uncertain significance, but has not been

fully explored in young-onset individuals. Using a cross-sectional cohort we sought to determine the phenotype of individuals with single antibody positivity.

Materials and methods: The MY DIABETES study recruits people diagnosed <30 years with any type of diabetes from white, south Asian & African-Caribbean ethnicity. Participants are phenotyped using fasting serum C-peptide (FCP) and anti-GAD-65, IA-2 & ZnT8 autoantibodies. We studied 3 groups; those with single antibody positivity (AB+), those with multiple antibodies positive (AB++) and those negative (AB-). We focussed analysis on people with residual beta cell function (FCP>99pmol/L). Clinical features of those with FCP>99pmol/L were compared across the 3 antibody groups.

Results: 229 of 701 (33%) individuals were AB+ at assessment (66% GAD, 17% IA-2 & 17% ZnT8), 165 (24%) were AB++ & 307 (44%) were negative to all three antibodies. Features of AB+: Of these 36 (17 %) had an FCP>99 pmol/L. People with FCP>99pmol/L were diagnosed older (24.5 vs 16 yrs, p<0.0001) with shorter durations (7.9 vs 22 yrs p<0.0001), had higher BMI (28 vs 25kg/m^2 p=0.02), were more likely to be from a non-white ethnic group (55% vs 20% p=0.003) & less likely to be insulin-treated (77% vs 100%) than those with FCP<100pmol/L. AB+ vs AB++: 39 of 165 (21%) individuals with AB++ had a FCP>99pmol/L. Compared to this group, people with AB+ & FCP>99pmol/L had longer durations of diabetes (7.9 vs 1.2 yrs p =0.0001), significantly higher FCP (467 vs 265 pmol/L p=0.005), less likely to be insulin-treated (77% vs 92% p=0.04) and no ethnic differences were observed. AB+ vs AB-: 127 of 307 (41%) individuals that were AB- had FCP>99pmol/L. Compared to this group, people with AB+ and FCP>99pmol/L had similar ages at diagnosis (21 vs 24.5 yrs p=0.22), durations (10.7 vs 7.9 yrs, p=0.42), BMI (28.1 vs 27.9 p=0.9) & FCP (580 vs 467, p=0.18). No differences in ethnicity or proportion insulin treated were observed. Key Findings: 1) An FCP cut-off of 100pmol/L identified two distinct AB+ groups; those with long duration who are FCP deficient and those with shorter durations who have preserved FCP. 2) Despite longer duration of diabetes, the AB+ group with preserved FCP has significantly higher FCP than the AB++. 3) The AB+ group with preserved FCP is non-significantly different from the AB- group.

Conclusion: Single antibody positivity in young-onset diabetes associates with two phenotypes; a long-duration FCP deficient phenotype similar to those with multiple antibodies positive & a shorter duration phenotype with preserved FCP. This latter group aligns more closely to the AB- group than the AB++ group with preserved FCP, suggesting mixed diabetes aetiology. This group requires further study to investigate if they represent a discrete phenotype of type 1 diabetes or non-type 1. In a multi-ethnic young-onset diabetes population, single antibody positivity may be associated with a non-type 1 diabetes phenotype. Individuals with single antibody positivity require more detailed follow-up and FCP assessment to determine subtype of diabetes.

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Disclosure: **S. Misra:** None.

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Overweight, obesity, genetic susceptibility and the risk of LADA: Latent Autoimmune Diabetes in Adults

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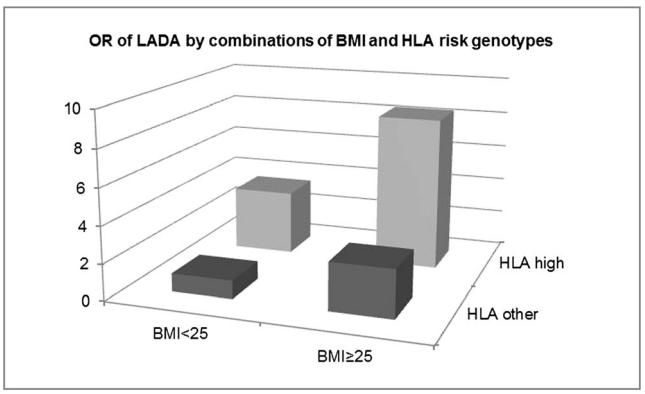
Background and aims: Excessive weight is a major risk factor for type 2 diabetes (T2D) but its role in the promotion of autoimmune diabetes is not clear. We investigated the risk of latent autoimmune diabetes in adults (LADA) in relation to overweight and obesity and the interaction with high risk HLA-genotypes using data from a large population-based case control study from Sweden.

Materials and methods: We used data from ESTRID (epidemiological study of risk factors for LADA and T2D), including incident cases of LADA (Glutamic acid decarboxylase antibodies (GADA) positive, n=425) and T2D (GADA negative, n=1420) from ANDIS/ANDIU and 1704 population-based matched controls. We present odds ratios (OR) with 95% confidence intervals (CI) adjusted for age, sex, family history of diabetes, physical activity, and smoking. HLA genotypes where categorized as high risk (DR4-DQ8, DR4/3-DQ8, DR3/4, DR3/3, DR4/4, and DQA1*0501-DQB1*0201) or other (DR3/X, DR4/X, DR4-DQ7, and DRX/X). Interaction between overweight (BMI ≥25 kg/m^2) and high risk HLA was assessed by attributable proportion due to interaction (AP).

Results: Obesity (BMI ≥30 kg/m^2) was associated with an increased risk of LADA (OR 2.93; CI 2.17-3.97) and even more so with T2D (OR 18.88; CI 14.29-24.94). The association was stronger in LADA with low GADA (<median) (OR 4.25; CI 2.76-6.52) than LADA with high GADA (OR 2.14; CI 1.42-3.24). The combination of overweight and high risk HLA yielded an OR 8.40; CI 5.04-14.00 with AP estimated at 0.39 (CI 0.13-0.64). Obese vs. normal weight LADA patients had lower GADA levels (51 vs. 250 vs. IU/ml, p<0.001), better β-cell function [HOMA] (26.1 vs. 60.8, p<0.0001) and less insulin sensitivity [HOMA] (32.1 vs. 54.1, p<0.0001).

Conclusion: Overweight/obesity is associated with increased risk of LADA, especially in carriers of high risk HLA genotypes. Obese LADA patients had a more type-2 like phenotype, but high BMI was also associated with more autoimmune LADA. These findings support the hypothesis that even in the presence of autoimmunity, factors linked to insulin resistance such as excessive weight will promote onset of diabetes.



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OP 46 Diabetic foot: from mechanism to therapy

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Early skin aging in diabetes is associated with a decrease in CB1 cannabinoid receptor expression

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Background and aims: The G protein-coupled metabotropic cannabinoid receptor type-1 (CB1R) is a major regulator of metabolism, growth and inflammation. Yet, its potential role in the skin is little understood. We found that CB1R expression was decreased in diabetic mouse skin. Our aim was to evaluate the role of CB1R in the skin alterations related to aging in diabetes with the use of a CB1R knockout mice model.

Materials and methods: We quantified by q-RT-PCR markers of proliferation, inflammation, angiogenesis, oxidative stress, collagen (COL1A2, COL3A1) in the skin of wyld-type control (WT), WT streptozotocin (STZ)-induced diabetic mice (DM WT), CB1R knockout (CB1RKO) and DM CB1RKO mice. We stained the skin collagen with trichrome masson. Also, we measure the levels of reactive oxygen species (ROS) and the macrophage phenotype, M1 and M2, in skin by immunohistochemistry.

Results: We found that the mRNA CB1R expression and the CB1R protein levels were decreased in the skin of diabetic mice (0.5±0.03 fold-change and 72.6±5.3% of WT, respectively, $p<0.05$). The expression of collagen was significantly decreased in diabetic and CB1RKO mice (COL1A2, 0.29±0.1 and 0.28±0.03 fold-change, respectively, $p<0.01$; and COL3A1, 0.24±0.1 and 0.19±0.04 fold-change, respectively, $p<0.01$), and similar alteration was found in DM CB1RKO mice, suggesting an early ageing process. The absence of CB1 receptors significantly augmented the expression of several inflammatory markers, interleukin-6 (IL-6, 5.6±1.5 fold-change, $p<0.01$), keratinocyte-derived chemokine (KC, 5.2±2.2 fold-change, $p<0.01$), and tumor necrosis factor-alpha (TNF-α, 3.7±0.6 fold-change, $p<0.01$), and similar levels were found when compared with DM WT and DM CB1RKO mice. Moreover, the ratio M1/M2 macrophage and the ROS levels were significantly higher under diabetic conditions (M1/M2: DM WT, 0.4±0.03 comparing with WT, 0.2±0.03, $p<0.05$, and ROS: DM WT, 189±16.9% of WT, $p<0.05$) and in CB1RKO mice (M1/M2: CB1RKO, 0.5±0.07 comparing with WT, 0.2±0.03, $p<0.05$, and ROS: DM WT, 176±21.3% of WT, $p<0.05$), which was consistent with the decrease in the antioxidant capacity of the skin, particularly a decrease in superoxide dismutase (SOD)-1 (DM WT: 0.58±0.1 fold-change, CB1RKO: 0.69±0.1 fold-change, DM CB1RKO, 0.39±0.1 fold-change, $p<0.05$), catalase (DM WT: 0.41±0.1 fold-change, CB1RKO: 0.49±0.1 fold-change, DM CB1RKO, 0.36±0.1 fold-change, $p<0.05$) and heme-oxigenase1 (Hox1) expression (DM WT: 0.35±0.1 fold-change, CB1RKO: 0.51±0.1 fold-change, DM CB1RKO, 0.1±0.03 fold-change, $p<0.05$).

Conclusion: Our results indicate that the lack of CB1R impairs the expression of markers involved in the control of inflammation and tissue regeneration. These lead to accelerated skin aging by the increased production of reactive oxygen species, a decrease in the antioxidant defenses and a higher pro-inflammatory environment. We conclude that the decreased CB1R expression may be a major pathologic mechanism in accelerated skin aging in diabetes.

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Relation to skin microvessel proliferation and vascular endothelial growth factor expression to microvascular complications in patients with type 2 diabetes

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Background and aims: Previous studies have indicated the involvement of vascular endothelial growth factor (VEGF) in the pathophysiology of diabetic neuropathy, retinopathy and nephropathy, but the expression of VEGF in skin microvasculature in relation to type 2 diabetic microvascular complications has yet to be fully explored.

Materials and methods: The current study enrolled 26 patients with type 2 diabetes (20 males/6 females; mean (SD) age, 51 (16) years; HbA1c, 76 (25) mmol/l), and 16 male non-diabetic subjects (mean (SD) age, 41 (10) years) with fasting plasma glucose levels of <5.6 mmol/l and HbA1c of <33 mmol/mol. All participants provided written informed consent. Three-mm skin biopsies were performed 10-cm above the lateral malleolus, and 60-μm-thick cryostat sections of the skin were double immunostained with type 4 collagen and VEGF-A or the pan-neuronal marker protein gene product (PGP) 9.5. Projected images of 30-μm optical sections at 2-μm increments were acquired by confocal microscopy to quantify the occupancy rate of subepidermal type 4 collagen-immunoreactive microvascular basement membrane area (ORT4C), the subepidermal VEGF-A/type 4 collagen immunoreactivity ratio (VEGF/T4C), and the linear density of PGP 9.5-immunoreactive intra-epidermal nerve fiber (IENF) in three dimensions. IENF density (IENFD) was deemed to be reduced if it was <7.4 fibers/mm. Diabetic retinopathy was detected by ophthalmoscopy and fluorescein angiography. Diabetic nephropathy was defined by the presence of either microalbuminuria or macroalbuminuria.

Results: IENFD was significantly ($P<0.001$) decreased in diabetic patients compared with non-diabetic subjects (median (interquartile range); 5.8 (0.4-9.6) fibers/mm vs. 13.9 (11.6-17.5) fibers/mm). Nine diabetic patients had reduced IENFD, 10 had retinopathy, and 15 had nephropathy. ORT4C was significantly ($P<0.001$) increased in diabetic patients compared with non-diabetic subjects (median (interquartile range); 3.90 (3.08-5.35) % vs. 2.43 (1.88-2.97) %) regardless of the presence or absence of reduced IENFD, retinopathy, or nephropathy. VEGF/T4C was significantly ($P=0.005$) decreased in diabetic patients compared with non-diabetic subjects (mean (SD); 0.13 (0.09) vs. 0.29 (0.20), regardless of the presence or absence of retinopathy or nephropathy, while it was significantly ($P=0.006$) decreased in diabetic patients with reduced IENFD, but not in those without.

Conclusion: The present study provided the first direct evidence of skin microvascular basement membrane thickening and its impaired expression of VEGF, which occur before the emergence of chronic microvascular complications and may represent part of the earliest manifestations of generalized microangiopathy in patients with type 2 diabetes. Whether basement membrane thickening and impaired VEGF expression in the skin microvasculature can be a novel therapeutic target to prevent late complications warrants further investigation.

Supported by: MHLW

Disclosure: **K. Sugimoto:** None.

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Local administration of microRNA-210 promotes wound healing in db/db diabetic mice

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Background and aims: Diabetic foot ulcers represent a major medical and socioeconomic challenge to the global healthcare system. The current therapeutic strategies are limited and specific treatment avenues are still being pursued. A better understanding of the pathogenic mechanisms underlying

the chronic foot ulcers would lead to novel therapeutic strategies. Besides hyperglycemia, chronic wound hypoxia has been recently shown to play important role in delayed wound healing. microRNAs (miRNAs) are a major class of small RNAs which are 20-24 nucleotide long oligonucleotides that inhibit gene transcription by translation inhibition or mRNA degradation. miRNAs have been recently implicated in various diseases and its therapeutic value is still evolving. There is increasing evidence for the role of various miRNAs in wound healing. miR-210 is a major hypoxia-responsive miRNA upregulated by Hypoxia Inducible Factors (HIF). miR-210 mediates various hypoxic responses such as mitochondrial metabolism, cell survival and angiogenesis reflecting HIF function in hypoxia. In this study, we intend to study the role of microRNA-210 in diabetic wound healing.

Materials and methods: The effect of diabetes on miR-210 expression in skin and wounds was studied in the type 2 diabetic mouse model db/db mice and controls. The wound model consists of full-thickness wounds made on the dorsum of the animals. The influence of miR-210 reconstitution in wounds was studied by transdermal injection with a mature miR-210 mimic on the wound edges. The wound area was determined every second day using a digital camera. *In vitro*, the modulation of miR-210 by glucose and oxygen was investigated in human dermal fibroblasts (HDF) cultured in normal (5mM) and high (30mM) glucose concentrations and exposed to normoxia (21% O_2) or hypoxia (1% O_2) for 24 hours. The effect of miR-210 on HDF migration was studied by transfecting miR-210 mimic in HDF cells and performing scratch assay. The expression of miR-210 was evaluated both *in vitro* and *in vivo* by qPCR.

Results: The expression of miR-210 in the skin of db/db mice was significantly reduced compared with the skin from the control mice ($p<0.01$, t-test; n=10). After wounding, miR-210 was induced by 1.7 folds in wounds compared with uninjured skin ($p<0.01$, t-test; n=10). However, miR-210 expression was markedly reduced in the wounds of db/db animals than controls ($p<0.05$, n=10). Wound healing was impaired in db/db mice compared with normoglycemic controls ($p<0.01$, two-way ANOVA, n=7). However, injection of miR-210 mimic locally at wound edges led to a significant improvement in wound healing in db/db mice ($p<0.01$, two-way ANOVA, n=7), but not in non-diabetic animals. *In vitro*, hypoxia increased miR-210 levels in HDF, but it was significantly inhibited in cells that were exposed to both hypoxia and hyperglycemia ($p<0.05$, t-test, n=5). Reduced miR-210 expression was accompanied with decreased migration and proliferation of HDF that were exposed to both hypoxia and hyperglycemia ($p<0.05$, t-test, n=4). Reconstitution of miR-210 in HDF restored cell migration and proliferation.

Conclusion: miR-210 expression in skin and wound is inhibited in diabetes. Reconstitution of miR-210 expression locally in wounds is a promising strategy to promote wound healing in diabetes.

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Disclosure: **X. Zheng:** None.

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Regulation of miRNA-146a and miR-29a during wound healing in a mouse model of type 1 diabetes

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Background and aims: Impaired wound healing of diabetes patients can result from a combination of inflammation, microvascular and macrovascular complications, leading to development of chronic diabetic foot ulcers. The role of microRNAs miR-146a and miR-29a, both increased by inflammation, is not well characterized in wound healing. We aim to evaluate the impact of miR-146a and miR-29a, in wound healing using a diabetic mouse model and also to characterize the role of these miRNAs on migration in an in vitro wound healing model (scratch assays of HaCAT cells).

Materials and methods: Diabetes was induced in C57Bl/6 mice using a multiple low-dose streptozotocin injections. Eight weeks later, 6-mm full-thickness excisional wounds were made and skin wound biopsies obtained at 3 and 10 days after wounding. MicroRNA-146a and miR-29a were measured in RNA extracted from skin at day 0, 3 and 10 after wounding. In vitro scratch assays in human keratinocyte (HaCAT) cells were made after transfection with miR-146a and miR-29a inhibitors. Predicted target genes in skin were investigated using TargetScan 7.1 and filtered using an mRNA expression array (E-GEOD-23006), and analyzed for Gene Ontology enrichment using DAVID tools.

Results: Relative to control (ctrl) mice, STZ diabetic mouse skin had 13.7 (95%CI: 2.9-24.7) fold increased miR-146a and 4.9 (1.9-7.9) fold increased miR-29a levels. Whereas ctrl skin had significantly increased miR-146a (3.1 fold) and miR-29a (2.0 fold) at day 3 of wounding, levels in diabetic skin were suppressed after wounding both at day 3 and 10. Scratch wound assays in HaCAT showed a faster scratch closure after 24hrs when miR-146a or miR-29a was inhibited. Gene Ontology analysis showed that predicted target genes of miR-29a were over-represented in the GO category 0030199~collagen fibril organization ($p=0.045$).

Conclusion: MicroRNAs miR-146a and miR-29a are dynamically regulated by wounding and differentially regulated during wound healing in a diabetic mouse model. Moreover, inhibition of miR-146a and miR-29a increased migration of HaCAT keratinocytes. These findings suggest that normalizing expression levels of miR-146a and miR-29a in diabetic wounds could improve healing, however, more studies are needed.

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Disclosure: **M. Petkovic:** None.

OP 47 Experimental and clinical immunology

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Gut inflammation and mucus layer alterations trigger intestinal activation of islet-reactive T cells and autoimmune diabetes

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Background and aims: Signs of intestinal inflammation and alteration of gut mucosal immunity are found in patients and animal models of Type 1 Diabetes (T1D). Low-grade intestinal inflammation precedes the clinical signs of diabetes thus suggesting that is mechanistically linked with T1D pathogenesis rather than being secondary to diabetes-induced metabolic alterations. While these results suggest a causative link between enteropathy and the pathogenic process of T1D, it is yet to be determined whether gut inflammation does promote β cell autoimmunity and, if it does, how this process occurs. The intestine has several means to maintain immune homeostasis and avoid chronic inflammation. Those include physical barriers such as the epithelial barrier and the vascular barrier and biological barriers such as the mucin protein barrier, a complex system of molecules released by intestinal cells that have crucial immune modulatory functions and anti-microbial activities. Our aim is to assess whether gut inflammation and functional loss of mucin barrier function promotes activation of diabetogenic T cells by increasing antigen trafficking and mucosal immune dysregulation.

Materials and methods: We induced chronic colitis by administration of low-dose DSS (two cycles of 2% DSS in drinking water with a two-week interval) to TCR transgenic BDC2.5XNOD mice that carry the rearranged TCR alpha (Valpha1) and beta (V beta 4) chain of a diabetogenic BDC2.5 T cell clone and do not normally develop autoimmune diabetes even if 90% of their T cell repertoire is constituted of islet-reactive T cells. In this chronic colitis model we studied modification of mucus layer structure by histological analysis and mucin protein barrier composition by RT-PCR. Moreover, we analyzed the activation state and cytokine-secretion phenotype (intracellular FACS analysis) of the diabetogenic T cell clone within the gut mucosa, pancreatic lymph nodes (PLN) and intra-islet lymphocytes (IIL) of BDC2.5XNOD mice with or without DSS-colitis. Incidence of T1D was assessed by weekly measurements of glycemia and microbiota composition by 16S rRNA analysis on the Illumina HiSeq2000 platform.

Results: Our data show that DSS-induced breakage of the mucus layer and alteration of mucin barrier composition lead to intestinal activation of islet-specific T cells within the gut mucosa and occurrence of autoimmune diabetes in TCR transgenic BDC2.5XNOD mice. Specifically, we found that loss of mucus barrier integrity with increased expression of pro-inflammatory mucin MUC-4 and reduction of immune-regulatory mucin MUC-1 leads to immune dysregulation with increased activation and effector phenotype of diabetogenic T cells in the gut mucosa, PLN and IIL. The presence of the microbiota is necessary for islet-reactive T cell activation in those mice thus suggesting the physical interaction between islet-reactive T cells and microbial antigens is required for triggering islet-reactive T cells within the gut mucosa.

Conclusion: Our data demonstrate that gut inflammation is not an epiphenomenon in T1D pathogenesis but it is directly responsible for triggering islet-reactive T cell activation by disrupting the mucus barrier integrity and favoring the interaction between microbiota components and islet-reactive diabetogenic T cells.

Supported by: JDRF

Disclosure: **M. Falcone:** Employment/Consultancy; San Raffaele Hospital. Grants; The Juvenile Diabetes Foundation.

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Beta cell expression of interleukin-35 attenuates autoimmune diabetes and allograft rejection

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Background and aims: Interleukin-35 (IL-35) has recently been identified as a cytokine expressed by T regulatory cells and B cells, with the ability to suppress conventional T cell proliferation and effector functions. Transgenic beta-cell expression of IL-35 protects non-obese diabetic (NOD) mice from autoimmune diabetes, with decreased insulitis and a reduction in infiltrating [or insulitic] CD4+ and CD8+ T cells. We sought to determine whether a gene therapy approach could be used to express IL-35 for prevention of autoimmune diabetes and protection against islet allograft rejection.

Materials and methods: We generated an adeno-associated virus serotype 6 expressing IL-35 downstream of the rat insulin promoter (AAV6-RIP-IL-35) to express IL-35 specifically in beta cells. NOD mice received AAV6-RIP-IL-35 (or AAV6-RIP-empty, as control) via injection into the pancreatic duct at 6 weeks of age. Beta-cell specific expression of IL-35 was confirmed by quantitative real-time PCR and immunofluorescence. To assess allograft rejection, Balb/c donor mice were given AAV-RIP-IL-35 (or AAV-empty) by pancreatic duct injection, and their islets isolated 3 weeks later and transplanted under the kidney capsule of streptozotocin-diabetic C57Bl/6 recipients. Blood glucose was measured weekly to monitor diabetes development and allograft rejection.

Results: Beta cell expression of IL-35 in NOD mice from 6 weeks of age prevented diabetes development compared to mice that received empty virus and untreated controls (0 % vs. 50% vs. 20% respectively, $p<0.05$). Peripheral beta cell-specific CD8+ T cells tended to be decreased in mice that received AAV6-RIP-IL-35. Allogeneic transplants of Balb/c IL-35-expressing islets into diabetic C57Bl/6 recipients had better survival than did empty control (33% vs 0% respectively; $p<0.05$).

Conclusion: These data demonstrate that beta-cell expression of IL-35 reduces diabetes incidence in NOD mice and protects against islet allograft rejection, suggesting that IL-35 may have therapeutic potential in autoimmune diabetes and islet transplantation.

Disclosure: **S. Alvarez:** Other; UBC 4 year fellowship.

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Virus infections affect the activity of regulatory T cells in young children

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Background and aims: Virus infections have been implicated in triggering islet autoimmunity and type 1 diabetes. Incidence of type 1 diabetes (T1D) differs significantly between Finland and Estonia, two populations with similar genetic risk for T1D. We studied the hygiene hypothesis by comparing Tregs in peripheral blood samples and viral RNAs from stool samples of infants from these two countries with different standards of living.

Materials and methods: Subjects: A subgroup of children participating in the DIABIMMUNE (*Pathogenesis of type 1 diabetes: testing the hygiene hypothesis*) study. These index cases from Estonia and Finland (n=161; 85/76, Estonia and Finland, respectively) carried HLA-conferred T1D susceptibility. We studied with flow cytometry (FACS) samples from 3, 6, 12, 24, and 36 month old infants. We also performed RT-qPCR analysis of stool samples collected monthly, from ages one month to one year. (715 samples / 84 children). Methods: FACS analysis of Treg cell frequencies and FOXP3 protein expression was performed in whole blood samples (CD4, CD127, CD25, and FOXP3 Fluorecent mAbs). Virus mRNA expression was analyzed from a 10% stool suspension from the sample and viral RNA was extracted and reverse transcribed and amplified with RT-qPCR.

Results: The Treg (CD4+ CD127-/lo CD25+) frequencies and FOXP3 protein expression remained relatively stable during the first year of life and then, in both countries, decreased clearly. When analyzing FOXP3 expression, we found a population of Tregs expressing high levels of FOXP3 (TregFOXP3High) as a "tail" in the histogram. Estonian children had significantly more TregFOXP3High cells compared to the Finnish children at 3 months of age (p=0.0023; Mann Whitney U-test), but not at the other time points. Frequent virus infections before the age of 12 months were clearly associated with higher FOXP3 expression in Tregs at the age of 36 months (P=0.002). FOXP3 expression was higher in children with at least one virus infection within 60 days prior to Treg sampling (P=0.02). Infants with only Rhinovirus infections had more FOXP3 expression in Treg cells (P=0.032) than children without, in TregFOXP3High cells, a similar trend could be seen. Interestingly, Enterovirus infected infants had lower FOXP3 expression in Tregs (P=0.22) and, more significantly in TregFOXP3High cells at 60 days (P=0.018) and 30 days (P=0.003) prior to sampling. Infections that occurred more than 60 days before Treg sample was taken had no such effect.

Conclusion: Our data show that virus infections during the first year of life are associated with high FOXP3 expression in Tregs. The overall viral exposure was associated with increased Treg activity both near the infection (during the following 60 days), and at older age (age of 36 months). The results suggest that early virus infections may promote the function of immunoregulatory cells.

Clinical Trial Registration Number: 228/13/03/03/2008

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Disclosure: **T. Ruohtula:** Employment/Consultancy; OV is an employee of AstraZeneca R&D. Grants; European Union Seventh Framework Programme FP7/2007-2013 (202063), the Academy of Finland Centre of Excellence in Molecular Systems Immunology and Physiology Research (250114), Sigrid Juselius Foundation, Medicinska understödsföreningen Liv och Hälsa.

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HERV-W-Env, an ancestral protein as novel therapeutic target in type 1 diabetes

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Background and aims: Human endogenous retroviruses (HERVs), known to represent 8% of the human genome, have been associated with several autoimmune diseases. In particular, the HERV-W family has been involved in the pathogenesis of Multiple Sclerosis (MS), a disease in which the envelope protein of HERV-W (HERV-W-Env) exerts immuno-inflammatory effects and promotes autoimmunity. These pathogenic properties have been shown to be mediated by TLR4, a receptor involved in innate immune response. As part of MS studies, cohorts of patients suffering from other autoimmune disorders have been assessed for the presence of HERV-W-Env protein in their sera. Only T1D patients were found to display significant HERV-W-Env expression in this pilot study, prompting further investigations on HERV-W-Env involvement in T1D pathogenesis.

Materials and methods: HERV-W-Env expression has been assessed in humans T1D patients and control individuals, in serum by ELISA, in PBMC by RT-qPCR and in pancreas by IHC. Macrophages infiltration in pancreas has been assessed through CD68 staining; HERV-W-Env and CD68 staining have been automatically quantified. In vitro properties of HERV-W-Env have been studied on primary human Langerhans islets, and *in vivo* effects have been evaluated in a transgenic mouse model expressing HERV-W-Env.

Results: We observed that HERV-W-Env protein is expressed in the sera of 60% of T1D patients (n=30 and $p < 0.0001$ vs controls), and that its RNA is upregulated in PBMC of 57% of T1D patients (n=23 and $p < 0.0001$ vs controls). An extensive immuno-histological study revealed that HERV-W-Env is also expressed in 75% of human T1D pancreas (n=20 and $p < 0.001$ vs controls). Quantification of HERV-W-Env and CD68 staining then revealed a significant correlation between HERV-W-Env expression within the pancreas and macrophages infiltrates ($p < 0.01$). Notably, this infiltration of macrophages was found in the exocrine pancreas ($p < 0.001$ vs controls), in accordance with recent studies supporting the importance of pancreatic exocrine abnormalities in T1D pathogenesis. This histological study further revealed that acinar cells is the cell type producing HERV-W-Env, raising the question of direct pathogenic effects on beta cells, which are known to expressed TLR4 receptor. To test this hypothesis, series of in vitro experiments on human primary Langerhans islets have been performed and revealed that insulin secretion is inhibited by HERV-W-Env in a dose dependent manner ($p < 0.01$ at 100ng/mL). Importantly, a treatment with a neutralizing monoclonal antibody allowed to maintain normal insulin secretion despite the presence of HERV-W-Env. We then developed and characterized a transgenic mouse model in which HERV-W-Env is expressed under the control of CAG promoter. In accordance with human histological data and *in vitro* results, transgenic mice also displayed immune cells infiltrates in their exocrine pancreas ($p < 0.01$), a feature associated with significant hyperglycemia ($p < 0.0001$) and decreased levels of insulin ($p < 0.05$).

Conclusion: Taken together, the combination of the pancreatic pro-inflammatory effects of HERV-W-Env and its direct inhibitory effects on insulin secretion indicates that HERV-W-Env is a potential pathogenic target in T1D, thus unveiling novel therapeutic perspectives.

Disclosure: **S. Levet:** Employment/Consultancy; Geneuro Innovation. Stock/Shareholding; Geneuro.

OP 48 Nephropathy: new approaches

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Evaluation of the effect of empagliflozin on improving hyperfiltration in diabetic nephropathy

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Background and aims: Diabetic kidney disease (DKD) is one of the most common vascular complications associated with diabetes mellitus (DM). Recently, sodium-glucose cotransporter 2 (SGLT2) inhibitor was reported to have renal protective effects. In the EMPA-REG OUTCOME trial, empagliflozin, which is a SGLT2 inhibitor, reduced the risk of cardiovascular events and suppressed the progression of DKD in type 2 DM patients. In this trial, the incidence of albuminuria occurred in 11.2% of the empagliflozin group and in 16.2% of the placebo group, which is a significant relative risk reduction. And this reduction also coincided with the reduction of estimated glomerular filtration rate (GFR). These results suggested that empagliflozin may reduce intraglomerular hyperfiltration such as renin-angiotensin system (RAS) inibitor. However, the molecular mechanism is unclear. We focused on tubuloglomerular feedback (TGF) and investigated by using in vivo imaging technique whether empagliflozin reduces the vascular tonus of the glomerular afferent artery via TGF activation.

Materials and methods: We used wild-type mice (WT; C57 BL/6 background) and type 1 diabetic Akita mice (Akita). We prepared five groups as follows: WT +Vehicle (Vehi), WT +Empagliflozin (5mg/kg; Empa), Akita +Vehi, Akita +Empa, and Akita +Insulin. The mice were sacrificed and blood and tissue samples were harvested after 4 weeks' treatment. Glomerular reactive oxygen species (ROS) and nitric oxide (NO) bioavailability were checked by an ex vivo study. To evaluated ROS and NO bioavailability, diaminofluorescein (DAF) and CellROX® Deep Red were perfused into the kidneys, and fluorescent images of NO and ROS were obtained using multiphoton microscope (MPM). Next, we established a new technique of intravital imaging to evaluate the single nephron GFR and any changes in the glomerular diameter and single nephron GFR were evaluated by using MPM before and 3 hours after the administration of hypoglycemic agents. Next, an nNOS inhibitor or a COX2 inhibitor was administered to these groups to inhibit vasodilator factors delivered from the macula densa. Finally, single nephron GFR were evaluated.

Results: The changing ratio in the diameter of a single nephron was decreased in the Akita +Empa group compared with the Akita +Vehi and Akita +insulin groups. Hyperfiltration was also decreased. Intra-glomerular ROS production was increased and intra-glomerular NO production was decreased in the Akita +Vehi group. These changes were improved in the Akita +Empa group much more than in the Akita +insulin group. More over these effects of Empa were impaired with administrations of nNOS inhibitor and COX2 inhibitor. These data indicated Empa could reduce glomerular hyperfiltration in DKD by activating TGF.

Conclusion: Empagliflizon improved glomerular hyperfiltration via TGF activation in DKD mice. The renoprotective effect of empagliflozin was, in part, independent of the plasma glucose.

Supported by: Boehringer Ingelheim

Disclosure: **Y. Sogawa:** Grants; Boehringer-Ingelheim.

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DPP4 inhibitor reduces urinary albumin excretion through the protection of glomerular endothelial function

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Background and aims: Diabetic nephropathy is a major microvascular complication of diabetes and a leading cause of end-stage renal disease. Recently, inhibitors of type 4 dipeptidyl peptidase (DPP-4) have been developed and approved for the oral treatment of diabetes mellitus (DM). In TECOS and SAVOR trials, DPP-4 inhibition did not reduce cardio-vascular events. However several pre-clinical studies suggested that DPP-4 inhibition not only reduces plasma glucose levels but also reduces the risk of cardio-vascular events (CVE) and shows kidney protection. We hypothesize that the DPP-4inhibitor linagliptin has the potential to prevent glomerular endothelial dysfunction beyond the reduction of plasma glucose levels.

Materials and methods: To prove our hypothesis, we used wild type mice (WT) and Akita diabetic model mice, which have a C57BL/6 genetic background. The mice were divided into three groups (Con, Akita, and Akita-Lina). Linagliptin (5 mg/kg/day) or PBS was administrated beginning at six weeks and continuing to 14 weeks of age. Blood samples were collected to determine HbA1c. Kidney tissues were harvested after sacrifies. Urine samples were collected 24 h before sacrifice. Urinary albumin and creatinin were quantified and expressed as the ratio of albumin to creatinine. Urinary 8-OHdG was detected by ELISA to assess the redox status in the kidney. Glomerular hyperfiltration was assessed using intravital microscopy. Data were expressed as mean ±s.e.m. Differences between the groups were assessed for statistical significance using one-way analysis of variance for multiple groups. Statistical significance was defined as $P<0.05$.

Results: The HbA1c levels in the Akita mice were same as those in the Akita-Lina (HbA1c 10.8 ±0.12% in Akita and 10.3 ± 0.17 % in Akita-Lina mean ±S.E.M.), while urinary albumin excretion/creatinin levels were significantly reduced in the Akita-Lina mice compared with the Akita (100.4 ± 14.6 mg/g in Akita and 56.5 ± 4.2 mg/g in Akita-Lina, $P<0.05$). This suggests that the DPP4 inhibitor can reduce urinary albumin excretion beyond lowering blood glucose effects. Glomerular size which is indicated for hyperfiltration in the Akita mice was larger than in the Akita-Lina mice (127 ± 2.7% in Akita and 116 ± 2.6% in Akita-Lina, $P<0.05$). Next, we addressed the endothelial surface layer, which can be detected with tomato lectin. The lectin staining areas remained more prevalent in the Akita-Lina than in the Akita mice. However Podocyte injury which is detected by podocin staining were not differ in Akita and in Akita-Lina. Next, we used intravital microscopy to assess glomerular filtration. Leakage of FITC-labeled dextran into the Bowman's capsule space was undetectable in the WT and Akita-Lina mice, whereas it was detectable in the Akita. Finally, we addressed oxidative stress, which is one of the most likely mechanisms to explain how the DPP-4 inhibitor attenuates glomerular hyperfiltration. Urinary 8-OHdG levels normalized to creatinine were significant lower in the Akita-Lina than in the Akita mice (30.3 ± 3.5 ng/g in Akita and 20.5 ± 1.5 ng/g, in Akita-Lina $P<0.05$).

Conclusion: Linagliptin reduces urinary albumin excretion beyond blood glucose lowering effects. These data suggest that linagliptin is a potential new drug to treat diabetic nephropathy via the prevention of endothelial dysfunction.

Supported by: Boehringer-Ingelheim

Disclosure: **N. Hajime:** Grants; Boehringer-Ingelheim.

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Renal outcomes with DPP-4 inhibitors compared with sulfonylurea as add-on to metformin among patients with type 2 diabetes

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Background and aims: Prior studies have demonstrated that compared with placebo, dipeptidyl peptidase-4 inhibitors (DPP-4i) may be associated with nephro-protective effects among people with type 2 diabetes (T2D) who also have mild to moderate renal impairment. This study compared the renal outcomes between patients with T2D using DPP-4i versus sulfonylurea (SU) as an add-on to metformin (MET) in the UK.

Materials and methods: Patients aged 18-79 years diagnosed with T2D who used MET only prior to initiating DPP-4i or SU in 2007-2014 were selected using the UK Clinical Practice Research Datalink. The first record with DPP-4i or SU in addition to MET after MET monotherapy was the index event.

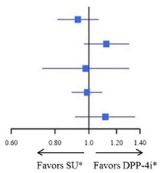
Patients were required to have ≥1 valid test result for ≥1 of the following tests during the 6 months before (baseline) and ≥12 months after (follow-up) the index date: urine albumin and urine creatinine reported separately or urine albumin-creatinine ratio (UACR), or urine microalbumin; serum creatinine (SCr), or estimated glomerular filtration rate (eGFR). Multivariate Cox proportional hazard models were used to compare incidence of the following outcomes: microalbuminuria, UACR <30 mg/day, doubling of SCr, worsening of kidney function (eGFR <60 mL/min/1.73m^2), and decrease in eGFR by ≥25% for at least 12 months after index. Models were adjusted for: age, gender, body mass index, baseline HbA1c, Charlson comorbidity index (CCI), duration of monotherapy, duration of diabetes, year of index date, baseline UACR/SCr/eGFR level (depending on the outcome), and use of antihypertensive agents or statins.

Results: Of the 23,341 patients included in the sample, 5,757 (24.6%) were DPP-4i users. Compared with SU users, DPP-4i users were significantly (p<0.05) younger (58.6 years vs. 59.7 years), were less likely to be men (60.2% vs. 62.1%), had lower mean HbA1c level (8.7% [71.6 mol/mol] vs. 9.1% [76.0 mmol/mol]), and had lower CCI (0.49 vs. 0.58) during baseline. The DPP-4i cohort had better renal function during baseline, as indicated by lower mean UACR (44.1 vs. 49.8) and SCr (79.4 vs. 87.1) levels, and higher eGFR (81.9 vs. 78.9 mL/min/1.73m^2). After adjusting for differences in patient characteristics, there was no statistically significant association between treatment cohort and incidence of any renal outcome during the follow-up period (Figure 1).

Conclusion: The findings of this study suggest that DPP-4i and SU have comparable effects on renal outcomes as add-on therapy among patients with T2D.

Figure 1: Likelihoods of incident microalbuminuria, UACR <30 mg/day, doubling of SCr, worsening of kidney function, and decrease in eGFR by ≥25% for at least 12 months after index among T2D patients using SU compared with DPP-4i

Outcome	Hazard ratio (SU relative to DPP4i)	95% CI	P-value
Microalbuminuria	0.93	0.81 - 1.07	0.2968
Improvement in albumin to <30 mg/day	1.12	0.97 - 1.30	0.1224
Doubling of SCr	0.98	0.74 - 1.30	0.8881
Worsening kidney damage	0.99	0.89 - 1.09	0.8075
Sustained decreases in eGFR ≥ 25%	1.11	0.92 - 1.36	0.2781



Notes:
*Except for 'improvement in albumin to <30 mg/day' where HR >1 indicates better outcome with SU than DPP-4i. DPP-4i users were the reference cohort for all comparisons.
CI = confidence interval; DPP-4i = dipeptidyl peptidase-4 inhibitor; eGFR = estimated glomerular filtration rate; SCr = serum creatinine; SU = sulfonylurea; T2D = type 2 diabetes; UACR = urine albumin-creatinine ratio.

Disclosure: **H.H. Teh:** Employment/Consultancy; I am employee of AstraZeneca which provided research funding to Analysis Group, Inc.

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Nephroprotective properties of vildagliptin and metformin combination in randomised, comparative, prospective clinical study

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Background and aims: Accumulating evidence suggests that the modulation of incretin signaling is beneficial in both glycemic control, as well as nephroprotection. Metformin has been reported to inhibit DPP-4 and stimulate GLP-1 secretion. Thereby, DPP-4 inhibitor vildagliptin in combination with metformin could additively improve glycemic control and metabolic status in type 2 diabetes (DM2) with attractive therapeutic option in regard to diabetic nephropathy (DN). A randomized comparative study assessed the effect of the combination of vildagliptin in daily dose 50 mg and metformin in daily dose 1500 mg added-on insulin therapy on renal function in type 2 diabetic patients.

Materials and methods: We investigated the dynamic of metabolic parameters, estimated GFR using creatinine (eGFRcr) and cystatin C(eGFRcys), UACR, and excretion of liver-type fatty acid binding protein (L-FABP) in 43 insulin-treated near-compensated patients with normal/mildly impaired renal function (CKD C1-2,A 1-2) randomizing either to continue insulin therapy (IG group, n=21), or to receive 6-month combined vildagliptin and metformin treatment added to insulin (VMIG group, n=22). Main non-inclusion criteria were allergy to metformin/vildagliptin, severe micro-/macrovascular complications of DM2, non-diabetic renal diseases, nephrotoxic drugs usage, uncontrolled hypertension/ dyslipidemia, inflammatory/oncological conditions.

Results: The mean age of patients in IG group was 60.1 ± 6.13 years, in VMIG - 63.7 ± 4.21 years, p=0.032, and majority were women (about 60 %). Duration of DM2 was approximately 10 years. Albuminuric stage of DN was present in 42.9 % of participants in IG group and in 54.6% in VMIG group, p=0.048. eGFR, UAER level, and main assessed metabolic parameters were comparable in both groups. After 6 months, there were no significant changes in main assessed parameters in IG group. VMIG group showed significant decrease in HbA1c (-5.5 %, from 7.2 ± 0.5% до 6.8 ± 0.7 %, p=0.0003), fasting plasma glucose (-12.9 %, from 6.9 ± 1.5 to 6.0 ± 1.22 mmol/l, p=0.014), and blood triglycerides (-21.4 %, from 2.2 ± 1.4 to 1.76 ±0.75 mmol/l, p=0.016) compared to IG group. Whiles eGFRcr and eGFRcys in VMIG group did not significantly change (eGFRcr +1.2 % from 76.4 ± 13.1 ml/min/1.73 m^2), UACR and urinary L-FABP decreased significantly (-11.8 % (from 33.7 [17.5;84.0] to 27.4 [16.5;63.9] mg/g, p=0.019) and -7.3% (p=0.028), respectively. Multivariate regression analysis revealed glucose- and metabolic-independent pattern of UAER changes. However, the dynamic of L-FABP in VMIG group was correlated with dynamics of fasting blood glucose (r = - 0.58, p=0.004) and triglycerides (r=0.66, p=0.001).

Conclusion: The study showed that 6-month combined vildagliptin and metformin treatment added on insulin therapy in near-compensated type 2 diabetic patients demonstrated nephroprotection regarding albuminuria and urinary excretion of tubular injury marker L-FABP. Moreover, UAER decreased beyond normalization of DM2 compensation parameters

Supported by: The study was provided by resource center Molecular&Cell Technologies SPbSU

Disclosure: **V. Bayrasheva:** None.

PS 001 Glycaemia, glycaemic control and diabetes

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Relative contributions of fasting and postprandial glycaemia to HbA_{1c} among persons with normal, pre-diabetes and diabetes HbA_{1c} levels

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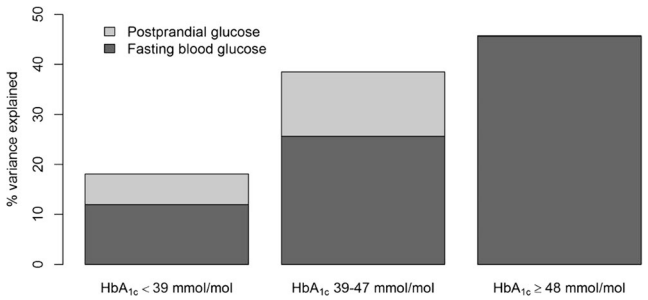
Background and aims: Lowering of postprandial glucose (PPG) is physiologically relevant for patients with diabetes, and possibly also beneficial for reducing diabetes risk in the general population. However, the contributions of PPG and fasting glucose (FG) to the HbA_{1c} level are not clear. We aimed to quantify the relative contribution of real-life PPG versus FG levels to HbA_{1c} among persons with different glycaemic status.

Materials and methods: Data from 80 individuals without diabetes and 94 non-insulin-treated patients with type 2 diabetes from the A1c-Derived Average Glucose Study were used. Continuous glucose monitoring in periods of at least two days and measurement of HbA_{1c} were performed at baseline and monthly over 3 months. PPG was estimated as the area under the curve 2 hours after meal intake. The study participants were sub-dived into groups with HbA_{1c} levels in the normal (<39 mmol/mol, n=86), pre-diabetic (39-47 mmol/mol, n=48) and diabetic range (≥48 mmol/mol, n=40). Associations of HbA_{1c} with FG and PPG were analysed by linear regression stratified by HbA_{1c} groups and adjusted for age, sex and BMI. In linear regression analysis using the same three HbA_{1c} groups, we calculated the proportion of variance in HbA_{1c} explained by PPG and FG.

Results: Each 1 SD higher level of FG (1.8 mmol/L) was associated with HbA_{1c} levels which were 1.8 (0.3-3.4), 2.1 (1.1-3.0), and 6.8 (4.6-9.0) mmol/mol higher among individuals with HbA_{1c}<39, 39-47, and ≥48 mmol/mol, respectively (P<0.05). Each 1 SD higher PPG (1.3 mmol/L) was associated with HbA_{1c} levels which were 1.5 (0.3-2.6) and 1.0 (0.2-1.7) mmol/mol higher in individuals with HbA_{1c}<39 and 39-47 mmol/mol, respectively (P<0.05). Among individuals with HbA_{1c}≥48 mmol/mol, PPG was not associated with HbA_{1c} (P=0.985). The figure shows the proportion of variance in HbA_{1c} explained by FG and PPG by categories of HbA_{1c}. Proportions are scaled to the total proportion of variance explained by FG and PPG combined.

Conclusion: Using real-life data, the relative contribution of FG to HbA_{1c} increases as HbA_{1c} levels increase from normoglycaemic levels to pre-diabetic and diabetic levels. The contribution of PPG is less, but most apparent in the non-diabetic HbA_{1c} range. These findings suggest that a reduction in PPG may be most advantageous for glycaemic control among healthy individuals and well-regulated type 2 diabetes patients with HbA_{1c} levels in the normal and pre-diabetic range.



Supported by: Unilever
Disclosure: **K. Faerch:** Grants; Unilever.

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Association of HbA_{1c} values with haemopoietic parameters in a selected population of healthy individuals

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Background and aims: Glycated hemoglobin (HbA_{1c}) values are influenced by a number of factors other than glucose concentration, those implicated in erythropoiesis being of critical importance. In previous studies, HbA_{1c} levels have been associated with markers such as hemoglobin, erythrocyte indices (MCV, MCH) and red cell distribution width (RDW) in individuals with or without diabetes mellitus. However, in these observations, subjects with a variety of hemopoietic defects that could potentially affect the overall results were usually included. This study aims to determine the association of HbA_{1c} levels with erythrocyte indices in a selected population of individuals without apparent hemopoietic defects.

Materials and methods: A total of 88 individuals (43 males, 45 females, mean age 31.7 years) with no past medical history having a complete blood count within normal range, were examined. Apart from a complete blood count, a number of laboratory parameters, possibly contributing to HbA1c variance (hemoglobin electrophoresis for detection of hemoglobinopathies, fasting serum glucose [FSG], creatinine, fructosamine, albumin, hsCRP and ferritin) were measured. Statistical analysis was carried out using SPSS statistical package version 21.

Results: In univariable regression analysis, HbA1c levels were positively correlated to age (r= 0.25, p=0.020), body mass index (BMI) (r= 0.38, p<0.001), serum creatinine (r= 0.24, p=0.023) and RDW (r= 0.33, p=0.002). There was also a trend towards negative correlation between HbA1c and both MCH (r=-0.19, p=0.08) and MCV (r=-0.17, p= 0.10). Four multivariable linear regression models were created to examine the association of HbA1c (dependent variable) with: (1) hemopoiesis (hemoglobin, erythrocyte indices and ferritin), (2) glycaemia (FSG, fructosamine and BMI), (3) inflammation (hsCRP, serum albumin, ferritin) and (4) all the above. All models were adjusted for age, gender and serum creatinine. The RDW was the only hemopoietic parameter significantly associated with HbA1c, both in the first model (B=0.09, p=0.01) and in the fully adjusted model (B=0.08, p=0.02). In addition, BMI was the only other parameter that was significantly associated with HbA1c in the fully adjusted model (B=0.02, p=0.03).

Conclusion: Our study verified the independent correlation of HbA1c to RDW, this time however, in a healthy population with apparently normal hemopoietic function and glycaemic levels. This may indicate that slight interindividual variations of normal hemopoiesis project an effect on HbA1c values.

Disclosure: **D. Tsilingiris:** None.

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Study of insulin and glucose parabolas and dysglycaemia timeline using oral glucose tolerance test

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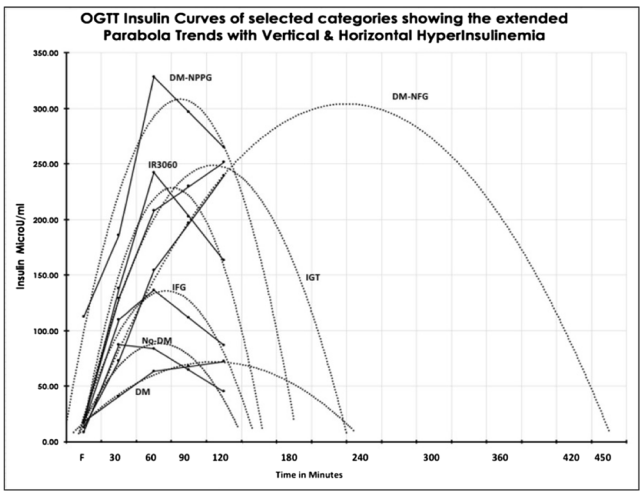
Background and aims: Type 2 Diabetes (T2DM) is characterised by progressive loss of Beta cell Function (BCF) with varying degree of obesity. Need was felt to study the interrelation between these etiological factors.

Materials and methods: A cross sectional study of 600, not previously diagnosed diabetic individuals with 'standard two hours and 75 grams' oral glucose tolerance test with addition of insulin levels using the time points of 0, 30, 60 and 120 minutes. These subjects were further grouped into 14 categories, based on their fasting(FG) and 120-minute post-glucose(PG) values, such as ' NoDM, MatIR, HepIR, IFG, IR30, IR60, IR3060, IGT, IFG-IGT, DM-IFG, DM-IGT, DM-NFG, DM-NPPG and DM. These categories actually represented the various stages on the timeline of T2DM. These categories were further grouped into Fasting (n=107)(FP), Prandial (n=161)(PP), Overlap

(n=192)(OP) pathways depending on the values in FG-PG pair and their Homa1-IR and Matsuda Indexes. The parabolas of glucose and insulin for all the fourteen categories were analysed and their trend-lines were extrapolated till they met the baseline again. The data was used to prepare a timeline of events.

Results: In FP categories, fasting insulin (FI) and BMI values were significantly correlated. (P <.0002) The Insulin and glucose parabolas followed quadratic distribution rules. Hence, increment in FI lead to exponential rise in post-glucose insulin values and manifested as post-glucose-load hyperinsulinemia (HI). During the first hour secretory phase of OGTT, all categories except the diabetes category ('DM') showed positive incremental change with proportional HI. The rising trend of insulin secretion reverses when the pancreatic beta cell reserve is exhausted, that is when the 'BetaMax' is reached. During the second hour of OGTT, the FP categories show downward trend whereas the PP categories continue their upward secretory trend past two hours. However, the insulin secretory defect (ISD) results in proportionately lower rate of rise of insulin and results in longer time interval to return back to the basal fasting level. This interval was slightly more than two hours for the NoDM category and almost eight hours for the 'diabetes with Normal Fasting Glucose' (DM-NFG) category. The insulin and glucose parabolas are interdependent and are also linked to the insulinogenic index (IGI), Incremental Area Under the Curve of insulin as well as the disposition index (ISSI-2) and showed a very typical 'inverted tick mark' distribution. Using this data, it was possible to develop a new timeline showing the evolution of 'Lipo-Diabesity'.

Conclusion: Evolution of T2DM occurs mainly via the fasting and prandial pathways or an overlap of these two. The FP is associated with calorie excess (CE) and obesity whereas PP with ISD and BCF loss. A timeline of dysglycemia with glucose and insulin levels, ISSI-2, rising BMI and continued CE is proposed. Continued CE is suggested to explain the relentless progressive nature of diabetic dysglycemia.



Disclosure: **S.N. Shinde:** None.

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Glucose energy spectrum as a function of dysglycaemia progression

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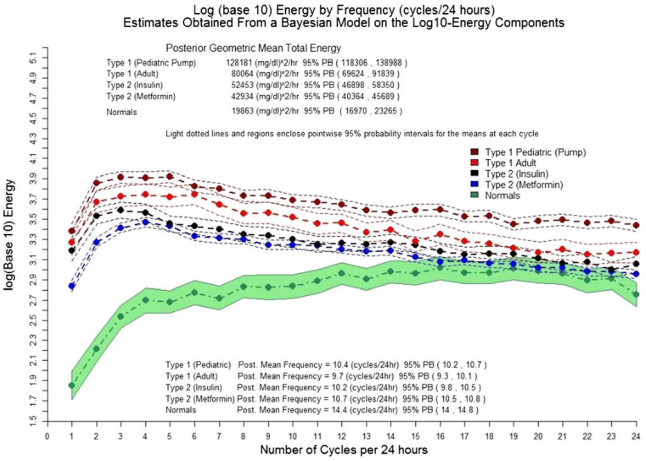
Background and aims: Glucose homeostasis is maintained physiologically by B-cell insulin secretion being sensitive to both absolute and increasing glucose concentrations. A glucose increase is rapidly followed by insulin secretion resulting in a fast return toward the baseline. With progression to Impaired Glucose Tolerance first or early phase insulin release diminishes and glucose return times become longer. In type 2 (T2) Diabetes Mellitus (DM) insulin secretory capacity is further compromised resulting in fasting glucose increases and prolonged elevated glucose following meals. In late type 1 (T1) DM insulin secretion is absent and glucose will continue to rise without exogenous insulin. As B-cells deteriorate glucose variability would thus be expected to increase and shift from fast to slower components. The total variability energy (TE) for a given 24 hour CGM curve f is defined as the area under the curve $(df/dt)^2$, or the summation over time of the glucose "velocity" (df/dt) squared. Using the finite Fourier transform, TE can be distributed across the cycles of oscillation, being the sum of the energy components for each of frequencies 1, 2, 3, .. per 24 hours describing successively faster glucose changes. This study summarizes the TE from the first 24 cycles, and the distribution of this energy by frequency over cycles 1 through 24, called the "glucose energy spectrum". Given the natural history outlined above and that we in the treatment setting measure the combination of the disease and the affected person's therapy, we predict TE would increase and shift to slower components through populations of normals, T2 DM on metformin only, T2 DM on insulin, well controlled T1 DM and finally pediatric T1 DM, reflecting more variability and slower glucose changes.

Materials and methods: Using baseline CGM profiles from 341 T1 and T2 DM subjects from 4 clinical trials, and a cohort of 25 normal subjects, the geometric mean TE's and corresponding energy spectra were estimated.

Results: Results as shown on the graph were consistent with the hypothesis. The mean TE for T2 DM was about double the corresponding mean TE for the normals, and even larger for T1 DM. The distribution of this energy, expressed as log(base 10) energy versus cycle, was similar for all DM, concentrated in slow cycles 3-5, while the normal energy peaked at cycles 16-20 resulting in a large mean frequency.

Conclusion: These results show the glucose energy spectrum reflects disease progression and may suggest DM therapy goals should include more rapid response to glucose changes in addition to lowering glucose.



Disclosure: **P. Strange:** None.

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Glycated haemoglobin is increased in critically ill patients with stress hyperglycaemia: implications for risk of diabetes in survivors of critical illness

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Background and aims: Stress hyperglycaemia occurs frequently in critically ill non-diabetic patients and typically resolves. It remains uncertain if stress hyperglycaemia is indicative of a predisposition to the development of type 2 diabetes. Glycated haemoglobin (HbA1c) is a strong predictor of future diabetes risk, particularly with HbA1c values in the range 5.4 - 5.6% (36 - 38mmol/mol). The aim of this study is to determine if stress hyperglycaemia is associated with an increased HbA1c and, by inference, a greater risk of diabetes.

Materials and methods: This is a single centre retrospective observational study, performed at a mixed medical/surgical intensive care unit (ICU) in a tertiary hospital in Adelaide, South Australia. All adult patients admitted between March 2014 and November 2016 were screened for inclusion into the study. Patients with diabetes or HbA1c ≥ 6.5% (48mmol/mol) were excluded. Stress hyperglycaemia was defined as a blood glucose ≥11.1 mmol/l on at least two occasions within a 24-hour period and/or having insulin administered via intravenous infusion for ≥ 2 hours for a blood glucose ≥10.0 mmol/l in patients without a history of diabetes. Patients who did not meet the criteria for stress hyperglycaemia were classified as normoglycaemic. HbA1c was measured in all patients between March and September 2014 and, thereafter, only on patients identified with stress hyperglycaemia until November 2016. The difference in HbA1c between the two groups was tested using an independent samples t-test. Multiple linear regression was used to adjust for differences in sex, age and illness severity (APACHE II score).

Results: Stress hyperglycaemia was identified in 63 of 631 patients without diabetes from March to September 2014, with the remaining 568 patients classified as normoglycaemic. A further 136 patients with stress hyperglycaemia were identified between September 2014 to November 2016 for a total of 199 patients with stress hyperglycaemia. There was no difference in age or sex between the two groups. Patients with stress hyperglycaemia had a higher HbA1c (5.57 ± 0.48% (37.4 ± 5.3 mmol/l) vs 5.45 ± 0.46% (36.1 ± 5.0mmol/l), P = 0.001), greater illness severity (APACHE II score 19.5 ± 6.8 vs 16.2 ± 6.7, P < 0.001), longer hospital length of stay (17.3, IQR: 10.6 - 36.6 days vs 13.3, IQR: 7.2 - 23.7 days, P < 0.001) and higher in hospital mortality (17.1% vs 10.2%, P = 0.01) compared to normoglycaemic patients. After adjusting for sex, age and illness severity, HbA1c was 0.14% ± 0.038 (1.5 ± 0.4 mmol/mol, P=0.001) higher in stress hyperglycaemia patients.

Conclusion: Stress hyperglycaemia is associated with increased HbA1c, indicative of an increased risk of incident type 2 diabetes.

Disclosure: **Y.T. Du:** None.

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Effect of resveratrol on glucose control and insulin sensitivity in subjects with type 2 diabetes: a systematic review and meta-analysis

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Background and aims: Although the regular consumption of polyphenol resveratrol has been considered to improve glucose homeostasis and reverse insulin resistance in type 2 diabetes mellitus (T2DM), the reported results are conflicting. The aim of this systematic review was to assess the effects of resveratrol on glycemic control and insulin sensitivity in patients with T2DM.

Materials and methods: A literature search was conducted of PubMed, the Cochrane Library and Web of Science through the reference lists of relevant articles until March 2017. Randomized controlled trials in T2DM patients in whom resveratrol were administered as an intervention were included. Using random or fixed effect model, standardized mean differences across trials were calculated for changes in fasting plasma glucose, fasting insulin, glycated hemoglobin A1c (HbA1c) and homeostasis model assessment of insulin resistance (HOMA-IR). We performed subgroup and sensitivity analyses to evaluate potential heterogeneity. Two reviewers independently selected trials, extracted data, and evaluated the methodological qualities and evidence levels.

Results: Nine randomized controlled trials involving 283 participants were included. Meta-analysis showed resveratrol significantly improved fasting plasma glucose [standardized mean difference (SMD)= -0.29, 95% confidence interval (CI): -0.51, -0.06, p = 0.013], fasting insulin (SMD = -0.64, 95% CI: -0.95, -0.32, p = 0.000), reduced HOMA-IR index(SMD = -0.52, 95% CI: -1.00, -0.04, p = 0.000), systolic blood pressure (SMD = -0.58, 95% CI: -0.86, -0.30, p = 0.000), diastolic blood pressure (SMD = -0.43, 95% CI: -0.70, -0.15, p = 0.003) in participants with T2DM. Changes in HbA1c, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol were negligible. A subgroup analysis comparing resveratrol supplementation dose of <100mg/d versus ≥100mg/d, exhibited a significant difference for fasting plasma glucose in favor of the latter subgroup.

Conclusion: The consumption of resveratrol appears to have a beneficial role in the management of T2DM; however, larger, longer term and high-quality randomized controlled trials are needed to inform the development of evidence-based treatment guidelines.

Disclosure: **X. Zhu:** None.

PS 002 Epigenetics

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The GLP-1RA exenatide LAR increases circulating miR-27b in patients with type 2 diabetes: an 8-month prospective study of cardiometabolic and epigenetic response

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Background and aims: The impact of once-weekly exenatide (long-acting release, LAR) on microRNAs (miRNAs), endogenous 21-25 nucleotides noncoding RNA, which regulate gene expression at the post-transcriptional level, is unknown. Specific miRNA-27b appears to exert pro-angiogenic effects on endothelial cells. We investigated whether exenatide LAR may improve expression of miRNA-27b and whether this effect is associated with the modulation of several evaluated cardio-metabolic parameters, including carotid intima-media thickness (cIMT), endothelial function and adipokines.

Materials and methods: Sixty subjects with T2DM (41 men and 19 women; 60±10 yrs) naïve to incretin-based therapies, were treated with exenatide LAR as add-on to metformin (from 1500 up to 3000 mg/day) for 8 months. Exclusion criteria included the presence of a previous major cardiovascular event, as well as moderate and severe renal and liver function. Fasting blood samples were collected at baseline and after 8 months. miRNA-27b was isolated from sera using the mirVana miRNA Isolation Kit (Ambion, Waltham, MA, USA), and then quantified by SYBR Green Real-Time (RT) polymerase chain reaction (PCR). cIMT was assessed by B-mode real-time ultrasound, while endothelial function by flow mediated dilation (FMD) of the brachial artery.

Results: Statistical analysis was performed by paired t-test and Spearman correlation. After 8 months of exenatide LAR therapy, significant improvements were observed in body weight (from 89±18 to 86±17 kg, p=0.0002), BMI (from 33±9 to 31±6 kg/m^2, p=0.0348), waist circumference (from 109±13 to 106±13 cm, p=0.0105), fasting glycaemia (from 8.8±2.8 to 7.3±2.2 mmol/L, p<0.0001), A1c (from 8.0±0.4 to 6.9±1.1 %, p<0.0001), total-cholesterol (from 4.4±0.9 to 4.2±1.0 mmol/L, p=0.0012), LDL-cholesterol (from 2.5±0.8 to 2.2 ±0.9 mmol/L, p<0.0001), HDL-cholesterol (from 1.2±0.3 to 1.3±0.3 mmol/L, p=0.0188), cIMT (from 0.98±0.14 to 0.87±0.15, p<0.0001) and FMD (from 5.8±1.3 to 6.8±1.7 %, p<0.0001). Adiponectin, leptin and resistin improved, although the statistical significance was achieved for adiponectin level only (3.6±2.7 to 4.3±2.9 μg/ml, p=0.0393). A cell adhesion molecule with a key role in endothelial function, soluble(s) L-selectin, improved significantly (from 0.49±0.17 to 0.55±0.22 μg/ml, p=0.0426). Levels of miR-27b were significantly increased after exenatide therapy (1.33 mean fold change, p=0.0355). By correlation analysis we found a significant association between changes in the miRNA-27b and those in adiponectin (r=0.269; p=0.0377), leptin (r=-0.307; p=0.0170) and L-selectin (r=0.436; p<0.0001), respectively.

Conclusion: Exenatide LAR, beyond cardio-metabolic control, may exert epigenetic effect in patients with T2DM by regulating miRNA-27b involved in the maintenance of endothelial cell homeostasis. A potential interplay between miRNA-27b and adipokines in endothelial angiogenesis seems to exist, however, further investigations are needed for better understanding.

Clinical Trial Registration Number: NCT02380521

Supported by: AstraZeneca

Disclosure: **D. Nikolic:** Other; I have participated in clinical trials sponsored by AstraZeneca and Novo Nordisk.

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Cell-free insulin and amylin DNA as biomarkers of progressive beta cell loss during first 12 months of type 1 diabetes in children and adolescents

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Background and aims: DNA methylation can be used as a biomarker of beta-cell loss in type 1 diabetes (T1D). This study was designed to measure the levels of demethylated insulin and amylin DNA in the sera of children and adolescents with new onset T1D during the first 12 months post-diagnosis. In addition, these findings were associated with residual beta-cell function (stimulated C-peptide [SCP]), glycaemic control and glucagon.

Materials and methods: Serum samples drawn 3 and 12 months after diagnosis from 126 children and adolescents with new onset T1D were analysed. DNA was extracted and treated with bisulfite prior to methylation analysis. The methylation pattern for insulin DNA was measured with methylation sensitive probes using droplet digital PCR (ddPCR), while methylation specific primers were used to quantify amylin DNA using nested qPCR.

Results: An increase of the demethylated insulin DNA was observed in 46% samples, whereas 47% had declining values from three to 12 months after diagnosis. Amylin DNA demethylation index (DMI) increased in 52% and decreased in 48% of patients. Gender and age had no significant effect on the level of demethylated insulin DNA or amylin DMI. No correlation was found between demethylated insulin DNA and SCP, Glucagon, HbA_{1c} or $IDAA_{1c,}$ whereas amylin DNA was significantly associated with SCP at 12 months (p=0.016). Demethylated insulin was correlated with amylin DNA at 3 months (p=0.024) after diagnosis.

Conclusion: Circulating demethylated amylin DNA correlated with biomarkers of disease progression 12 months post-diagnosis whereas insulin DNA did not. These findings support the results of a previous report, which showed a loss of insulin but not amylin expression in beta cells following T1D presentation. The observation suggests that demethylated amylin DNA patterns may serve as more sensitive biomarker of beta-cell loss than demethylated insulin DNA during the first 12 months of T1D progression.

Supported by: JDRF

Disclosure: **N. Samandari:** None.

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Insulin resistance, BMI and cholesterol levels associate with DNA methylation in human myoblasts from obese individuals

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Background and aims: Reduced skeletal muscle mass and insulin resistance are risk factors for type 2 diabetes (T2D). Skeletal muscle repair and maintenance are dependent on proper proliferation and differentiation (myogenesis) of muscle stem cells (satellite cells). Satellite cells from obese and T2D individuals show impaired glucose and lipid metabolism after in vitro differentiation, suggesting a memory of the in vivo environment. Recently, we mapped the DNA methylome and transcriptome in human myoblasts before and after differentiation into myotubes for the first time in a human cohort of controls and obese individuals. We showed that myoblasts from obese are epigenetically and transcriptionally reprogrammed. In the same dataset we observed that a number of CpG sites showed a high variability within only obese or non-obese. The obese population is heterogeneous and the current aim is to look at CpG sites with high variation in methylation in myoblasts from obese

individuals. We hypothesize that demographics associated with obesity, such as insulin resistance, BMI and physical activity, may contribute to this variation.

Materials and methods: Human satellite cells were isolated from vastus lateralis biopsies and the myoblasts harvested before being 50% confluent. DNA methylation was analyzed with the Infinium HumanMethylation450 BeadChip Kit in primary human myoblasts from 14 non-obese and 14 obese individuals. The relative standard deviation (RSD) (standard deviation/mean) was calculated for methylation of each CpG site in obese and non-obese, respectively. Methylation of CpG sites with a high RSD in only the obese group were correlated to the homeostatic model assessment of insulin resistance (HOMA-IR), BMI, plasma cholesterol levels and VO_2-max using spearman correlations.

Results: DNA methylation data were obtained for 477,226 CpG sites in myoblasts from 14 obese and 14 non-obese individuals. 41,538 CpG sites had an RSD of more than 30% in the obese group and 31 of these sites had an RSD of less than 10% in the non-obese group. The majority of the 31 sites (28) had a low degree of methylation (<17%) and 21 were annotated to CpG islands. Methylation of 5 of the 31 sites correlated significantly with HOMA-IR, 3 with BMI, 2 with LDL, 4 with HDL, and 1 with VO_2-max. Interestingly, several of these CpG sites are annotated to genes coding for transcription factors important for development and differentiation. For example, methylation of a CpG site in the gene encoding the anti-adipogenic factor GATA3 correlates with BMI and methylation of CpG sites in SOX5 and ONECUT1 correlate with both HOMA-IR and BMI.

Conclusion: This study shows that the variation in DNA methylation at a number of CpG sites increases with obesity and may be explained by differences in insulin resistance, BMI, cholesterol levels and physical fitness. The results implicate that several factors associated with obesity influence the DNA methylation pattern in primary human myoblasts and may contribute to the epigenetic and metabolic reprogramming observed during myogenesis in obese individuals.

Supported by: Diabetes Wellness Sweden, Royal Physiographic Society of Lund, Sweden

Disclosure: **C. Davegårdh:** None.

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Induction of permanent epigenetic changes in Treg signature genes after repeat BCG vaccination of subjects with type 1 diabetes

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Background and aims: Increasing the potency or numbers of active regulatory T cells (Tregs) may be beneficial in the treatment of autoimmunity and is therefore a goal of many clinical trials. In a published study, BCG-treated subjects with type 1 diabetes (T1D) had statistically significant increases in Treg numbers for 4-6 weeks after repeat BCG vaccination. A longer-lasting effect would be optimal and may be able to stably reverse diabetic autoreactivity. Chronic Mycobacteria infections evade host recognition on a cellular level via measurable Treg increases and induction of host Tregs. For BCG's virulent counterpart, tuberculosis, epigenetic imprinting of host genes is a mechanism for host Treg production and infection chronicity. We therefore investigated if the effect of repeat BCG vaccinations on Tregs could be permanent and driven by host epigenetic modifications to Treg signature genes.

Materials and methods: BCG's impact on methylation was studied at methylation sites on 6 Treg signature genes (Foxp3, TNFRSF18, IL2RA, IKZF2, IKZF4, CTLA4) by profiling transcriptional start site (TSS) clusters located within the Treg-specific demethylation region in T1D subjects before and 8-weeks after in vivo BCG dosing.

Results: BCG induced demethylation at almost all TSS in all 6 signature genes. In vivo documented epigenetic changes correlated with increased mRNA expression of all 6 Treg signature genes in isolated CD4 T cells.

Conclusion: Overall, repeat BCG vaccinations reset the immune system by consistent and rapid demethylation of all 6 key Treg genes for enhanced mRNA expression, as monitored via CD4 T cells in blood from BCG-treated T1D subjects. This suggests that not only are Treg cell numbers transiently elevated after BCG vaccination, but also that permanent epigenetic expression of Treg genes that control Treg potency is stably re-established by BCG treatment. BCG vaccination, like tuberculosis, powerfully modulates Treg induction. Studies are underway to follow vaccinated T1D subjects for long-term beneficial clinical effects of Treg upregulation by BCG.

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Disclosure: **D.L. Faustman:** None.

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Epigenetic modifications of the ZNF423 gene control adipogenic commitment and are dysregulated in human hypertrophic obesity

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Background and aims: Subcutaneous adipocyte hypertrophy (SAT) is associated with insulin resistance and increased risk of type 2 diabetes (T2D) and predicts future development of T2D independent of obesity. In humans, SAT hypertrophy is a consequence of impaired adipocyte precursor cell recruitment into the adipogenic pathway rather than lack of precursor cells. Recently, the zinc-finger transcription factor Zfp423 was identified as a major determinant of preadipocyte commitment and maintained white adipose cell function. While its levels do not change during adipogenesis, ectopic expression of Zfp423 in non-adipogenic cells is sufficient to activate Pparγ expression and to increase the adipogenic potential of these cells. Here, we have investigated whether the Zfp423 gene is under epigenetic regulation and whether this plays a role in the restricted adipogenesis associated with hypertrophic obesity.

Materials and methods: 3T3-L1 and NIH-3T3 cells were used as fibroblasts committed and uncommitted to the adipocyte lineage, respectively. Human preadipocytes were isolated from the Stromal Vascular Fraction (SVF) of SAT of 20 lean non-diabetic individuals characterized by a wide adipose cell size range. mRNA levels were measured by Real-time PCR, while methylation levels were analyzed by bisulfite sequencing. Chromatin structure was analyzed by Micrococcal nuclease protection assay and DNA-methyltransferases were chemically inhibited by 5-azacytidine (AZA). Adipocyte differentiation rate was also evaluated by Oil Red O staining.

Results: Comparison of uncommitted (NIH-3T3) and committed adipose precursor cells (3T3-L1) revealed that *Zfp423* expression is increased in parallel with the ability of the cells to differentiate into mature adipocytes due to both decreased promoter DNA methylation and nucleosome occupancy in the 3T3-L1 compared to NIH-3T3 cells. Interestingly, non-adipogenic epigenetic profiles can be reverted by treatment of NIH-3T3 cells with 5-Azacytidine (AZA), as AZA increases *Zfp423* mRNA levels, reduces DNA methylation at a specific CpG site, decreases its nucleosome occupancy and induces adipocyte differentiation. These epigenetic modifications can also be initiated in response to changes in the adipose cell microenvironment, and where BMP4 plays a key role. We finally show that, in human adipocyte precursor cells, impaired epigenetic regulation of the *ZNF423* (human ortholog of the murine *Zfp423*) is associated with inappropriate subcutaneous adipose cell hypertrophy. As in the NIH-3T3 cells, normal *ZNF423* epigenetic profile can be rescued by AZA exposure.

Conclusion: Our results show that epigenetic events regulate the ability of precursor cells to commit and differentiate into mature adipocytes by modulating the *ZNF423* gene and indicate that dysregulation of these mechanisms accompanies SAT hypertrophy in humans.

Disclosure: **M. Longo:** None.

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Asymmetry in fingerprints as an early indicator of type 2 diabetes

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Background and aims: Early screening tools, coupled with an effective intervention, could help reduce the growing problem of type 2 diabetes mellitus. Current models for determining risk of T2DM include phenotypic characteristics and are based on characteristics that are often manifested after the development of hyperglycemia (high BMI, waist circumference). Models that incorporate genetic variants can provide insights into risk prior to the development of associated health problems. However, the costs involved for the models that involve genetic testing can be prohibitive. Fingerprints form during gestation, and are potentially influenced by the same environmental factors that influence the expression of genes involved in T2DM. In a previous study in Southeastern Ohio (100% White/Caucasian; 165 prints), asymmetry scores for finger pairs IV and V significantly predicted T2DM controlling for gender and age. In this study we tested the hypothesis that fluctuating asymmetry in fingerprints can be used as an early predictor of T2DM by assessing asymmetry scores for each finger pair using a wavelet-based analysis in an ethnically diverse population from Northern California.

Materials and methods: The study population was recruited from a series of public health primary clinics that provided a focused diabetes consultation service. The participants were eligible if they were adults (18 years or older) and had medically confirmed type 1 diabetes, type 2 diabetes, or were above the age of 40 and had no personal or family history of type 2 diabetes, metabolic syndrome, PCOS or any insulin resistant condition. People with chromosomal syndromes (Down's, Turner's, Klinefelter's), cleft palate or those with cystic fibrosis were also excluded. Rolled fingerprints were collected at the time of medical care using a Crossmatch Verifer 320 LC scanner at a resolution of 500 ppi and 256 level grayscale. The prints of all fingers on both hands were stored as uncompressed digital images on a dedicated laptop with encryption. The fingerprints were scored for similarity between corresponding fingers on each hand using a wavelet-based analysis.

Results: This study population (n=64) was a more ethnically diverse population (41% White/Caucasian, 26.5% African American, 14.5% Asian American, 18% Hispanic). In the multivariate model that best fit our data (Likelihood Ratio Chi-square = 29.3, df = 12, P = 0.004), asymmetry in finger pair IV was significant (P = 0.036), as well as interactions between finger pair IV and ethnicity (P = 0.046) and finger pair IV and age (P = 0.040).

Conclusion: These results add additional support to the hypothesis that asymmetry in fingerprints has the potential to be a powerful, early predictor of propensity to develop T2DM. In addition, fingerprint asymmetry could compliment genetic studies to improve current understanding of the genetic and environmental interactions involved in T2DM.

Supported by: Internal grants from Ohio University and Touro University CA

Disclosure: **J.H. Shubrook:** None.

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Paternal exercise increased the susceptibility to develop type 2 diabetes by trigger an incapacity of beta cell to compensate insulin resistance

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Background and aims: The pancreatic beta cell failure is a hallmark in the type 2 diabetes development and susceptibility , and the risk to develop it is dependent on genetics and environmental factors. The epigenetic mechanism helps to explain the link between environment alterations and transcriptional activity. The effect of paternal endurance exercise training upon offspring pancreatic islet function as well as DNA methylation profile have not been investigated yet.

Materials and methods: Female were kept in sedentary conditions as well as half of male progenitors, whereas the other half was subjected to 10 weeks of endurance exercise training. During the last week of the exercise protocol, the progenitors were mated. At weaning, the offspring were kept on high fat diet and distributed into two groups: male offspring from sedentary progenitors (H) and male offspring from trained male progenitors (HT). We measured glucose and insulin tolerance, as well as pancreatic islet insulin secretion and, calcium influx. Gene expression was realized by Real-time PCR, and DNA methylation by BisPCR^2 on pancreatic islets of offspring. To analyze the data, we used students'-t test. $p \leq 0.05$

Results: The insulin resistance was worst in HT compared to H mice (1.8 ±0.51 %/min from K_{ITT} H vers. 0.8± 0.44 HT, $p \leq 0.05$). HT insulin secretion was lower than H mice (21±3.32 ng/mL H vers. 8.22 ± 5.54 HT, $p \leq 0.05$), which trigger a very high glucose intolerance compared to offspring from control fathers (2000±20.02 AUC H vers. 2700±32.90 HT, $p \leq 0.05$). The reduced glucose stimulated insulin secretion by isolated pancreatic islets observed in the offspring from trained fathers was also associated with reduced pancreatic islet calcium influx (7.53±1.33 AUC H vers. 5.44±1.78 HT, $p \leq 0.05$). Due to knew function of HNF4-a gene to control the beta cell capacity to adapt its function in the context of insulin resistance, we look at HNF4-a gene expression in the pancreatic islet from offspring fed on high fat diet, and as we expected the HNF4-a gene expression was reduced in the offspring from trained fathers (1.00±0.44 arbitrary unit (UA) H vers 0.50±0.32 HT, $p \leq 0.05$), and the DNA methylation level in the HNF4-a gene promoter was higher explaining, at least in part how paternal exercise may impact the pancreatic islet function and HNF-4a gene expression (0.8±0.02 UA H vers. 0.95±0.01 HT, $p \leq 0.05$).

Conclusion: Taken together, paternal exercise training seems to increased the susceptibility to develop type 2 diabetes through beta cell reprograming, probably by orchestrated HFN4-a gene expression through DNA methylation modifications.

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Disclosure: **J.M. Costa-Júnior:** Grants; FAPESP.

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Expression of leptin receptor gene in HUVECs related to degree and duration of intrauterine hyperglycaemia

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Background and aims: Gestational diabetes mellitus (GDM) is associated with an increased incidence of type 2 diabetes and obesity in offspring. However, the mechanisms through which the GDM in the mother affects the development of these diseases in the offspring has not been studied thoroughly yet. The reason for those disorders can be either genetic predisposition or epigenetic influence of intrauterine hyperglycemia. One of the important regulators of energy homeostasis and the action of insulin is known to be leptin. Our aim was to study the effect of the degree and duration of maternal hyperglycemia on the level of expression of leptin and its receptor genes in HUVECs of newborns from women with gestational diabetes mellitus.

Materials and methods: The study included 22 women with GDM treated for GDM starting before 30-th week of gestation (GDM1), 6 women treated for GDM after 34-th week of gestation (late treatment group or GDM2) and 10 women without GDM (control group). All women underwent an OGTT at 24-28 weeks of gestation. The diagnosis of GDM was based on International Association of Diabetes and Pregnancy Study Groups criteria. HUVECs were isolated end expanded in vitro up to passage 2 and tested for viability and

replicative senescence. Samples with viability > 85% and low level of senescent cells (<10%) were used. Immunophenotype was determined by Flow Cytometry analysis. The level of genes expression was determined by RT-PCR. Women with GDM kept electronic nutrition and glycemic control diaries with the help of a specially developed mobile application and sent data to the doctor. According to the personal diaries automatic calculations of the integral indicators characterizing the self-control of glycemia (mean fasting and postprendial glycemia, the frequency of exceeding the target levels of glycemia) were accomplished. Statistical analysis included Kruskal-Wallace test, Mann-Whitney test and Spearman correlations.

Results: The increase in the expression of the leptin receptor (LEPR) gene in GDM2 group ($p < 0.001$) and in GDM1 group ($p = 0.039$) compared with the control group appeared to be 23.8 ± 6.8, 17.0 ± 3.7 and 4.5 ± 0.77, respectively. Notably, LEPR gene expression was higher in GDM2 than in GDM1 group though the difference didn't reach statistical significance (p= 0,1). The expression of the leptin gene was observed as below the detection threshold. Age and pregestational BMI did not differ among the groups. There was a positive correlation between the level of LEPR gene expression and the fasting blood glucose (BG) elevation rate above 5.3 mmol / L at 35 weeks gestation period ($r = 0.507$, $p = 0.032$), and the BG elevation rate above 7.0 mmol / L 1 hour postprandially at 35 weeks of gestation ($r = 0.488$, $p = 0.040$) and the value of 2 hour BG in OGTT ($r = 0.503$, $p = 0.004$).

Conclusion: The expression of LEPR gene in HUVECs of newborns was positively associated with the degree and duration of maternal hyperglycemia.

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Disclosure: **P. Popova:** Grants; The study was funded by Russian Science Foundation (project №15-14-30012).

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Correlation between circulating microRNAs and chronic kidney disease in patients with and without type 2 diabetes

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Background and aims: MicroRNAs (miRNAs) are small non-coding RNAs that usually function as intracellular repressors of target genes involved in many pathophysiologic processes. MiRNAs can also be detected outside cells including circulating cell-free body fluids. Circulating miRNAs have been proposed repeatedly in the literature as new attractive biomarkers in many diseases, including type 2 diabetes (T2DM) and chronic kidney disease (CKD). T2DM is closely linked to the development of CKD and it is unclear if putative associations between miRNAs and CKD differ in patients with or without T2DM.

Materials and methods: We therefore investigated the association between a panel of 40 candidate-miRNAs and CKD (based on estimated glomerular filtration rate) in 120 angiographied coronary patients with (n=65) and without (n=55) T2DM, respectively. P-values of less than 0.05 were regarded as statistically significant after correction for multiple testing.

Results: In the total patient cohort, miR-320a and miR-320b were significantly increased and miR-451a, miR-106b-5p, miR-25-3p, miR-20a-5p, miR-19b-3p, miR-16-5p, and miR-140-3p were significantly decreased in patients with CKD (corrected p-values ranging between 0.003 and 0.045). In the subpopulation of patients without T2DM associations between miR-451a, miR-106b-5p, miR-25-3p, miR-20a-5p, miR-19b-3p, miR-16-5p, and miR-140-3p and CKD were still significant (corrected p-values ranging between 0.011 and 0.039). In addition the association between miR-19a-3p and miR-99b-5p with CKD was significant in non-diabetic patients (corrected p-values = 0.039 and 0.021, respectively). None of the investigated miRNAs were significantly associated with CKD in patients with T2DM, at least after correction for multiple testing.

Conclusion: We conclude that numerous circulating miRNAs are significantly associated with CKD and that this association may be masked by the prevalence of T2DM.

Disclosure: **K. Geiger:** None.

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Plasma circulating microRNAs: novel clinical parameters to monitor autoimmune disease progression in children with type 1 diabetes

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Background and aims: Type 1 Diabetes (T1D) is an autoimmune disorder characterized by lymphocyte-mediated destruction of the insulin producing β cells of the pancreatic islets. Triggers of islet autoimmunity, the time course and the mechanism beyond the progressive β cell failure have not been completely clarified and we are in urgent need of novel biomarkers able to predict the rate of disease progression and to trace the progressive immune-mediated loss of insulin-secreting islet cells. One highly suitable source of blood-associated biomarkers is represented by extracellular microRNAs that, released by most cells in the body, reach the circulation, remain very stable, and may be used to assess cell activity at distance. Our published work shows that blood circulating microRNAs can be used as biomarkers of human lymphocyte activation in health and disease: in particular, our group has identified a specific microRNA signature that is massively released by activated CD4+ T lymphocytes in vitro, significantly deregulated in vivo in blood of individuals affected with auto-immunity (psoriasis) and then reverts to healthy donors' levels upon effective treatment with anti- inflammatory drugs. The aim of the present work was to test whether the quantification of plasma microRNAs may help to monitor and improve the prediction of T1D disease course.

Materials and methods: To test our hypothesis, we have retrospectively screened plasma samples from children who received the diagnosis of T1D (n=65) and healthy control subjects (n=26). microRNAs were quantified by RT qPCR and upon normalization with the average cQ value, differentially expressed microRNAs in diabetic children compared to the healthy counterpart were identified. We also performed a correlation analysis to evaluate the relationship i) among the differentially expressed microRNAs and ii) between the microRNAs and available clinical data of our patients, including C-peptide, hemoglobin A1c and the numbers of blood circulating lymphocyte subpopulations.

Results: The majority of the identified differentially expressed microRNAs are indeed part of the specific microRNA signature released by activated T lymphocytes, suggesting their blood modulation may be directly connected to disease-associated lymphocyte deregulation. Moreover, the subsequent correlation analysis was able to highlight complex microRNA co-modulation patterns, and potentially important relations between microRNAs and the traditionally used disease markers.

Conclusion: In conclusion, the present project has led to identifying blood circulating microRNAs as novel and potentially useful clinical parameters, directly connected to the pathological deranged activity of T cells in T1D patients.

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Disclosure: **P. de Candia:** None.

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A single dose of dapagliflozin, an SGLT-2 inhibitor, induces higher glycosuria in GCK and HNF1A-MODY than in type 2 diabetes

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Background and aims: Maturity Onset Diabetes of the Young (MODY) is estimated to account for 1-2% of all cases of diabetes. Dietary intervention is generally sufficient to maintain good glycemic control in subjects with Glucokinase (GCK) gene mutation. Hepatocyte Nuclear Factor 1 Alpha (HNF1A) gene mutations affect insulin secretion to a greater extent. For patients with genetically confirmed HNF1A-MODY sulfonylurea therapy should be considered as the first-line treatment. The extra-pancreatic features of HNF1A-MODY include glycosuria due to a low renal threshold for glucose, which has been linked to decreased sodium/glucose co-transporter 2 (SGLT2) expression in tubular cells. SGLT2 inhibitors are widely used in type 2 diabetes (T2DM). Their effectiveness in MODY is unknown. It can hypothesized that response to SGLT2 inhibitors, such as dapagliflozin, may be altered among HNF1A-MODY individuals, thus influencing their efficacy in these patients. We aimed to assess the response to a single dose of 10 mg dapagliflozin in patients with HNF1A-MODY, GCK-MODY, and T2DM.

Materials and methods: We examined 14 HNF1A-MODY, 19 GCK-MODY, and 12 T2DM patients. All studied individuals received a single morning dose of 10 mg of dapagliflozin added to their current therapy of diabetes. To assess the response to dapagliflozin we analyzed changes in urinary glucose to creatinine ratio (GCR), serum 1,5-Anhydroglucitol (1,5-AG) level and fasting plasma glucose (FPG) level. All parameters were measured in the morning of the dapagliflozin administration day and the day after.

Results: T2DM patients were older (61.8±5.6 vs. 34.1±11.0 and 40.3±10.8 years, p=0.00), more obese (31.3±5.3 vs. 24.4±4.9 and 23.4±2.8 kg/m^2, p=0.00), and were characterized by worse glycemic control (HbA1c: 6.9±0.9 vs. 6.0±0.7 and 6.4±0.4%, p=0.03) than HNF1A-MODY and GCK-MODY subjects what is in line with the way how groups were defined. There were no differences between study groups in terms of sex distribution and kidney function. There were only 4 patients with positive urine glucose before dapagliflozin administration (1 with HNF1A-MODY, 2 with GCK-MODY, and 1 with T2DM), whereas after SGLT-2 inhibitor use, glycosuria occurred in all studied participants. Considerable changes in mean GCR after dapagliflozin administration were observed in all three groups (20.51±12.08, 23.19±8.10, and 9.84±6.68 mmol/mmol for HNF1A-MODY, GCK-MODY, and T2DM, respectively, p<0.001 for all comparisons). Post-hoc analysis revealed significant differences in mean GCR change between T2DM and each monogenic diabetes in response to dapagliflozin (p=0.02, p=0.003 for HNF1A and GCK-MODY, respectively), but not between the two MODY forms (p=0.7231). Significant change in serum 1,5-AG was noticed only in T2DM (-6,57±7,34 mg/ml, p=0.04). Subgroup analyses revealed significant mean FPG reduction in GCK-MODY (0.50±0.72 mmol/l, p=0.007), but not in HNF1A-MODY (0.14±0.90 mmol/l, p=0.56) and T2DM (0.23±1.20 mmol/l, p=0.52).

Conclusion: A single dose of dapagliflozin, an SGLT-2 inhibitor, induces higher glycosuria in GCK- and HNF1A-MODY than in T2DM. Whether flozins are a valid therapeutic option in these forms of MODY requires long-term clinical studies.

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Disclosure: **J. Hohendorff:** None.

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Modelling of ER-stress in beta cells using genome edited patient-derived iPSC carrying insulin gene mutations

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Background and aims: Permanent neonatal diabetes is caused by mutations in genes important for the function of the pancreatic beta cell. Insulin mutations result in the misfolding and endoplasmic reticulum (ER) retention of the insulin protein. Long-term accumulation of aberrant insulin eventually leads to ER-stress, activation of the unfolded protein response pathway and apoptosis of the beta cells. ER-stress is also associated with the progression of T1 and T2 diabetes. To establish a new model for ER-stress induced diabetes, we derived iPSC lines from two Finnish families carrying different heterozygous dominant insulin mutations that result in neonatal diabetes onset at 3-4 months of age.

Materials and methods: Differentiation efficiency towards the beta cell varies between cell lines with different genetic background. This may obscure the disease phenotype when studying the specific effect of the insulin mutation in beta cells. To overcome this, we generated corrected isogenic cell lines by repairing the insulin gene mutations using CRISPR/Cas9. Corrected cells were differentiated in parallel with mutant cells using an optimized suspension differentiation protocol for 30 days. The resulting islet-like 3D clusters were transplanted under the kidney capsule of immunodeficient mice to complete the beta cell maturation in vivo.

Results: We analyzed the islet-like clusters before and three months after transplantation. Increased ER-stress was detected specifically in the INS+ mutant beta cells, with strong BIP (HSPA5) expression progression from a few BIP+INS+ cells before transplantation to virtually all INS+ after transplantation. Corrected cell lines did not present increased ER-stress markers at any stage. Insulin-reporter cell lines were generated using CRISPR/Cas9 from mutant and corrected cell lines, enabling INS+ cells FACS-sorting followed by RNA-seq. We also conducted single cell RNA-seq of islet-like clusters before transplantation to study the transcriptional changes at the cell level.

Conclusion: We envision this model will provide new insights into the molecular mechanisms behind ER-stress based demise of the beta cells and serve as a drug-screening platform for novel antidiabetic agents.

Supported by: Novo Nordisk Foundation, Academy of Finland, Sigrid Jusélius Foundation

Disclosure: **D. Balboa:** None.

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StarSeq, an innovative method based on NGS for accurate detection of punctual mutations and copy number variants in obese children

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Background and aims: Although targeted or exomic next generation sequencing (NGS) is now widely used for the detection of punctual coding mutations, DNA microarrays (SNPs or CGH) are still the current methods for the detection of copy number variants (CNVs) in the molecular diagnosis of genetic disorders. We have developed a groundbreaking, NGS-based, one-step tool for an accurate and cost effective detection of both punctual mutations and CNVs. We assessed its usefulness and accuracy for the molecular diagnosis of patients with monofactorial metabolic diseases.

Materials and methods: We used a custom capture (NimbleGen SeqCap EZ Choice XL) which included probes regularly spaced in the entire genome, in combination with a capture targeting the whole exome (NimbleGen SeqCap EZ MedExome Enrichment Kit). The resulting probes covered >80 Mb of the genome. Probes were placed every 10 kb apart in the regions known to be affected by CNVs (based on the literature and public database), and probes were placed every 25 kb in the rest of the genome. We tested this capture in a cohort of 100 obese children with developmental delay and 20 controls, whose 50% had already been analyzed by DNA microarrays (CGH Agilent 60k v2) for CNV detection. The sequencing was performed through Illumina HiSeq

4000, using 100 bp paired-end reads, and a mean sequencing depth higher than 85× was achieved for each individual.

Results: We detected all the CNVs previously identified by DNA microarrays and we found additional CNVs which were not found by DNA microarrays in our patients, probably due to the better resolution of our method. In parallel, we were also able to detect novel punctual mutations with the same accuracy as a standard exome sequencing. We were able to diagnose more than half of the patients, establishing causality to either punctual mutations and/or CNVs.

Conclusion: We demonstrated that our custom capture in combination with NGS enables an accurate, inexpensive detection of CNVs and punctual mutations in only one step. StarSeq dramatically improves genetic diagnosis and thus has the potential to replace standard exome sequencing and DNA arrays as this method is cost-effective and fast.

Disclosure: **A. Bonnefond:** None.

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A novel mutation in the IPF-1/PDX-1 gene identified in a Japanese family with MODY

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Background and aims: Maturity onset diabetes of the young (MODY), a subtype of diabetes mellitus, is a single gene disorder. We have been studying for one large Japanese family with MODY. Although screening for mutations in *MODY1*, *2*, *3*, and *5* of the proband was performed, and non-parametric linkage analysis had disclosed marginal peaks on chromosome 6, 7 and 22, the pathogenic variant has not yet been identified. Hence, the aim of this study was to identify the causative mutation in this family.

Materials and methods: The family consisted of 31 individuals with three generations. Standard 75g OGTT was performed to determine the phenotype and genomic DNA were obtained from 31 members. Whole exome sequencing were performed for two individuals with MODY (the proband and his daughter) and proband's wife for the reference. Exomes capture was performed with solution-based hyblidization using Sure-Select Human All Exon Kit Ver. 5 (Agilent Technologies, Santa Clara, CA, USA), and captured DNA fragments were subsequently sequenced using the HiSeq2000 system (Illumina, San Diego, CA, USA) by paired-end 100-bp reads. Reads were mapped to the Genome Reference Consortium human build 37 using the BWA-MEM. Variants were identified by single-sample calling with UnifiedGenotyper using GATK ver. 3.5 and annotated using ANNOVAR. Functional effects of the detected variants were assessed using SIFT, PolyPhen-2 and CADD v1.3.

Results: The variant c.443G>T:p.R148L in IPF-1/PDX-1 (MODY4) gene was found in the proband and his daughter in heterozygous form, but not in the spouse. Although R148G on the same position was reported as rs193922355 and interpreted as likely pathogenic in ClinVar, R148L is not presented in Exac, gnomAD or T2D portal. This variant was located in the homeodomain (Exon 2). SIFT and PP2 predicted Damaging, and CADD score was 34.0. Those data strongly suggested that this variant could be pathogenic. Sanger sequence in the member of this family disclosed that the variant was segregated with diabetes except for one individual with MODY. Interestingly, he was identified to have p2 prompter c.-79C>T (hetero) in HNF4A (MODY1) gene, which was inherited from his father who did not have diabetes. Therefore, p2 promoter c.-79C>T in HNF4A gene does not seem to be pathogenic even CADD score was 18.11. We could not find any meaningful signal at least in 13 known MODY genes in this patient.

Conclusion: We identified the MODY4 family in Japanese with a novel substitution. Moreover, one phenocopy has a possibility to carry the different mutation within a single family.

Supported by: Grant-in-Aid from MEXT, and a Grant from MHLW, Japan

Disclosure: **N. Iwasaki:** None.

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Genome wide association search for variants influencing age of diagnosis in HNF1A-MODY diabetes

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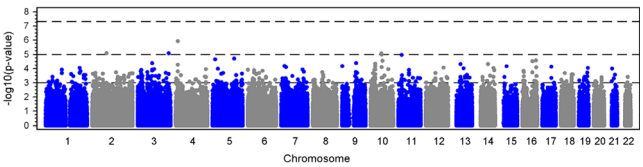
Background and aims: Diabetes caused by mutations in the HNF1A gene is one of the most frequent forms of monogenic diabetes. Since the age of diagnosis is variable we aimed to examine whether common genetic variants influence the age of onset of the disease.

Materials and methods: A genome-wide association study (GWAS) on 814 HNF1A diabetic mutation carriers was performed. Numbers of study participants from different countries were as follows: 257 patients from France, 247 from the UK, 90 from the USA, 86 from Czech Republic, 78 from Poland, and 56 from Slovakia. The GWAS was performed using Illumina Human Core chip. In total, 222,662 autosomal polymorphisms with minor allele frequency > 5% were eligible for the analysis. The analysis was performed with R version 3.3.1 and PLINK 1.07 and 1.9.

Results: We identified 5 loci on chromosomes 4, 2, 3 and 10 showing suggestive evidence of association with age at diabetes diagnosis ($p < 10^{-5}$). The most significant association was for LOC105374493 on chromosome 4. Its rare allele G postponed diabetes diagnosis by an average of 2.8 years. Similar effect was seen for another intronic variant rs2070633 and one regulatory region variant (rs1033287). Twenty seven variants were associated with age of diagnosis at the level $p < 10^{-4}$. Most of them were intronic variants, 7 intergenic, 3 downstream or upstream gene variants, 1 non coding transcript exon variant and 1 missense variant.

Conclusion: There is a suggestive evidence that common polymorphic variants contribute to age of diabetes onset in HNF1A-MODY. We were however unable to find any high impact loci associated with age of diagnosis. It is possible, that rare genetic variants influence this phenotype.



Supported by: Z/IPZ/00175

Disclosure: **A.H. Ludwig-Galezowska:** None.

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A novel pathogenic variant mutation of MODY type 3

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Background and aims: Maturity onset diabetes of young is a type of diabetes inherited which has autonomous dominant inheritance

Materials and methods: Case report: We report a case of lean 37 year male who came to us with history of diabetes for 19 years diagnosed at age of 18 years. When seen first he was on 4 OHA, metformin 2 gm , canaglifozin 100 mg, sitagliptin 100mg, pioglitazone 15 mg his fasting blood glucose was 160

and post prandial blood glucose was 280 and a HbA1c of 9.4 %. On carefully reviewing the history we found that he had family history diabetes involving three generation at similar age. Patient was shifted on glimepride 3 mg and all the other OHA was stopped. He had good response the same and his fasting blood glucose came to 110 and post prandial blood glucose 160 and HbA1c after three months was 6.7%. Genetic analysis was done by Sanger sequencing. His report came out to be positive for novel Pathogenic variant c.599G>A p>Arg200Gln in HNF1A gene. This variation is located in a conserved region and the bioinformatic analysis using Polyphen_2, Sorting Intolerant From Tolerant (SIFT), mutation tester predict this variant as probaly damaging. This mutation has not been described by exome sequencing project or exome aggregation consortium.

Results: N/A

Conclusion: Therefore, this novel variation with a previous reports of mutation at this codon and damaging in silico predictions suggest that this variant is clinically relevant and is likely pathogenic based on American College of Medical Genetics and Genomics (ACMG) 2015 guidelines

Supported by: CMC Vellore endocrinology department for genetic anslysis

Disclosure: **A.K.R. Pande:** None.

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Predicting the effects of glucokinase mutations: Are we there yet?

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Background and aims: Inactivating mutations of the glucokinase (GCK) are causative for monogenic diabetes (*GCK*-MODY), permanent neonatal diabetes mellitus and insulin-deficient hyperglycemia, while the activating mutations lead to the persistent hyperinsulinemic hypoglycemia of infancy (PHHI). Characterization of inhibitory mutations is a laborious and time-consuming task, which prevents large-scale analyses conducted for clinical purposes, particularly when considering a structural perspective. Recently, computational evolution- and structure-based prediction analyses were suggested to be used in order to predict the effects of particular GCK mutations but were never matched with the data obtained *in vitro* or with clinical data.

Materials and methods: We analyzed the usefulness of eight most frequently used *in silico* methods, which have a potential to predict the effects of GCK mutations (PolyPhen2, SIFT, SNAP2, PoPMuSiC 2.1, I-Mutant 3.0, SNPs&GO, PhD-SNP and AlignGVGD). These methods were based on biophysical characterization, evolution-based sequence information, and structural data. The resulting data were calculated using either neural networks or Bayesian methods and mathematical operations. We compared these predictions with outcomes of direct measurements of enzyme kinetics *in vitro* (126 mutations) and reported previously or reported newly in course of this study and with the clinical information known from patients carrying these mutations (161 MODY-associated mutations, 14 PHHI-associated mutations, 14 neutral mutations, and polymorphisms).

Results: The clinical data and the data on enzyme kinetics of GCK constructs carrying particular disease-associated mutations corresponded with the *in silico* predictions to only a limited extent. The prediction methods were particularly ineffective when attempting to identify the activating mutations known to cause PHHI, and were unable to distinguish between the activating and inhibitory mutations at all. Some prediction methods had high sensitivity when predicting MODY-associated mutations (e.g., the sensitivity of Align GVGD was 93%). However, their sensitivity for neutral mutations was low, usually below 80%, or with a high ratio of false positive predictions of the neutrality among the disease-associated mutations (up to 29%). Similar results were obtained when matching the outcomes of the prediction methods to the GSIR-T or Hill coefficient.

Conclusion: This study provides the first robust evidence allowing to choose the best-fit model for a prediction of effects of GCK mutations, for which the *in vitro* data are still absent. Low precision of the available methods still suggests that the laborious *in vitro* functional analyses of the enzyme kinetics of disease-associated GCK mutations are still needed.

Supported by: Czech Science Foundation 15-03834Y

Disclosure: **P. Heneberg:** Grants; Czech Science Foundation 15-03834Y.

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Developing high throughput assay for functional classification of novel missense variants in HNF1A

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Background and aims: Variants in the transcription factor gene *HNF1A* are associated with different diabetes phenotypes and can be identified in subjects with maturity-onset diabetes of the young (MODY) type 3, type 2 diabetes and children with apparent type 1 diabetes. With increasingly effective sequencing methods identifying variants with unknown significance in these subjects, tools are needed to determine the true pathogenicity of such variants. Bioinformatics based programs have limitations. Current functional assay are low throughput and laborious. Furthermore, these assays utilize artificial transcriptional reporter constructs that may not have fidelity with in vivo function. In order to accurately discriminate human disease causing and benign variants, we aim to develop a high throughput cell-based HNF1A functional assay based on multiple endogenous target genes in multiple cellular contexts.

Materials and methods: To develop assays with higher fidelity with in vivo function, we assessed the full complexity of *HNF1A* transcriptional response in different cellular contexts. For this purpose we generated *HNF1A*- free cell lines by knocking out endogenous *HNF1A* in HUH7 and Hep3B hepatoma cells using CRISPR/Cas9. *HNF1A* was re-introduced into the knock-out cell lines at varying doses, then gene expression profile analysed by RNA sequencing. We selected target genes with the highest expression fold-change in response to *HNF1A* induction. Significance of differential expression was assessed by the Wald test. mRNA level expression of selected targets will use in RNA-Fish FACS-based system that allows segregation of cell populations based severity of *HNF1A* dysfunction

Results: Transcriptomic profiling identified multiple target genes; some were cell line specific while others were common across both cell lines. Of the most significantly differentially expressed genes ($q \leq 7.2 \times 10^{-31}$) the highest fold changes for HUH7 was *CLDN2* (fold-change 24), *AKR1B10* (fc=22) and *HABP2* (fc=21). In Hep3B the highest fold-changes were *KRT39* (fc=133), *FOLR1* (fc=100) and *KL* (fc=61). *LIPC*, *CLDN2*, *CYP3A* genes showed significantly increased expression in both cell lines ($q \leq 1.7 \times 10^{-28}$). Some canonical HNF1A target genes showed different expression profile between the two cell lines. For example *TMEM27* and *TM4SF4* were expressed only in Hep3B and *AFP* and *FGB* only in HUH7. The mRNA expression levels of multiple target genes will characterize in single cells by RNA-FISH. The level of expression is indicative of HNF1A function

Conclusion: In conclusion we have identified multiple novel, endogenous cell-specific targets for *HNF1A* that can be used for developing a high throughput functional assay. RNA-FISH in combination with flow cytometry enables us to measure multiple targets simultaneously in single cells, thus leading the way towards high-throughput disease-relevant assays of gene function.

Supported by: KG Jebsen

Disclosure: **L. Najmi:** None.

PS 004 Cardiovascular outcomes in diabetes

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Reduction of cardiovascular event risk by a pragmatic lifestyle intervention in rural India

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Background and aims: India has a substantial burden of cardiovascular disease (CVD). Evidence regarding the effect of pragmatic strategies targeting the burden of CVD in India is lacking. The Kerala Diabetes Prevention Program (K-DPP) was a clustered randomised controlled trial of a pragmatic lifestyle intervention program for prevention of type 2 diabetes among individuals at high risk for diabetes recruited using a diabetes risk score from rural communities in Kerala, India. This presentation aims to answer the following question: Whether a pragmatic lifestyle intervention program could reduce CVD risk compared with the control arm among K-DPP participants?

Materials and methods: The intervention arm of K-DPP received a 12-month group-based peer support lifestyle intervention. Control arm received a booklet on lifestyle modification advice. Participants were assessed at the end of intervention and 12 months post-intervention. The analysis set included those aged ≥40 years at baseline. The risk of having a CVD event within 10 years was estimated for every participant at baseline and at 12 and 24 months, using the "GloboRisk" equation. Participants with a predicted risk of at least 20% were defined as being at high risk for a CVD event. Due to the clustered and repeated nature of the data, a logistic regression model was fitted using a generalised estimating equation to estimate the odds ratio (OR) and 95% confidence interval (CI) for having a high predicted CVD risk in lifestyle intervention arm compared to control one. The models included predicted CVD risk status (≥20% vs. <20%) as the outcome and intervention status, time point, and the interaction term between intervention status and time point as well as baseline values of age, sex, total cholesterol, systolic blood pressure, current smoking status, fasting plasma glucose, 2-hour plasma glucose, and HbA1c as independent variables. The estimated coefficients of interaction term at 12 and 24 months were used to interpret the effect of the lifestyle intervention on CVD risk.

Results: Overall, 387 participants (47% female) in the control arm and 375 (46% female) in the intervention arm with the mean (standard deviation) age of 49.7 (6.0) and 50.5 (5.7) years, respectively, were included in the analysis set. There was no clinically important difference in baseline characteristics between the study arms. The median (25th -75th percentile) of estimated CVD risk in control and intervention arms were 8.6 (4.9; 14.5) and 8.9 (5.6; 14.6) at baseline, 8.9 (5.2; 14.2) and 8.9 (5.7; 15.1) at 12 months, and 9.9 (6.1; 16.4) and 9.8 (6.4; 15.3) at 24 months, respectively. The proportion of participants with high predicted CVD risk in the control arm was 10.1% and 12.0% in the intervention arm at baseline. In the control arm, the proportion of those with CVD risk increased to 12.4% at 12 months and 17.2% at 24 months and the corresponding proportions in the intervention arm were 12.8%, and 13.8%, respectively. The OR [95% CI] of having a high CVD risk in the intervention compared to control was 0.80 [0.38, 1.67] at 12 months and 0.45 [0.20, 1.00] at 24 months.

Conclusion: In the population that the study represents, the risk of CVD and proportion of those with high CVD risk increased over a period of 2 years in absence of any intervention. Compared to this, a pragmatic lifestyle intervention showed a reduced risk.

Clinical Trial Registration Number: ACTRN12611000262909

Supported by: the NHMRC, Australia

Disclosure: **M. Lotfaliany:** None.

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Effect of depression and/or anxiety on the presentation of cardiovascular events in a large cohort with metabolic syndrome

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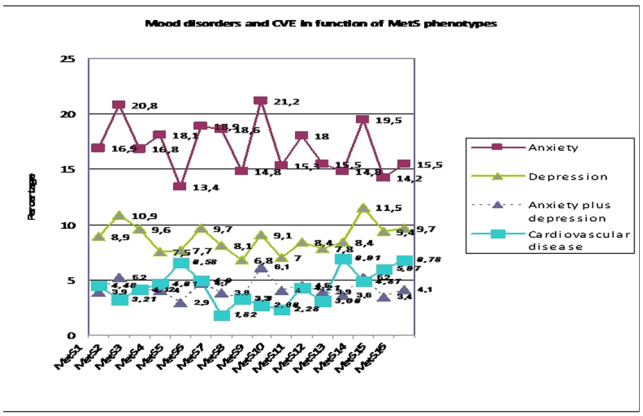
[1]Catalan Health Institute, Salou, [2]Catalan Health Institute, Reus, [3]Catalan Health Institute, L'Ampolla, [4]Catalan Health Institute, Constantí, Spain.

Background and aims: Metabolic syndrome (MetS) is a common condition in all developed countries. The effect of anxiety and/or depression as manifestations of stress on the adrenal axis, gluconeogenesis, cardiovascular disease or glucose tolerance has been widely demonstrated. But association of stress with poor prognosis and cardiovascular disease remain still unclear. To determine the role of anxiety and depression on the incidence of cardiovascular events (CVE) in a Catalonian population with MetS over a five-year follow-up according to the number/type of MetS criteria.

Materials and methods: Prospective study to determine the incidence of CVE according to the presence of anxiety and depression disorders among individuals with different combinations of clinical traits of the MetS. Setting: Primary Care, Catalonia (Spain). Subjects: 35-75 years old fulfilling MetS criteria without CVE at the initiation of follow-up (2009). We studied 16 MetS phenotypes [NCEP-ATPIII criteria] based on the presence of depression/anxiety. The primary endpoint was the incidence of CVE at five years.

Results: We analyzed 401,743 people with MetS (17.2% of the population); 8.7% had depression, 16.0% anxiety and 3.8% both. 14.5% consumed antidepressants and 20.8% tranquilizers. At the 5-year follow-up, the incidence of CVE was 5.5%, being 6.4% in men and 4.4% in women. On comparing individuals with and without depression the incidence of CVE was 6.7% vs. 5.3%, respectively ($p<0.01$), being 5.5% in both groups in relation to anxiety.The graphic shows data about mood disorders and CVE according to phenotypes; then, anxiety was more frequent in the MetS9, MetS2 and MetS14 phenotypes; depression in the MetS14, MetS2 and MetS6 phenotypes; and the MetS9, MetS14 and MetS2 phenotypes were more often found in both anxiety and depression.

Conclusion: Unlike other European cohorts the predominant MetS phenotypes in Catalonia do not include obesity as a criterion. Depression and anxiety play a role in the poor prognosis of patients with MetS.



Supported by: Grant SEMFYC 2009; Grant to Research CAMFiC 2010; Grant SIDIAP 2013

Disclosure: **Y. Ortega:** None.

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Indexes of body fat, but not BMI, are associated with cardiovascular (CV) mortality in subjects with diabetes (DM) during 17 year follow-up in the Norwegian HUNT2 study

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Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital, Oslo, Norway.

Background and aims: Obesity may be associated with increased CV risk and mortality, but the best measure to quantify metabolic unhealthy obesity is still debated. In a large Norwegian population-based cohort, we aimed to explore the association between four different indexes of obesity and CV mortality in subjects with or without DM.

Materials and methods: We used data from the Nord-Trøndelag Health Study (HUNT2), a Norwegian,population-based cohort study of 63 978 subjects, of whom 1955 had DM. Baseline assessments were done in 1995-1997 and validated markers of adiposity were calculated: Body mass index (BMI), waist-hip-ratio (WHR), index of central obesity (ICO=waist /height) and estimated total body fat ($eTBF_{men}$: 100*(-98.42 + [4.15*WC] - [0.082*weight])/ weight; $eTBF_{women}$:100*(-76.76 + [4.15*WC] - [0.082*weight])/weight). Causes of death were retrieved from the Norwegian Cause of Death registry from August 15th 1995 to December 31st 2015 with a mean±SD follow up of 17.2±4.8 years. The associations between adiposity markers and CV death were explored by Cox regression.

Results: At baseline, mean ± SD of the fat markers in the DM population were: BMI: 28.8±4.6 kg/m^2, WHR: 0.89±0.08, ICO: 0.57±0.07, and eTBF: 29.6±8.8%. During follow-up 560 (28.6%) and 4803 (7.7%) CV deaths occurred in the DM and non-DM subgroups, respectively. The marker with the strongest association to CV death in DM was eTBF (HR (95% CI) per 1 SD increase: 1.35 (1.25-1.45), p<0.0001), followed by ICO (1.26 (1.17-1.35), p <0.0001) and WHR 1.17 (1.07-1.27), p=0.022), while BMI was not significant (1.01 (0.94-1.09), p=0.792). In the non-DM group, findings were similar for eTBF, ICO and WHR, with HR per 1 SD increase eTBF (95% CI): 1.91 (1.86, 1.96), ICO: 1.90 (1.86, 1.95), and WHR: 1.75 (1.70, 1.80), but in this group also BMI was significant: 1.24 (1.21, 1.28). Of interest is that the curves that describe the relationship between eTBF and proportion of CV death differ between DM and non-DM groups indicating that CV mortality does not increase in the highest eTBF categories (Figure).

Conclusion: In this population-based cohort study, eTBF was the fat marker with the strongest association to CV death in subjects with DM, whereas BMI was not significantly associated. eTBF may be suitable for risk stratification in a clinical setting.

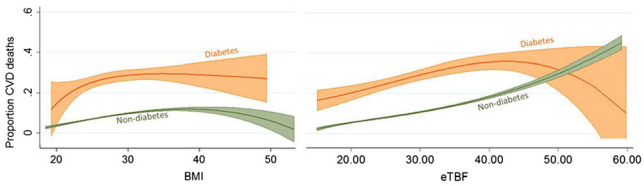

Figure: Relationship between BMI (kg/m2) and estimated total body fat (eTBF, %) and estimated mean (95% CI) proportion of CV deaths in the diabetes and non-diabetes population.

Disclosure: **A.P. Ofstad:** Employment/Consultancy; Boehringer Ingelheim Norway.

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Sex differences in major cardiovascular events by diabetes status in England: a nationwide study 2004-2014

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Background and aims: Secondary prevention of cardiovascular disease (CVD) has improved immensely during the past decade but controversies persist on cardiovascular benefits among women. While secondary prevention has become more equitable between men and women, women are still less likely to achieve treatment targets. We investigated recent trends in hospital admission rates for acute myocardial infarction (AMI), stroke, percutaneous coronary intervention (PCI), and coronary artery bypass graft (CABG) by sex and diabetes status in England.

Materials and methods: We identified all hospital admissions for major CVD events and procedures among people aged ≥17 between 2004 and 2014 in England. We calculated diabetes-specific and non-diabetes-specific rates for study outcomes by sex. To assess temporal changes, we fitted negative binomial regression models.

Results: Age- and sex-standardised rates were higher in men compared with women in people with and without diabetes for all outcomes except for stroke in the non-diabetes group. During the 11-year follow-up time, diabetes-related admission rates remained unchanged for AMI (rate ratio 0.99 [0.98-1.01]), increased for stroke by 2% (1.02 [1.01-1.03]) and PCI by 3% (1.03 [1.01-1.04]) and declined for CABG by 3% (0.97 [0.96-0.98]) annually. Trends did not differ significantly by diabetes status. Women with diabetes had significantly lower rates of AMI (0.46 [0.40-0.53]) and stroke (0.73 [0.63-0.84]) compared with men with diabetes. However, sex differences in AMI rates attenuated in diabetes compared with the non-diabetic group. While diabetes tripled admission rates for AMI in men (3.15 [2.72-3.64]), it increased it by over 4-fold among women (4.3 [3.8-4.93]). Proportional changes in rates were similar in both sexes for all outcomes, leaving the relative risk of events unchanged.

Conclusion: Diabetes still confers a greater increase in risk of AMI in women relative to men. However, the absolute risk remains higher in men. These results call for intensified CVD risk factor management among people with diabetes and treatment intensity to align with risk.

Disclosure: **E.P. Vamos:** None.

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Life years lost and cardiovascular risk in type 2 diabetes patients requiring glucose-lowering treatment 2008-2015: nationwide data from Norway and Sweden

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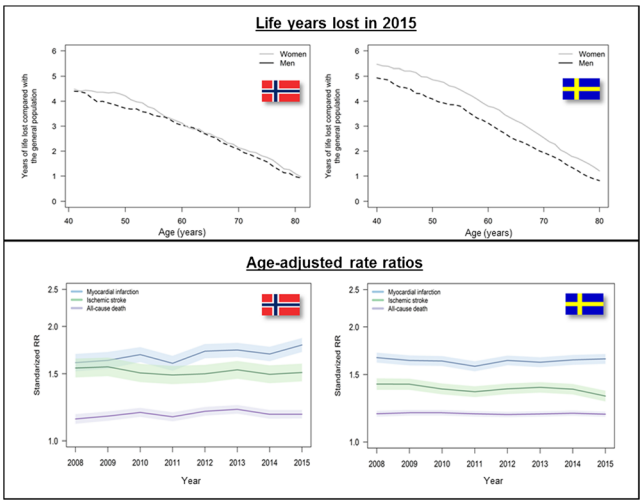
Background and aims: Type 2 diabetes (T2D) carries severe long term consequences for the patient by shortening life expectancy and increasing risk of cardiovascular (CV) disease. Considering recent T2D treatment strategies, it is vital to fill the lack of current data on changes in patient related disease consequences. The aim was to examine life-years lost and age-adjusted rate ratios of myocardial infarction (MI), stroke and all-cause mortality (ACM) in Norway and Sweden, two neighbouring countries with similar health care systems.

Materials and methods: All patients dispensed with glucose lowering drugs (GLDs) during 2008—2015 were identified in nationwide registries in Norway and Sweden. Patients aged <18 years, and those with polycystic ovarian syndrome, type 1- or gestational diabetes were excluded. Life-years lost was estimated by age calculating the difference between life expectancy in a general population vs the T2D population. Rate ratios were calculated by comparing age adjusted rates in the T2D population with rates in the corresponding general population.

Results: In Norway and Sweden, 604,936 T2D patients were identified in 2015. In 2015, younger patients lost more life years and results were similar in both countries (figure, upper panels). In Sweden, women had a slightly higher life-year loss compared to men, while no gender differences were seen in Norway. With increasing age, life-years lost showed a linear decrease up to 80 years of age. In 2015, age adjusted rate ratios were 1.72, 1.41 and 1.17 for MI, stroke and ACM, respectively (figure, lower panels). The rate ratios for MI, stroke and ACM showed small but non-significant changes over time in both countries, except for slight increase for MI in Norway.

Conclusion: T2D patients on glucose lowering drugs and in the age of 40 years, are expected to have about 5 years shorter life expectancy than the general population in Norway and Sweden. The frequency of severe CV complications showed similar patterns over time in the two countries. These nationwide results highlight the importance to prevent

diabetes development at early ages and improve CV prevention when T2D is present.



Supported by: AstraZeneca

Disclosure: **H.L. Gulseth:** Grants; Astra Zeneca.

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Epidemiological characteristics of lower extremity arterial disease in Chinese diabetes patients: a prospective, multicenters, cross-sectional study

X. Zhang[1], Z. Xu[2], X. Ran[3], L. Ji[1,4], China DIA-LEAD Study Investigators; [1]Department of Endocrinology, Peking University International Hospital, [2]Department of Endocrinology, The 306th Hospital of PLA, Beijing, [3]Department of Endocrinology, West China Hospital, Chengdu, [4]Department of Endocrinology and Metabolism, Peking University People's Hospital, Beijing, China.

Background and aims: Lower extremity arterial disease (LEAD) is associated with a 5.9-fold increased risk of cardiovascular mortality in type 2 diabetes patients. However, LEAD is often neglected because of asymptomatic intermittent claudication in real-life settings. This study was designed to determine the epidemiological characteristics of LEAD and identify practical gaps in LEAD management in China.

Materials and methods: This cross-sectional study consecutively enrolled 7113 inpatients and 3568 outpatients aged 50 years and older who had been diagnosed with diabetes from 30 hospitals across China between June 2016 and January 2017. Patient demographics, medical history, recent blood glucose measurements, and medications were recorded. The Ankle-Brachial Index (ABI) for each leg was separately calculated, and the lower value was used for analysis. All patients were assessed for LEAD by ABI and/or lower limb ultrasonography according to 2013 Chinese guidelines on LEAD screening and management. Multivariate logistic regression was used to identify risk factors for LEAD.

Results: A total of 10,681 eligible patients, 5714 men (53.5%) and 4967 women (46.5%), were included in the present analysis. The mean age was 64.2 years; 16.1% of patients were obese; and 26.6% were smokers. The mean fasting blood glucose and glycosylated hemoglobin (HbA1c) levels were 8.9 mmol/L and 8.4%, respectively. Only 28.2% (n=3012) of patients achieved the goal of HbA1c <7.0%. The median duration of diabetes was 9.0 years (range: 0.1-60 years). The overall prevalence of LEAD was 21.2%, with 10.6% (n=1134) of patients diagnosed with LEAD before enrollment and 11.8% (n=1131) newly diagnosed at the present visit. Most cases of newly diagnosed LEAD were easily confirmed by ABI (defined as ABI >1.3 or ABI ≤0.9). Interestingly, the percentage of patients with an ABI >1.3 was significantly lower among newly diagnosed patients than among patients with a prior diagnosis (7.6% *vs.* 51.0%, $p<0.001$). There was no gender disparity regarding LEAD diagnosis. A significantly greater percentage of inpatients (25.5%) were diagnosed with LEAD compared with outpatients (12.6%, $p<0.001$). Of patients with newly diagnosed LEAD, the percentages of patients receiving antiplatelet, antihypertension, lipid modification, and vasodilator therapies were 42.8%, 54.4%, 46.4%, and 39.3%, respectively. In multivariate logistic regression analysis, older age, abnormities on skin examination, higher systolic blood pressure, cerebrovascular comorbidity, and diabetic retinopathy were independent risk factors for LEAD among both inpatients and outpatients.

Conclusion: LEAD is highly prevalent in Chinese diabetes patients, and half of cases are underdiagnosed. The noninvasive ABI measurement can easily identify most patients with previously unrecognized LEAD. The current treatment approach for diabetes patients with LEAD is suboptimal. Efforts are need to optimize both diagnosis and therapy to further bridge the gap between guideline recommendations and real practice.

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Disclosure: **X. Zhang:** None.

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Relationship between weight change and incident stroke based on Korean National Health Screening Database

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Background and aims: The association between overweight and risk of ischemic stroke is well known. However, it remains controversial how weight change affects the stroke development, especially in Asians. In our study, we used Korean Nationwide Health Screening Database to study the association between weight change and incidence of stroke.

Materials and methods: Our study enrolled 11,084,683 Koreans who participated in National Health Screening Program from 2009 to 2012, and we measured weight change by checking the weight of four years ago. The occurrence of ischemic stroke, which was diagnosed for the first time in life, was observed until 2012. We categorized the study population according to weight change and analyzed the risk of ischemic stroke with Cox regression hazard model.

Results: 113,591 subjects (1.02% of total) were newly diagnosed with stroke during the mean observation period of 5.2 years. After multivariate-adjusted subgroup analyses, the hazard ratio of stroke was higher in subjects who had weight loss (≤-5%) or weight gain (≥+5%) compared with those who maintained weight (-5%~+5%) {1.152 (1.135~1.17) and 1.087 (1.069~1.106) vs. 1.000}. When the analyses were performed in 8 groups according to weight change, the risk for stroke showed a U-shaped curve with those who had weight changes within ±5% showed the lowest risk and gradually increased as weight loss or gain. When the incidence of stroke was analyzed according to the pre- and post-analysis body mass index (BMI), those who were obese and became lean, and those who were lean and became obese showed the higher incidence of stroke compared with those who maintained their BMI.

Conclusion: The risk of ischemic stroke showed a U-shaped curve with the group of maintaining weight (-5%~+5%) having the lowest risk, and the risk increased in those who had weight loss or gain. Further follow-up studies are required to analyze the long-term effects of weight change on the incidence of ischemic stroke.

Clinical Trial Registration Number: KBSMC 2017-02-031

Disclosure: **J. Cho:** None.

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Rationale and design of a longitudinal nationwide study on management and real-world outcomes of diabetes in India (LANDMARC): a 3-year Pan-India cohort

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Background and aims: India, the second most populous country of the world, has been severely affected by the global diabetes epidemic. While the incidence and prevalence of diabetes is being studied through cross-sectional studies, there are only limited longitudinal studies to understand the development of diabetes complications over time and to explore their regional occurrence. LANDMARC (A **L**ongitudin**A**l **N**ationwide Stu**D**y on **M**anagement **A**nd **R**eal-world Out**C**omes of Diabetes in India) is by design, the first prospective, long-term, longitudinal and observational study conducted in India, investigating a large cohort of patients with type 2 diabetes (T2D) across the country. The study is primarily designed to document and analyse the management of diabetes and its complications over time.

Materials and methods: Overall, 6300 adult patients, with diagnosed T2D from 450 sites across India, who are currently on 2 or more anti-diabetic agents (either controlled or un-controlled on HbA1c) are planned to be enrolled. Study participants will be prospectively followed-up for 3 years. Data collection will include key baseline patient characteristics, comorbidities, glycaemic parameters, use of oral and injectable anti-diabetic agents, and any cardiovascular or other diabetes related event occurring during the observational period.

Results: This study will identify the proportion of patients developing macrovascular complications during 3 years follow-up. Other endpoints include microvascular complications (composite of renal and retinal), glycemic control, and time to treatment adaptation or intensification during 3 years. We will also be able to note the changes in physician preferences, how new drugs have changed the treatment paradigm in India. LANDMARC is also expected to help us in understanding the common trends of diabetes management in India (e.g. emergence of complications at a relatively earlier age, delayed insulinization, high use of OAD combinations, dose titration patterns).

Conclusion: The LANDMARC study aims to to capture and understand the progression of the disease, its control and treatment, and complications over time.

Supported by: Sanofi India Ltd

Disclosure: **A. Mithal:** Employment/Consultancy; Sanofi India Ltd.

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White blood cell count is associated with incident cardio-renal complications in Chinese patients with type 2 diabetes

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Background and aims: Previous studies revealed that chronic inflammation is one of the major mechanisms responsible for the development of diabetic complications. This study aims to investigate the relationship between white blood cell count (WBCC), a biomarker of inflammation, with cardio-renal complications in a prospective cohort of Chinese patients with type 2 diabetes (T2D).

Materials and methods: We included 7893 patients with T2D (47.0% male, mean age 57.8 ± 13.2 years and BMI 25.2 ± 4.1 kg/m^2) who had undergone a comprehensive assessment of complications and cardiometabolic risk factors based on the European DIABCARE protocol. The WBCC was measured and we removed outliers beyond 4 standard deviations from the mean. In the analysis for each incident diabetic complication, patients with history of the particular complication at baseline were excluded. Sex-specific associations between WBCC and incident diabetic complications were assessed by Cox proportional hazard regression adjusted for conventional risk factors including age, duration of diabetes, body mass index, HbA_{1c}, smoking status and the use of drugs at baseline. The sex-specific association results were meta-analyzed using the inverse-variance weighting approach. Cochran's Q test was used to assess heterogeneity of effects between sexes. We conducted a 4-knots restricted cubic spline regression analysis to test for the non-linear relationship between WBCC and complications.

Results: During a mean follow up period of 10.7 years, 734 (10.1%) patients developed coronary heart diseases (CHD), 711 (9.3%) developed stroke, 1232 (18.4) developed cardiovascular diseases (CVD), 648 (8.4%) developed congestive heart failure (CHF), 2422 (36.1%) developed chronic kidney disease (CKD), 976 (12.5%) developed end stage renal disease (ESRD), and 762 (9.9%) developed cancer. In the meta-analysis, each 1-unit (1×10^{-9}/l) increment of WBCC was associated with increased risk of incident CHD by 5.3% (95% CI 1.4-9.5), incident stroke by 5.1% (1.0-9.3), incident CVD by 5.7% (2.5-8.9), incident CHF by 9.7% (5.4-14.2), incident CKD by 7.0% (4.7-9.4), and incident ESRD by 10.3% (6.8-13.8). We further observed a sex-specific association for incident stroke in female patients (P for heterogeneity test = 0.0322). Moreover, we observed a non-linear relationship between WBCC and cancer (P for non-linearity test = 0.022 and 0.029 in male and female, respectively).

Conclusion: High WBCC is associated with increased risk of incident cardio-renal complications. Our findings support the role of low grade inflammation in the development of diabetic complication in T2D.

Supported by: RGC of HKSAR and FIS from CUHK

Disclosure: **C.H.T. Tam:** None.

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Cardiovascular events before and 15 years after diagnosis of adult onset type 1 and type 2 diabetes and levels of C-peptide and mortality

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Background and aims: Cardiovascular disease (CVD) is the main threat to health in type 2 (T2D), and also for adults with type 1 diabetes (T1D). Treatment goals and means have changed the last decades. We report baseline and 15-year follow-up incident CVD from the Diabetes Incidence in Kronoberg study 1998-2001, where diabetes type was determined by serology.

Materials and methods: All 1666 with new diabetes diagnosis during 3 yrs in Kronoberg. In onset/15yrs follow-up analyses/fasting (C-peptide, FCP) 1580/1299/1110 (95%) were included, 109/88/80 (7%) had T1D by serological criteria: GADA+, ICA+, or C-peptide <0.25 nmol/mol) and 1471/1211/1030 (93%) had T2D. Patients were followed until 20141231. We report prevalence of hypertension (HT), myocardial infarction/ischemic heart disease (IHD), and stroke/TIA by physician report at diagnosis and these + congestive heart failure (CHF) after 15 years follow up according to compulsory National Registers of Hospital discharge diagnoses according to ICD-10, I20-I25, I50-51 and I60-69. Several models for simple and multiple (MRA), linear and logistic regression and cox proportional hazards models, were applied to explore associations, including with different levels of FCP at onset. R^2 given are adjusted Nagelkerke, <2% (0.02) not shown; CIs are 95%.

Results: At onset of diabetes present in patients with new T1D / T2D were HT in 26% (22/86) / 46% (548/1201); IHD 17% (15/86) / 26% (309/1191); stroke/TIA in 1% (1/85) / 7% (84/1201). FCP was associated with the risk of having had a CVD event already at/before the onset of diabetes (OR (CI)) of HT 4.1 (1.5-10.9), p 0.005, R^2 0.14 / 1.3 (1.1-1.4), p 0.002; IHD 3.9 (1.3-11.3), p 0.013, R^2 0.12 / 1.3 (1.1-1.5), p< 0.0001; Stroke/TIA ns / ns. The influence of BMI was much smaller than that of age at diagnosis for the risk of already having CVD, R^2 <2% for both diabetes types, vs for age 0.13 for T1D; 0.10 for T2D, p all< 0.001. In MRA including age at diagnosis, BMI and FCP their combined R^2 was, 0.26 in T1D / 0.16 in T2D, mostly driven by age. The influence of BMI was ns in T1D / 1 (< 0.02, p < 0.001) in T2D. First HbA1c (T1D mean 8.1±2.1 / T2D 7.1±1.7% DCCT) was not related to risk of IHD or any CVD in T2D, but in T1D R^2 was 0.04, p 0.038, and for any CVD 0.06, p 0.017, but lost significance to age in MRA. After 15 years of follow-up were registered 2003 episodes of the following types of CVD: 1082 (54.0%) first

episodes of any kind of IHD (I20-25), of which 49.7% (538) were angina pectoris (I20) and 50.3% (544) myocardial infarction or close complications to this (I21-I25). There were 565 (28.2%) episodes of CHF; and 356 (17.8%) of Stroke/TIA (I60-69). FCP predicted time to death in T1D R^2 0.07, p 0.09; and T2D R^2 0.08, p< 0.0001; FCP for dead after 15 yrs in T1D OR 10.1 (3.2-31.6) R^2 0.31, p<0.0001; in T2D OR 2.0 (1.7-2.4) R^2 0.07, p<0.0001; and FCP <0.60 nmol/l T1D OR 6.4 (2.1-19.1), p<0.001; T2D OR 1.8 (1.2-2.6), p 0.007.

Conclusion: At diagnosis of adult-onset diabetes and after 15 yrs, especially in T2D, hypertension and other CVD were frequent, 50% of T2D patients had died, and many T2D patients had CVD, while prognosis was better in adult-onset T1D. FCP influenced prevalence of CVD, both at diabetes onset and follow up; time to death, and OR of being dead within 15 years, as did age at onset, but the influence of BMI was very limited

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Dsiclosure: **M. Thunander:** None.

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Risk of anaemia with long-term metformin use in a community setting

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Background and aims: The DPP Outcomes Study showed that metformin use in individuals with impaired glucose tolerance was associated with increased risk of anaemia at 5 years, independent of vitamin B12 status. In people with type 2 diabetes (T2D), anaemia is a common finding but the impact of long-term metformin use on anaemia risk has not been studied. We examined whether cumulative exposure to metformin in T2D is associated with an increased risk of anaemia using data from a large population-based cohort containing longitudinal prescribing and repeated haematology measures.

Materials and methods: We utilised the electronic medical records from patients in the Genetics of Diabetes Audit and Research (GoDARTS) cohort, Scotland. Anaemia was defined as haemoglobin (Hb) <11g/dL. Microcytic anaemia was defined as a mean corpuscular volume <80fl and macrocytic anaemia as >100fl. Individuals with T2D diagnosed from 1996 onwards who had a baseline Hb measured and were not anaemic were followed until they became anaemic, died, left the area or to 30th September 2015 as the end of the follow-up period. Discrete-time failure analysis was used to model the effect of cumulative drug exposure on anaemia risk. This model is set up as a logistic regression model in which each patient contributes one observation for each 28 day time interval from T2D diagnosis to study end. Observational pharmacoepidemiological studies are often prone to allocation bias. To be satisfied that an estimate of a drug's potential causal effect cannot be due to time invariant between person confounding we include two time-updated terms in our model: one for ever-exposure and one for cumulative exposure. We focus the inference of causality on the cumulative term. In addition to metformin exposure, we also evaluated sulfonylureas as a comparator group. The model also included age at diagnosis, sex, baseline Hb and year of T2D diagnosis as fixed covariates, and diabetes duration, social class, eGFR, and BMI as time dependent covariates.

Results: Of 3,485 individuals studied, 2,487 had accumulated some exposure to metformin by the end of follow-up. Median (IQR) follow-up time and number of Hb measures per individual was 8.3 (5-11.5) years and 11 (6-20) respectively. A total of 1,458 (41.8%) individuals became anaemic: 745 in current users, 194 in ex-users and 519 in never-users. Anaemia risk was higher with age at diagnosis (OR 1.03 (95% CI 1.02-1.04) per year) and duration of diabetes (1.05 [1.03-1.08] per year) and decreased with higher baseline Hb (0.70 [0.66-0.74] per 1g/dL) and higher eGFR (0.98 [0.98-0.99] per ml/min/1.73m^2). Cumulative metformin exposure was independently associated with increased risk of anaemia (1.06 [1.02-1.09] per year), but there was no anaemia association with sulfonylureas use. Of those who developed anaemia in the metformin exposed patients, microcytic anaemia was more frequent (12.1% vs. 7.3%) and macrocytic anaemia less frequent (7.6% vs. 12.3%), compared with the non-metformin exposed group.

Conclusion: In this large, observational, population-based study with a maximum follow-up period of almost 20 years, we show that metformin treatment was associated with a 6% higher risk of anaemia for every cumulative year of metformin exposure. As this increased risk was not associated with increased macrocytosis it is unlikely that it relates to vitamin B12 deficiency.

Disclosure: **L.A. Donnelly:** None.

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Impact of pioglitazone on stroke outcomes: a real world database analysis

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Background and aims: RCT data from the PROactive trial demonstrated a potent effect of pioglitazone in reducing recurrent stroke in patients with T2DM. More recently, the IRIS Trial showed pioglitazone decreased the risk of vascular events following stroke or TIA in those with insulin resistance without diabetes. The purpose of this study was to investigate this important pleiotropic effect in people exposed to pioglitazone as a glucose-lowering therapy.

Materials and methods: Routine data from the Clinical Practice Research Datalink for people with T2DM were extracted from January 2000 to December 2012, with follow-up to 2016. Cases were selected if exposed to pioglitazone >=90 days. The index date was first prescription for pioglitazone. Patients with prior history of heart failure were excluded. Two sets of non-exposed controls were matched on age, sex, HbA1c, diabetes duration, stroke history, co-morbidities, and prior glucose- lowering regimen. Control set 1 were patients who initiated a new therapy at a comparable time as their respective case initiated pioglitazone. Control set 2 were those patients who continued on the same regimen as their respective case. Outcomes were risk of incident stroke, proportion of strokes resulting in death within 30 days, hospital length of stay and stroke recurrence. Two time-frames were used; duration of treatment and full follow up within the database. Time to events was evaluated using the Cox proportional hazards model adjusted for potentially confounding factors, including atrial fibrillation. Proportion of strokes resulting in death and discharge to usual residence were compared using logistic regression, length of hospital stay was compared using ANCOVA.

Results: 4,267 patients were matched to control set 1 and 3,629 to control set 2. For the primary outcomes there was a trend for a reduced hazard ratio for cases versus controls which was statistically significant for control set 1; 0.627 (0.404-0.972) during therapy period and 0.640 (0.485-0.843) over the entire database period. There was no significant difference between cases and controls in either the 30 day mortality rate or rate of recurrent stroke. For events resulting in hospital admission, there was no difference in overall mean length of stay but there was a significant difference when considering those admissions that resulted in discharge to the patient's usual place of residence (6.3 days versus 12.4; p<0.001) for control set 1. However there was no difference in the proportion of admissions resulting in immediate discharge to the patient's usual residence in either control set.

Conclusion: In support of evidence from two randomized trials, these observational data show that pioglitazone has an apparent potent effect in reducing stroke events in people with T2DM.

Table: Number, crude rate and adjusted hazard ratio for incident stroke for patients initiated with pioglitazone and matched controls.

	Case		Control			
	Events	Rate	Events	Rate	Hazard ratio* (95% CI)	p
Control set 1						
On therapy	32	3.1	55	5.2	0.627 (0.404-0.972)	0.037
Until end of follow up	86	4.3	126	6.9	0.640 (0.485-0.843)	0.002
Control set 2						
On therapy	25	2.8	44	4.4	0.641 (0.382-1.074)	0.091
Until end of follow up	73	4.3	82	5.4	0.809 (0.584-1.122)	0.204

*Adjusted for age, gender, Charlson index, prior 12 month primary care contacts, smoking status, body mass index, systolic blood pressure, total cholesterol, HbA1c, prior history of atrial fibrillation.

Supported by: Takeda

Disclosure: **C.L. Morgan:** Grants; Takeda.

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Glycaemic, lipid and blood pressure control according to guidelines in patients initiating second-line glucose-lowering therapy: results from the global DISCOVER study

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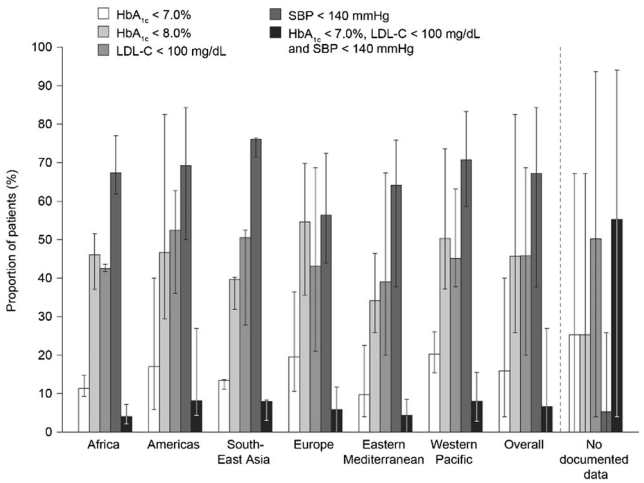
Background and aims: In addition to glycaemic control, guidelines recommend management of LDL cholesterol (LDL-C) and BP in patients with type 2 diabetes mellitus (T2DM). Global patterns of HbA_{1c}, LDL-C and BP control among patients with T2DM are currently not well described.

Materials and methods: DISCOVER is a 3-year observational study of patients with T2DM initiating a second-line therapy, conducted in 37 countries. Clinical variables at baseline were measured according to routine clinical practice at each site, and recorded using a standardized electronic case report form.

Results: Of a total of 14 391 patients, HbA_{1c}, LDL-C and systolic BP (SBP) levels were documented in 74.7%, 49.8% and 94.8%, respectively. All three variables were documented in 44.7%. Mean HbA_{1c} levels varied greatly across countries, but were consistently high (8.44%; across country range [ACR] 7.37-9.20%). Overall, 15.9% of patients had HbA_{1c} < 7.0% (ACR 3.9-40.0%), and 45.7% had HbA_{1c} < 8.0% (ACR 25.7-82.5%). A total of 45.8% of patients had LDL-C < 100 mg/dl (ACR 20.0-68.7%) and 42.4% were taking statins (ACR 14.3-78.4%). Overall, 67.2% of patients had SBP < 140 mmHg (ACR 37.7-84.3%) and 48.2% were taking an antihypertensive drug (ACR 19.1-84.4%). Only 6.6% had HbA_{1c} < 7.0%, LDL-C < 100 mg/dl and SBP < 140 mmHg (ACR 0.0-26.9%). Regional differences are shown in the Figure.

Conclusion: Management of cardiovascular risk factors is inadequate in most patients initiating a second-line glucose-lowering therapy; large proportions of patients are not reaching guideline-recommended targets for LDL-C and SBP. Very few patients achieve target levels for all three parameters (HbA_{1c}, LDL-C and SBP), although elevated HbA_{1c} is expected in this population of patients initiating second-line therapy.

Figure. Proportion of patients attaining recommended goals for HbA_{1c}, LDL-C and SBP.



Whiskers represent across-country ranges. LDL-C, LDL-cholesterol; SBP, systolic BP. Africa: Algeria and South Africa; Americas: Argentina, Brazil, Canada, Colombia, Costa Rica, Mexico and Panama; South-East Asia: India and Indonesia; Europe: Austria, Czech Republic, Denmark, France, Italy, Netherlands, Norway, Poland, Russia, Spain, Sweden, Turkey; Eastern Mediterranean: Bahrain, Egypt, Jordan, Kuwait, Lebanon, Oman, Saudi Arabia, Tunisia and United Arab Emirates; and Western Pacific: Australia, China, Malaysia, South Korea and Taiwan.

Clinical Trial Registration Number: NCT02322762
Supported by: AZ

Disclosure: **M.B. Gomes:** Honorarium; AstraZeneca, Merck-Serono.

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Glycaemic control in 14,005 patients with type 2 diabetes initiating second-line therapy in 36 countries: the DISCOVER study

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Background and aims: There is a lack of data on the extent of glycaemic control among patients with type 2 diabetes in many countries. DISCOVER, a global, prospective, observational study, recruited patients initiating second-line glucose-lowering therapy. Here, mean HbA_{1c} levels at baseline are presented from 36 countries.

Materials and methods: In total, 14 005 patients were evaluated. HbA_{1c} levels were measured in 74.8% of patients according to standard clinical practice in each country.

Results: Patients had a mean HbA_{1c} level of 8.45%, with an across-country range of 7.37-9.20%. A total of 45.5% of patients had an HbA_{1c} level < 8.0%, 24.7% had an HbA_{1c} level ≥ 8.0 and < 9.0%, and 29.8% had an HbA_{1c} level ≥ 9.0%. The proportions of patients in each country with HbA_{1c} ≥ 9.0% at initiation of second-line therapy are shown in the Figure.

Conclusion: HbA_{1c} levels at initiation of second-line therapy varied greatly across countries but were consistently high, suggesting that intensification of glucose-lowering treatment is currently delayed in many people. In some countries, HbA_{1c} levels are not routinely measured; this may be compensated for by assessment of fasting plasma glucose.

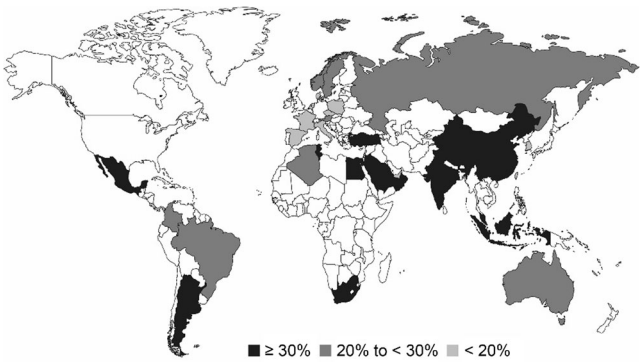

Figure. Proportion of patients with HbA_{1c} ≥ 9.0% at baseline (initiation of second-line glucose-lowering therapy)

Clinical Trial Registration Number: NCT02322762
Supported by: AZ
Disclosure: **L. Ji:** Grants; Roche, Sanofi. Honorarium; Bayer, Boerhinger Ingelheim, AStraZeneca, Bristol-Myers Squibb, Eli Lilly, Merck, Sharpe & Dohme, Novartis, Novo Nordisk, Roche, Sanofi, Takeda.

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Temporal changes in inpatient and outpatient hypoglycaemia among patients treated with sulfonylureas or dipeptidyl peptidase-4 inhibitors in the US

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Background and aims: Recent evidence suggests hypoglycemia rates, mainly in inpatient setting, have not changed much in recent years. Less is known about hypoglycemia in other healthcare settings and by treatment groups with known differential hypoglycemia risk. This real world study aimed to assess changes in annual rates of both inpatient and outpatient hypoglycemia among patients either on sulfonylureas (SU) or dipeptidyl peptidase-4 inhibitors (DPP-4i) during 2007-2013.

Materials and methods: This retrospective cohort study using MarketScan Commercial Claims database included adult patients with type 2 diabetes mellitus using either SU (n=245,201) or DPP-4i (n=176,786) (mono- or combination therapy with other drugs, excluding insulin). Hypoglycemia, defined by ICD-9 diagnosis codes, was assessed during 12-month follow-up period after drug initiation. Poisson regressions were used to calculate Poisson models were used to generate 95% confidence interval (CI) for hypoglycemia rates. Patient demographic and clinical characteristics were assessed within one year prior to drug initiation. (Figure)

Results: Outpatient hypoglycemia rates (100 person-years) increased from 2007 to 2013: 4.4 to 9.2 in SU; 3.0 to 6.0 in DPP-4i users. Inpatient hypoglycemia rates ranged 1.0-1.3 in SU; 0.2-0.4 in DPP-4i users. (Figure)

Conclusion: There was apparent increase outpatient hypoglycemia rate in recent years in both SU and DPP-4i, which might be related to improved awareness and coding/reporting. Changes in inpatient hypoglycemia overtime were relatively small. In both settings, hypoglycemic events were consistently higher among SU than DPP-4i users.

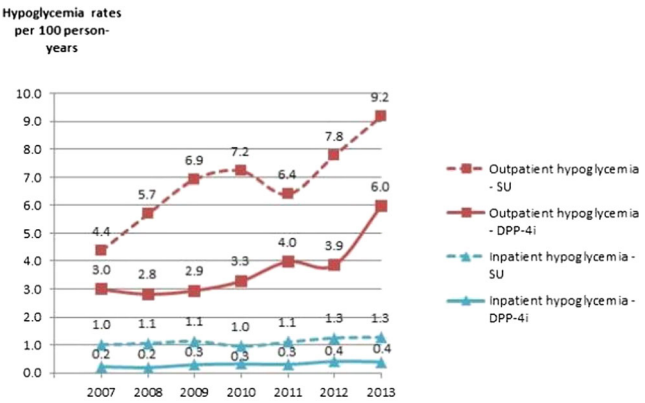

Figure. Inpatient and outpatient hypoglycemia rates in SU and DPP-4i users.
Notes: SU: sulfonylurea; DPP-4i: dipeptidyl peptidase-4 inhibitor.
SU group: N = 245,201; age=51.9 years, female=43.5%, Charlson Comorbidity Index=1.50;
DPP-4i group: N = 176,786; age=52.8 years, female=45.5%, Charlson Comorbidity Index=1.59.
Poisson models were used to generate 95% confidence interval (CI) for hypoglycemia rates:
Outpatient hypoglycemia rates from 2007 to 2013: 4.4 (95% CI: 4.1-4.7) to 9.2 (95% CI: 8.7-9.7) in SU; 3.0 (95% CI: 2.7-3.3) to 6.0 (95% CI: 5.5-6.4) in DPP-4i;
Inpatient hypoglycemia rates from 2007 to 2013: 1.0 (95% CI: 0.9-1.2) to 1.3 (95% CI: 1.1-1.5) in SU; 0.2 (95% CI: 0.2-0.3) to 0.4 (95% CI: 0.3-0.5) in DPP-4i.

Supported by: Merck & Co., Inc., Kenilworth, NJ USA
Disclosure: **S. Rajpathak:** Employment/Consultancy; Merck & Co., Inc. Stock/Shareholding; Merck & Co., Inc.

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Metformin regulates mitophagy through AMPK signalling pathway in human-derived mononuclear cells

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Background and aims: Metformin is recommended as a first-line drug in the management of T2DM. Besides the dual defects in T2DM namely; insulin resistance and insulin deficiency, the role of autophagy has also been implicated in β-cell function and health. Recent evidences report that metformin, apart from being a classical insulin sensitizer, also induces autophagy. However, its effect on mitophagy (selective autophagy of mitochondria) has

not been explored so far. The present study aims to elucidate the effect of metformin and its underlying molecular mechanism involved in the regulation of mitophagy.

Materials and methods: Peripheral blood mononuclear cells (PBMCs) were obtained from the healthy volunteers. The cells were cultured and treated independently with rapamycin (autophagy-inducer), bafilomycin (autophagy-inhibitor) and metformin. For exploring the role of AMPK signaling pathway, cultured PBMCs were pretreated with AMPK inhibitor (AMPKi), followed by metformin treatment. Acridine orange (AO) staining, transcriptional and translational expression studies of mitophagy-related genes (*PINK1, PARKIN, MFN2, NIX, LAMP2* and *LC3II)* and transmission electron microscopic (TEM) studies were performed.

Results: Metformin treatment led to a significant increase in the number of autophagolysosomes ($p< 0.05$) as shown by AO staining. Also, TEM studies revealed an increased percentage of mitophagy in metformin-treated cells ($p< 0.01$). This was also strengthened by the real-time qPCR studies, which showed an augmented expression of mitophagy markers in response to metformin treatment ($p < 0.05$). Further to elucidate the molecular mechanism of metformin-induced mitophagy, our observations revealed a significant up-regulation in the mRNA and protein expression of mitophagy markers in metformin-treated cells ($p < 0.05$), whereas, in the presence of AMPKi, the expression of mitophagy-related genes was significantly down-regulated ($p < 0.05$).

Conclusion: Metformin induces mitophagy and the pharmacological inhibition of metformin-activated AMPK phosphorylation suppresses the expression of mitophagy markers, suggesting metformin regulates mitophagy in an AMPK-dependent manner. Besides its classical role as an insulin sensitizer, metformin also promotes mitophagy, which might be beneficial in maintaining β -cell function, as mitochondria are involved in ATP generation and consequent insulin biosynthesis and secretion.

Disclosure: **S. Bhansali:** None.

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Acute and long-term effects of saxagliptin on post-prandial glycaemic response and cardiovascular parameters in obese patients with impaired glucose tolerance

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Background and aims: Impaired glucose tolerance (IGT) is associated with a high cardiovascular risk. Diabetes prevention should be proposed in this situation. In obese patients, cardiac output (CO) is increased and autonomic cardiovascular changes are common. The issue of cardiovascular safety concerns all new anti-diabetic treatments. The aim of this study was to examine the acute and long-term effects of saxagliptinon plasma glucose and various cardiovascular parameters before and after a breakfast in patients with IGT.

Materials and methods: We included 24 IGT patients, normotensive or with well-controlled hypertension, without any history of cardiovascular disease. The patients were randomized in a double blind trial with saxagliptin 5mg (S) *vs* placebo (P). The tested treatment was taken from the first to the second visit, during 12 weeks. The biological and physiological investigations were performed every hour before and after a standardized breakfast (75g carbohydrates), during 4 hours, at visit 1(day 0) and visit 2 (day 90). Sympatho-vagal balance (LF/HF-HR) was assessed by spectral analysis of heart rate variations; cardiac output (CO) and left ventricular ejection time (LVET) by thoracic impedance (Task Force Monitor®) at controlled breathing rate (12/min); mean cutaneous blood flow (CBF) was measured on the forearm during 6 minutes by laser doppler (Periflux®); and endothelial function using iontophoretic cutaneous infusion of acetylcholine (area under curve AUC-CBF).

Results: Sex-ratio (Females S: 83% *vs* P: 75%), age (S: 49.8±14.6*vs*P:40±10.7 years) and BMI (S: 36.2±5.6*vs*P: 37.5±4.1kg/m²) were similar in the two groups. Compared to the placebo group, patients treated by saxagliptin had similar level of plasma glucose at fasting at day 0 and day 90 but lower plasma glucose at 1 and 2 hours after breakfast by day 0 and at day 90 as compared to placebo-treated patients ($p<0.01$ to $p<0.004$). There was no significant difference for sympatho-vagal activity, CO, LVET, mean CBF and AUC-CBF between the 2 groups at day 0 and day 90, before and after breakfast.

Conclusion: Saxagliptin has a beneficial effect on post-prandial plasma glucose in obese patients with IGT without altering the cardiovascular parameters such as CO, cardiovascular autonomic activity or cutaneous microcirculation and endothelial function. Saxagliptin lowered immediately post-prandial plasma glucose values.

Clinical Trial Registration Number: P101105

Supported by: Astra Zeneca, Bristol-Myers-Squibb

Disclosure: **A. Rezki:** None.

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Factors associated with type 2 diabetes disease progression in a longitudinal cohort (2006 - 2014)

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Background and aims: There is little real world evidence available on the relationship between outcomes for type 2 diabetes (T2D) and different treatment pathways. This longitudinal study of a cohort of newly diagnosed T2D patients with 8 continuous years of enrollment examined the relationship over time between disease progression based on the Diabetes Severity Complications Index (DCSI) score, patient demographics, and changes in prescription drug use and adherence from 2006-2014.

Materials and methods: This was a retrospective study using an administrative claims database in the US. It included newly diagnosed T2D patients in 2006 with DCSI score of 0. DCSI score ranges from 0 to 13, the higher the score, the more severe the disease. Inclusion criteria were: ≥2 diagnoses for T2D or ≥1 T2D diagnosis + ≥1 OAD claim, and no more than 1 T1D diagnosis according to ICD-9 codes, ≥18 years of age, and continuous enrollment to 2014. Based on the year 8 DCSI score, patients were divided into three cohorts: DCSI of 0, 1-2, and ≥3. In addition, sub-group analyses based on 3 age groups (18-44, 45-64, and 65+ yrs) were conducted. Comparisons among the 3 DCSI cohorts were conducted using an ANOVA model for continuous variables and a logistic regression model for categorical variables.

Results: 16,950 newly-diagnosed T2D patients in 2006 were included in this study. By year 8, the proportions of patients with DCSI score 0 was 29.9% (N=5,070), 1-2 36.1% (N=6129), and ≥3 33.8% (N=5751), respectively. The most prominent difference among the 3 DCSI groups was age at diagnosis: 49.6, 52.3, and 59.8 yrs, $p<0.001$. The percentage of males was higher in the DCSI ≥3 group: 49.4%, 48.4%, 53%, $p<0.001$. Insulin usage was observed to increase over the course of the study and was significantly different among the 3 DCSI groups at year 8 (6.2%, 8.8%, 11.5%, $p<0.001$). Drug adherence rate over the whole study period with any antidiabetic medication was assessed by proportion of days covered (PDC): 58%, 62%, and 62%, $p<0.001$. When assessing the DSCI related comorbidities for 3 age groups (18-44, 45-64, and 65+ yrs), the two comorbidities which progressed the most were neuropathy/retinopathy for age 18-44 group, cerebrovascular/neuropathy for age 45-64 group, and cerebrovascular/cardiovascular for age 65+ group.

Conclusion: This study using real world data shows a relationship between T2D disease progression and age at diagnosis. Higher drug adherence rates did not appear to reduce DCSI score worsening. Insulin usage was observed to increase over the course of the study, and more so in the group with DCSI ≥3.

Disclosure: **W. Weng:** Employment/Consultancy; Employee of Novo Nordisk Inc. Stock/Shareholding; Novo Nordisk Inc.

PS 006 Lifestyle, obesity and diabetes

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Trajectories of obesity by spousal diabetes status in the English Longitudinal Study of Ageing

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Background and aims: People with a spouse with diabetes have an increased risk of diabetes, most likely due to assortative mating and their shared home and social environment. Spousal concordance in cardiometabolic risk factors has been observed across different populations, but has not been studied longitudinally. We examined whether the development of obesity with age was different for people with and without a spouse with diabetes.

Materials and methods: We analysed age-related trajectories of obesity by spousal diabetes status in the English Longitudinal Study of Ageing. We fitted quadratic age-trajectories using mixed-effects models. Outcomes of interest were body mass index (BMI) and waist circumference (WC). Our main exposure was having a spouse with diabetes defined by either self-reported medical diagnosis or screen-detected with fasting glucose or glycated hemoglobin during one of the follow-up waves. We restricted this analysis to opposite-sex spouses (based on self-report of marriage or cohabitation) of index individuals without diabetes.

Results: Our analysis included 7,156 individuals (49% women). At age 50, BMI levels were not different between individuals with and without a spouse with diabetes (Figure). Across all groups, BMI increased with age until 70 years and then showed a slight decline. Men with a spouse with diabetes experienced a steeper increase in BMI than men with a spouse without diabetes up to the age of 60 years. Women with a spouse with diabetes had a similar BMI trajectory to women who did not have a spouse with diabetes but their average BMI levels were higher. We observed similar shapes in waist circumference trajectories by spousal diabetes status for men and women (data not shown). Individuals with a spouse with diabetes had higher levels of waist circumference throughout follow-up. This difference was only statistically significant among women (2.6cm larger (95%CI: 0.5, 4.8) waist circumference at age 50 years in women with a spouse with diabetes compared to women whose spousal diabetes).

Conclusion: Individuals with a spousal history of diabetes have higher levels of obesity beyond the age of 55 years compared to individuals with no spousal diabetes. Middle-age spouses of individuals with diabetes may benefit from assessment of their weight status.

Supported by: OSR, AH, RKS and DRW are supported by Danish Diabetes Academy

Disclosure: **O. Silverman-Retana:** None.

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Associations between socioeconomic positions and lifestyle and motivation for change of lifestyle among individuals with diabetes in Denmark

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[1]Department of Clinical Research, Nordsjællands Hospital, Hillerød, [2]Master of Science in Public Health, University of Southern Denmark, Esbjerg, [3]National Institute of Public Health, University of Southern Denmark, Copenhagen, Denmark.

Background and aims: The increasing prevalence of diabetes mellitus (DM) constitutes an important public health problem. The risk of development of complications related to DM is a major burden for the individual and for the society. The risk of complications is influenced by lifestyle factors. Whilst the burden of complications may increase with a declining socioeconomic position (SEP), SEP has been found to be lower among individuals with DM than among non-DM individuals. The aim of this study was to examine the association between SEP and lifestyle factors: smoking, alcohol consumption and physical inactivity, and motivation for change of lifestyle among individuals with DM in Denmark.

Materials and methods: This cross-sectional study analyzed SEP based on educational level stratified into three categories: low, middle and high, and lifestyle factors including smoking status, alcohol consumption and physical activity. The data were provided by the Danish Health and Morbidity Survey, a nationwide survey in the general population (n=~15,000). Individuals with DM and age ≥ 40 years were included. All data were self-reported. Multiple logistic regression analyses were performed to analyze the associations and were adjusted for age and gender.

Results: The study population included 612 individuals with DM. The mean ± SD age were 64.3 ± 10.8 years and included 328 (53.6%) males and 284 (46.4%) females with a body mass index of 29.1 ± 5.4 kg/m^2. Among the participants 17.6% smoked, 15.6% consumed alcohol above the maximum limit of recommendations (7 and 14 alcohol units for women and men, respectively) and 23.1% were physically inactive. There were no association between SEP and smoking status. The odds ratio (OR) for exceeding alcohol consumption above the national recommendations was 2.78 (95% CI: 1.32-5.84) in individuals with high educational level compared to those with low educational level. In individuals with middle educational level OR was 0.50 (0.27-0.74) of being physically inactive (also adjusted for body mass index) compared to low educational level. There were no association between SEP and motivation for smoking cessation. Being motivated to decrease the alcohol consumption for individuals with high educational level compared to low educational level was OR 3.68 (1.03-13.19). No association was found between SEP and motivation for being more physically active.

Conclusion: Higher SEP assessed as educational level was associated with an alcohol consumption that exceeded the national recommendations and associated with an increased motivation for a reduction of alcohol consumption. Furthermore, higher SEP was associated with a reduced risk of being physically inactive. The results may have clinical implications in terms of a focus on new physical activity programs to persons with diabetes and low SEP. In general, there were limited associations between SEP and motivation for change of lifestyle among individuals with DM.

Disclosure: **A.D. Bjørkman:** None.

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Long term consequence of breastfeeding on maternal obesity in middle-aged elderly women

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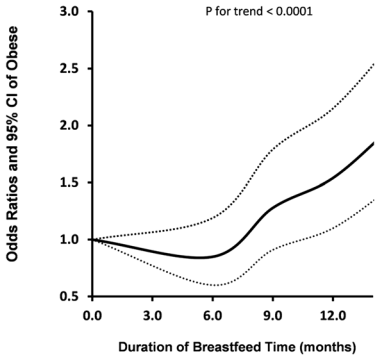
Background and aims: The relationship between breastfeeding and body composition changes among reproductive-aged women is highly variable in different nations. The longer term effect of breastfeeding history on bodyweight, especially for women around perimenopausal period, is still not clear. Our objective was to evaluate the long-term effects of breastfeeding on maternal obesity in developing country.

Materials and methods: We performed a population-based study in 6,430 eligible women aged 40 years or older. Information on breastfeeding and its duration was self-reported. Obese was defined as body mass index (BMI) equal or greater than 28 kg/m^2 while overweight was defined as BMI equal or greater than 24 kg/m^2 and less than 28 kg/m^2. Central obesity for women was defined as waist circumference (WC) equal or greater than 80 cm.

Results: Compared with normal weight women, the proportion of breastfeeding was significantly higher in overweight, obese and central obesity women (all $P < 0.05$). Breastfeeding duration was significantly correlated with both BMI and WC (all $P < 0.0001$). Compared with no breastfeeding women, those ever breastfeeding have a significant increased odds of overweight (OR 1.19, 95% CI, 1.04 - 1.37), obese (OR 1.39, 95% CI, 1.08 - 1.80) and central obesity (OR 1.22, 95% CI, 1.08 - 1.39) after multiple adjustments. A nonlinear relationship between breastfeeding duration and adiposity was also detected and longer breastfeeding period seems to increase prevalent overweight, obese and central obesity (all P for trend < 0.001).

Conclusion: Breastfeeding and its duration were associated with increase of prevalent overweight, obese and central obesity in middle-aged and elderly Chinese women. Dietary and exercise guidelines for breastfeeding women should include ways to prevent postpartum obesity in China. As there were many other factors associated with postpartum weight change and many positive benefits to breastfeeding, further prospective studies are necessary to verify our findings in other developing countries.



	Breastfeed druation (months)				
	0	0.1 - 6	6 - 9	9 - 12	> 12
Obese	1	0.85 (0.60 - 1.19)	1.28 (0.91 - 1.79)	1.54 (1.10 - 2.15)	2.00 (1.47 - 2.73)

Disclosure: **S. Kan:** None.

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Associations of dairy products intake with fat and lean mass distribution in the Fenland Study, UK

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Background and aims: Accumulating evidence suggests that fermented dairy products, including yoghurt are inversely associated with type 2 diabetes risk, though findings are less consistent for other types of dairy products. The mechanisms of the link between dairy products and metabolic disease have not been established, but effects on body composition have been proposed as one pathway. Our aim was to investigate the association between dairy products intake and objectively measured markers of body composition.

Materials and methods: We evaluated 12,065 adults (55% women) aged 30 to 65 years recruited to the Fenland study between 2005 and 2015 in Cambridgeshire UK. Diet was assessed with a validated food frequency questionnaire. Markers of body composition were derived from dual energy X-ray absorptiometry and ultrasonography including visceral-to-subcutaneous adipose tissue ratio (VAT/SCAT), total and peripheral body fat mass, and total and appendicular body lean mass. Cross-sectional associations were investigated between total and subtypes of dairy products (servings/day) and markers of body composition using MM-robust regression adjusting for socio-demographic, lifestyle, dietary factors and BMI. Missing values were imputed and the false discovery rate (FDR) correction was used to account for multiple testing. Interactions with sex and BMI were examined.

Results: The mean±SD dairy product intake was: total dairy 372±193 g/day; milk 288±176 g/day; yoghurt 52±64 g/day; and cheese 18±18 g/day. Total and high-fat dairy were not related to any body composition marker, but low-fat dairy products intake was inversely associated with VAT/SCAT [% difference per serving (95% CI): -2.58 (-3.91, -1.23)]. Among dairy subtypes, no association was observed for yoghurt, cheese, butter or ice-cream for any outcome. Milk intake was associated with body lean mass, with a glass of milk per day being associated with higher lean mass by 0.33kg (95% CI: 0.19, 0.46) and both low- and full fat milk showed similar associations. There were no significant interactions by sex and BMI for any associations, with exception for the interaction by BMI for the association of high-fat dairy consumption with appendicular lean mass. Appendicular lean mass was lower by 0.11 kg (95% CI: -0.22, -0.001) per one serving of high-fat dairy among adults with normal body weight, but not significant among overweight (+0.05 kg; -0.07, +0.18) or obese adults (+0.02 kg; -0.16, +0.20).

Conclusion: These findings suggest that total low-fat dairy products and milk intake may be associated with metabolic risk through the distribution of visceral fat relative to subcutaneous fat and body lean mass respectively, as potential pathways. Further research is warranted to confirm these findings in prospective analyses.

Supported by: Core MRC Unit, Programmes MC_UU_12015/1, MC_UU_12015/5, the Cambridge Trust

Disclosure: **E. Trichia:** Grants; The Fenland Study is supported by the Medical Research Council. Core MRC Unit support through Programmes MC_UU_12015/1 and MC_UU_12015/5. Other; The first author, Eirini Trichia is funded by the core MRC Unit and a scholarship from the Cambridge Trust.

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Eating out increases the risk of being overweight and deteriorates glucose control in patients with type 2 diabetes: a cross-sectional study

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Background and aims: In patients with type 2 diabetes (T2D), weight gain increases insulin resistance and exacerbates poor glycemic control. Unfortunately, it is quite difficult to manage weight in patients with T2D. Several studies have revealed a relationship between the habit of eating out and weight gain. However, the effects of the frequency of eating out on weight status and glucose control in patients with T2D are unclear. Therefore, we investigated the effects of the habit of eating out on weight and glucose control in patients with T2D.

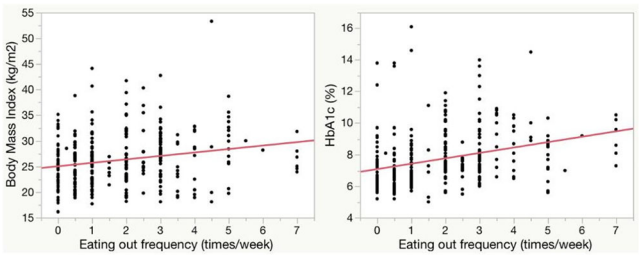
Materials and methods: We performed a cross-sectional study of patients with T2D (June 2010 to March 2017), and assessed eating out frequency (times/week) and diabetes-related information using a medical chart review. Univariate and multivariate regression analyses were performed to determine the association of eating out frequency with BMI (overweight status: BMI ≥ 25 kg/m^2), and HbA1c levels.

Results: After the screening, we included 324 consecutive patients with T2D in our analysis. Their mean eating out frequency was 1.8 ± 1.6 times/week,

mean BMI was 26.3 ± 5.2 kg/m^2, and mean HbA1c was 7.7 ± 1.8 %. In the univariate regression analysis, eating out frequency was positively related to BMI levels (β = 0.67, standard error (SE): 0.17, 95% confidence interval (CI): 0.33 to 1.02, p < 0.001). In the multivariate regression analysis, the relationship between eating out frequency and BMI levels was preserved even after adjustment for potential confounders including age, sex, estimated glomerular filtration rate (eGFR), diabetes duration, and diabetes treatment (adjusted β = 0.39, SE: 0.17, 95% CI: 0.06 to 0.71, p = 0.02). Eating out ≥ 2 times/week increased the risk of being overweight by 72% (adjusted odds ratio: 1.72, 95% CI: 1.06 to 2.83, p = 0.03). In the univariate and multivariate regression analysis, the eating out frequency was also positively related to HbA1c levels (univariate: β = 0.34, SE: 0.06, 95% CI: 0.23 to 0.46, p < 0.001, multivariate: β = 0.36, SE: 0.06, 95% CI: 0.24 to 0.49, p < 0.001). In the receiver operating characteristic (ROC) analysis, the cut-off value of eating out frequency for having an HbA1c level ≥ 7% was 2 times/week (sensitivity: 65%, specificity: 78%).

Conclusion: In this cross-sectional study, eating out frequency was positively related to BMI, being overweight, and HbA1c levels in patients with T2D. These findings suggest that encouraging eating at home for T2D patients might reduce the risk of being overweight, and improve glucose control.

Figure 1. Univariate regression analysis between eating out frequency, body mass index, and HbA1c



β = 0.67, SE 0.17, 95% CI 0.33 to 1.02, p < 0.001

β = 0.34, SE 0.06, 95% CI 0.23 to 0.46, p < 0.001

Disclosure: **Y. Kondo:** None.

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The effectiveness of a community lifestyle intervention progamme in the improvement of perceived health status and health-related quality of life. The DE-PLAN study

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[1]First Department of Propaedeutic/Internal Medicine, University of Athens Medical School, Laiko General Hospital, Athens, Greece, [2]Catalan Diabetes Prevention Research Group, Jordi Gol Primary Care Research Institute, Catalan Health Institute, Primary Health Care Division, Reus-Barcelona, Catalonia, Barcelona, Spain, [3]Department of Endocrinology, Jagiellonian University, Medical College, Krakow, Poland, [4]Lithuanian University of Health Sciences, Institute of Endocrinology, Kaunas, Lithuania.

Background and aims: Apart from the objectively diagnosed and managed complications of diabetes, the subjective evaluation of health status and quality of life (HRQOL) by the patient is often overlooked. Therefore it is important to include assessment of these parameters in all interventions aiming to improve quality of care or prevent the disease. Our aim was to examine the impact of a community based lifestyle intervention on perceived health status and HRQOL in individuals at high risk for type 2 diabetes (T2D) from four European countries participating in the DEPLAN study, through a post-hoc analysis.

Materials and methods: The DE-PLAN was a European multicentre study, designed and implemented with the primary objective of testing whether a community lifestyle modification program could serve as a means of primary prevention of T2D in high-risk individuals. Each centre was allowed to follow any intervention strategy-assisted, self-managed, group-based or individual-based consultation- of one-year duration. Anthropometric measurements and clinical evaluation, including an oral glucose tolerance test and lipid profile were performed at baseline and after one year. Health-related quality of life was assessed using the validated HRQOL-15D questionnaire before and after the intervention. This questionnaire contains 15 dimensions, each having five different levels of functional status and can be presented as a 15-dimensional profile or as a one-index score (15D). In the present analysis, individuals from four centres (Athens, Barcelona, Krakow and Kaunas) with complete data regarding the HRQOL during the study were included.

Results: Data from 919 participants (494 females) were analyzed (Athens: 125, Barcelona: 551, Krakow: 165, Kaunas: 78). The mean age and BMI of the population were 61.8±11.2 years, and 31.6±4.6 respectively. After 1 year, a significant weight reduction was observed (-0.84±4.3kg, P< 0.01). A significant overall improvement in HRQOL, as depicted by the 15D score, was shown (from 0.89 to 0.91, P<0.01). Improvement was consistent among all centers, albeit in different degrees. Significant positive changes in functionality were found in breathing, sleeping, excretion, usual activities, mental function, discomfort and symptoms, depression, distress and vitality (all p<0.01). In multivariable analysis, improvement in HRQOL was positively associated with lower 15D score at baseline, female gender, higher baseline fasting plasma glucose, younger age and amount of weight loss.

Conclusion: A community-based lifestyle intervention programme aiming at T2D prevention, even when implemented in diverse populations and by different centers, is a realistic mean to improve overall health-related quality of life, achieve weight reduction and thus serve in the primary prevention of the disease burden in Europe.

Supported by: the Commission of the European Communities

Disclosure: **G. Karamanakos:** None.

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Impact of sugar sweetened beverages on incidence of type 2 diabetes in Ireland

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Background and aims: Habitual sugar sweetened beverage (SSB) consumption is associated with increased incidence of type 2 diabetes (T2D). The rising prevalence of T2D is a significant public health concern. Our aim is to estimate the impact of SSB consumption on T2D incidence in Ireland and subsequently the potential impact of public health interventions targeting SSB consumption.

Materials and methods: Data from the nationally representative Survey of Lifestyle and Nutrition 2007 (SLAN) were analysed. Food frequency questionnaires were used to measure SSB consumption. A risk prediction algorithm (QDScore2015) was applied to the data to estimate individual risk of developing T2D and absolute event rate. Population attributable fraction was calculated using these ten-year risk estimates of developing T2D, SSB consumption and previously published relative risk of incident T2D diabetes per SSB serving. The absolute event rate and population attributable fraction were applied to census data, estimating absolute numbers of incident diabetes cases attributable to SSB consumption in the Irish population.

Results: Of the 7272 participants in SLAN, 53.3% consumed SSBs. The mean SSB consumption was 44.1(SD106.3) g/day. Applying the risk prediction algorithm, the absolute rate of diabetes over ten years from 2007 was estimated to be 4.5%. The population attributable fraction calculated for SSB consumption was 1.8%. When applied to the national adult population, this equates to 129,664 incident diabetes cases over the previous ten-year period with 2,334 of these cases attributable to SSB consumption.

Conclusion: Assuming a causal association, a notable number of incident cases of diabetes over the previous ten years may be attributed to SSB consumption in Ireland. Interventions aimed at reducing SSB consumption in the population have the potential to prevent up 1.8% of diabetes cases over a ten-year period.

Supported by: HRB Ireland
Disclosure: **K.N. O'Neill:** None.

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The association between multiple sleep-related characteristics and the metabolic syndrome in the general population: the New Hoorn study

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Background and aims: Previous studies have investigated the association between sleep duration, insomnia, day-time napping and metabolic syndrome individually, but never conjointly and the association with sleep medication use has yet to be investigated. Our aim was to examine the associations between these sleep-related characteristics and the metabolic syndrome in a population-based cohort, individually and conjointly.

Materials and methods: We used cross-sectional data of 1679 participants from the New Hoorn study, 52.6% women and age 60.8±6.4y. Sleep duration, insomnia and day-time napping were measured using validated questionnaires. Use of sleep medication was measured by registration of dispensing labels. Metabolic syndrome was defined according to ATP III. Linear and Poisson regression were used and all analyses were adjusted for age, sex, education level, job status, smoking, physical activity, depression and BMI.

Results: In our population-based cohort, 447 (26.6%) persons had the metabolic syndrome. The individual associations showed that after correction, day-time napping for ≤30 minutes and >30 minutes was associated with a prevalence ratio for the metabolic syndrome of 1.28 (95% CI: 1.1-1.5) and 1.74 (95% CI: 1.4-2.2), respectively, compared to participants who did not nap. Sleep duration, insomnia, and sleep medication use were not associated with the metabolic syndrome individually. The conjoint analysis, showed after correction that having ≥2 sleep-related characteristics was associated with a PR of 1.36 (95% CI: 1.0-1.8) of having the metabolic syndrome, compared to having no sleep-related characteristics.

Conclusion: Sleep-related characteristics were associated with a higher prevalence of the metabolic syndrome in the general population.

Disclosure: **F. Rutters:** None.

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Increasead HbA_{1c} in non diabetic night shift workers and former night shift workers with a self reported bad sleep quality

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Background and aims: Sleep has a regulatory effect on glucose metabolism and on hormones regulating appetite, and therefore disturbances in sleep may promote precocious glucose disturbance among shift workers. Recent data suggested that shift working activity increases the prevalence of diabetes although the effect of prolonged nightshift work pattern is still under investigation

Materials and methods: This is a cross-sectional work. The aim of the study is to identify whether sleep disturbances might mediate the relationship between night shift work and alteration in glucose metabolism in individuals without metabolic cardiovascular risk factors. Nurse hospital employees working a shift schedule of four up to seven 12 h nights per month, followed by 2 days off (NSW, n=111) were compared with former-night shift workers (ex-NSW, n=98) and with day-only workers (controls, n=64). We acquired anthropometric data and dosed glucose and lipid levels, and inflammation among the 3 groups. We used the Pittsburgh Sleep Quality Index (PSQI) global score as a potential mediator in the relationship between night shift work and precocious unknown glucose disturbances.

Results: Night shift and former-night shift work status was significantly associated with higher diastolic blood pressure (74.8±9.3 and 73.6±8.4 mmHg) respect to control group (71.0±9.5 mmHg, p:0.024). In addition, NSW and ex-NSW had higher A1c levels (5.3±0.3% for both) compared with controls (5.1±0.2%, p<0.001 controlled for age, BMI and gender) but not C-Reactive Protein, fasting glucose levels or insulin resistance (HOMA). Similarly, good sleepers (PSQI global score <6) compared to bad sleepers (PSQI global score ≥6) showed only lower levels of A1c (5.2±0.3% vs 5.3±0.3%, p=0.025). Next, we created 6 groups according to PSQI global score and shift work status (NSW-bad sleepers, NSW-good sleepers, ex-NSW-bad sleepers, ex-NSW-good sleepers, controls-bad sleepers and controls-good sleepers). Again, both NSW and ex-NSW with a self reported bad sleep quality had significantly higher A1c levels, but not inflammation, glucose or insulin resistance, respect to control groups either good or bad sleepers.

Conclusion: In a cohort of nightshift and non nightshift workers we observed that dysfunctional sleep pattern in night shift workers is associated with subtle differences in glucose tolerance as measured using A1C. This difference is also observed in former night shift workers suggesting that a metabolic memory may remain independently from age, sex and BMI. Whether the subtle defect in A1c depends from postprandial glucose levels as well as the role of sleep quality affects glucose tolerance are still under investigation.

Supported by: Eurhythdia project
Disclosure: **S. Rizza:** None.

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The effect of smoking and smoking cessation on the development of peripheral vascular disease in patients with type 1 diabetes

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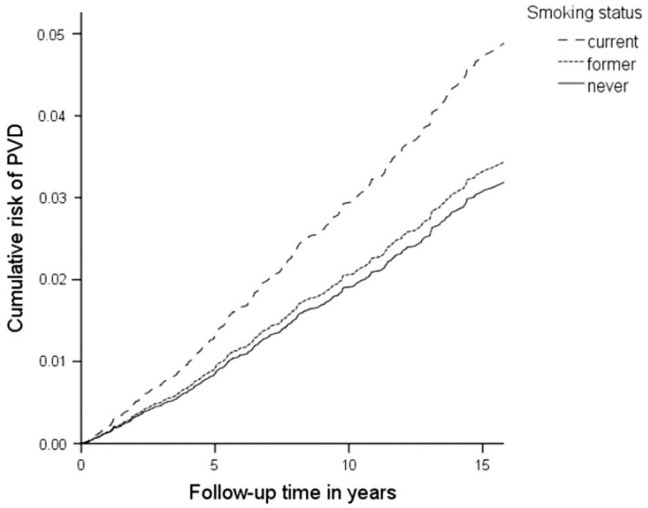
Background and aims: Previous studies regarding smoking and peripheral vascular disease (PVD) in diabetes have mainly included patients with type 2 diabetes. Furthermore, the effect of smoking cessation on the development of PVD is poorly studied. Our aim was therefore to study the effect of smoking and smoking cessation on the development of PVD in patients with type 1 diabetes.

Materials and methods: The study included 4,352 patients with type 1 diabetes participating in the FinnDiane (Finnish Diabetic Nephropathy) Study. Based on baseline smoking status, patients were divided into three groups: never, former or current smokers. Incident PVD was defined as lower extremity amputation, radiological or surgical revascularization. Follow-up data for the development of PVD were based on hospital discharge registers until end of 2014. Overall follow-up time was 54,086 person years and median follow-up time 13.7 (IQR 10.9-15.8) years. Risk of PVD was estimated by Cox-regressions. Smoking status was used as categorical variable, and years since smoking cessation as continuous variable. Never smokers or current smokers were used as reference groups. Sex, age, HbA_{1c}, hypertension, BMI, duration of diabetes, HDL-cholesterol, log triglycerides and baseline nephropathy status were used as covariables.

Results: Compared with never smokers, the risk of PVD was increased in current smokers with a HR of 1.55 (95% CI 1.15-2.07). The corresponding HR for former smokers was 1.08 (0.81-1.44) and did not differ from never smokers after multivariable adjustments (Figure). Smoking cessation decreased the risk of PVD in former smokers with a HR of 0.70 (0.51-0.95) compared with current smokers. Finally, when years since smoking cessation was used as continuous variable, the risk of PVD was reduced in former smokers with a HR of 0.979 (0.961-0.999) per each year without smoking before the baseline visit.

Conclusion: Smoking is a significant risk factor for PVD in patients with type 1 diabetes. After smoking cessation, the risk of PVD is gradually decreases in former smokers and finally reaches the level seen in never smokers.



Supported by: Folkhälsan Foundation, Stockmann Foundation, Diabetes Research Foundation

Disclosure: **M. Feodoroff:** Grants; Folkhälsan Research Foundation, Stockmann Foundation and the Diabetes Research Foundation.

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Use of EZScan test for differentiation of normal, diabetes and prediabetes state

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Background and aims: The early detection of prediabetes and diabetes is an important part for successful treatment and control of the disease. EZScan is a novel screening methodology based on detection of peripheral nerve complications that could be used for screening and disease control. The aim of our observation was to assess the potency of EZScan to discover disordrs in carbohydrate metabolism.

Materials and methods: 187 patients (95 women, 92 men, mean age 49.4±8.3years) were screened by using EZScan test to differentiate normal, diabetic and prediabetic state. Each patient underwent oral glucose tolerance test (OGTT) with 75 g glucose with assessing glucose level on 0 minute and 120 minute. Results from EZScan were compared with results from OGTT to find the coincidence of defined carbohydrate disorder.

Results: Among patients with normal carbohydrate metabolism diagnosed by EZScan, 70 patients (93%) were confirmed with OGTT and 5 patients (7%) were with impaired carbohydrate metabolism. Among patients with prediabetes, impaired glucose tolerance or impaired fasting glucose were found in 64 patients (96%) but 3 patients (4%) had already diabetes. In patients described as diabetics by EZScan, only 2 patients (4%) had prediabetes and the other 43 (96%) patients were with diabetes based on EZScan test.

Conclusion: EZScan shows good coincidence in defining carbohydrate disorder state compared with OGTT and is reliable method for assessing prediabetes/diabetes state. As it is easy to be performed, it is a promising tool that could be used in diabetes screening and prevention programs.

Disclosure: **I. Daskalova:** None.

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A simple screening model for type 2 diabetes in primary care patients treated for hypertension or dyslipidaemia

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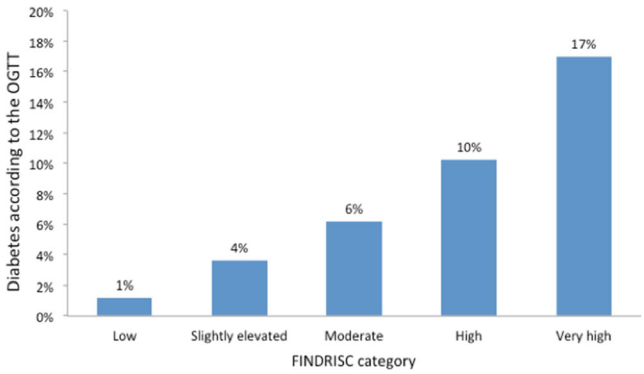
Background and aims: People with hyperlipidemia, hypertension and abdominal obesity are at increased risk for type 2 diabetes (T2DM) as part of the metabolic syndrome (MetS). In the presence of other cardiovascular (CV) risk factors T2DM becomes an even more important cause of premature CV morbidity and mortality. Still the presence of T2DM often remains undetected until the time of a coronary event. Since the MetS is a strong predictor of glycaemic perturbations there may be many individuals with undetected T2DM among people, who due to hypertension and/or hyperlipidemia are cared for in a primary care setting. A feasible screening model for T2DM would be of great value. To test the hypothesis that a combination of the simple Finnish Diabetes Risk Score (FINDRISC) and an oral glucose tolerance test (OGTT) would decrease the need for the more time consuming laboratory test when screening for T2DM in people at increased risk for but without established CV disease in a primary care setting

Materials and methods: The study cohort comprised 2395 patients (age 18-80 years) in the primary care arm of the EUROASPIRE IV survey without (i) a history of CV disease or TDM2, (ii) prescribed blood pressure lowering and/or lipid lowering drugs and (iii) complete information of FPG, 2hPG, HbA1c and FINDRISC questionnaire.

Results: According to the OGTT 22% (n=527) of the studied population had T2DM. The distribution of participants on FINDRISC risk levels to develop T2DM was: low 10% (n=244), slightly elevated 36% (n=857), moderate 29% (n=686), high 23% (n=549) and very high 3% (n=59). As demonstrated in the figure there was an almost linear relationship between FINDRISC category and the proportion of patients diagnosed with TDM2 by an OGTT increasing from 1% of those in the low risk to 17% in the very high risk category respectively.

Conclusion: About more than one fifth of people without CV disease treated for hyperlipidemia and/or hypertension had T2DM. Screening for this condition is simplified by the use of the FINDRISC questionnaire as a tool to select those in demand of an OGTT.



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Disclosure: **L. Rydén:** Grants; European Society of Cardiology, The Swedish Heart Lung Foundation.

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Validation of the diabetes screening methods suggested by the American Diabetes Association in an aging Chinese population

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Background and aims: Diabetes is a serious global health problem with increasing prevalence worldwide. Many diabetes subjects are undiagnosed or have already developed chronic diabetic complications at the time of diagnosis. Opportunistic diabetes screening for high risk subjects appears more practical in China, a populous country being threatened by an alarming increase in diabetes prevalence. A simple and effective screening tool for identifying at-risk subjects for diabetes testing should have substantial public health benefit. We therefore investigated the performance of the latest diabetes screening methods suggested by the American Diabetes Association (ADA), namely the age- and body mass index-based screening criteria (screening criteria) and ADA diabetes risk test (risk test), in our aging Chinese population.

Materials and methods: Subjects free of diabetes who returned for the 4th Hong Kong Cardiovascular Risk Factors Prevalence Study (CRISPS) in 2010-2012 were evaluated for the probability of having diabetes with reference to the screening criteria and the risk test, respectively, and the conclusion drawn was compared to their measured glycaemic status. Diabetes was defined by fasting glucose ≥ 7 mmol/L, or 2-hour post oral glucose tolerance test glucose ≥ 11.1 mmol/L or HbA1c ≥ 6.5% at CRISPS4.

Results: 1434 Hong Kong Chinese without known diabetes, aged 58.1 ±10.2, were evaluated. 157 (11.1%) were tested to have diabetes. The risk test showed good accuracy in screening for diabetes. The area under the receiver operating curve was 0.725 and the optimal cut-off score was 5, being identical to the cut-off suggested by the ADA. Compared to the screening criteria, the risk test had significantly better specificity (0.658 vs. 0.414, p<0.001), positive predictive value (PPV) (0.205 vs. 0.142, p<0.001) and positive diagnostic likelihood ratio (2.068 vs. 1.326, p<0.001) but no significant difference in sensitivity (0.707 vs. 0.777, P=0.101), negative predictive value (NPV) (0.947 vs. 0.937, P=0.282) and negative diagnostic likelihood ratio (0.445 vs. 0.538, P=0.266) To diagnose 1 case of diabetes, only 7 high risk subjects were needed to screen using the risk test as the screening tool, comparing to 13 subjects if the screening criteria was adopted.

Conclusion: Our findings suggested that, as a screening method for diabetes, the ADA diabetes risk test might be more effective than the ADA screening criteria in our aging Chinese populations, with good NPV, better specificity and PPV. It is simple to use in community or clinic setting involving only 7 questions and can be easily adopted as a public health strategy for identifying people with undiagnosed diabetes for early and appropriate treatment.

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Disclosure: **Y.C. Woo:** None.

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Haemoglobin A_{1c} as a screening tool for type 2 diabetes and prediabetes in populations of Swedish and Middle-East ancestry

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Background and aims: To explore and compare sensitivity for HbA1c ≥48mmol/mol as a predicitor for type 2 diabetes mellitus (T2DM) in populations with Swedish and Middle-Estern ancestry and to examine the predicitive value of two levels of HbA1c (≥42mmol/mol and ≥39mmol/mol) for prediabetes in these populations.

Materials and methods: Populations from four different cohorts of Iraqui, Turkish (defined as Middle-Eastern) or Swedish ancestry were included in this study. 1) The MEDIM study (n=1991 individuals of Swedish and Iraqui ancestry); 2) The Skaraborgs Project (n=1327 individuals of Swedish ancestry) 3) The 4-D study (n=354 individuals of Swedish and Turkish ancestry) 4) The Flemingsberg study (n=208 participants of Turkish ancestry). All participants were examined with an oral glucose tolerance test and a careful physical examination. T2DM was defined by one fasting plasma glucose ≥7.0 mmol/l or one venous 2-hour glucose tolerance test ≥11.1 mmol/l.

Results: HbA1c ≥48 mmol/mol had a sensitivity for T2DM of 31% and 25% respectively in individuals of Middle-East and Swedish ancestry. The positive and negative predictive value was high in both populations (70.3, 96.4 and 96.2, 97.6 respectively). Using HbA1c ≥42mmol/mol as a predictor for prediabetes gave a sensitivity of 17% in individuals of Middle-East and 15% in individuals of Swedish ancestry whereas HbA1c ≥39mmol/mol increased the sensitivity to 36% and 34% respectively. Sensitivity for impaired glucose metabolism was age-dependent and slightly higher in individuals with Middle-East ancestry.

Conclusion: Even if HbA1c ≥48mmol/mol is a valuable diagnostic tool, it is a blunt and insensitive tool for screening and would exclude most people with T2DM, independent ancestry and age. HbA1c is an inefficient way to detect individuals with prediabetes independent of ethnicity and age and thus glucose tolerance tests and fasting plasma glucose measurements are still important.

Supported by: ALF, SLS, LUDC, Exodiab, ANDIS, VGR, SI, VR, SCC, KI

Disclosure: **M.I. Hellgren:** None.

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Optimal cut-points of different anthropometric indices and their performance in prediction of type 2 diabetes

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Background and aims: Like many developing countries, Iran has a high burden of the type 2 diabetes (T2D). The first step for preventing T2D is identifying high-risk individuals. Non-invasive and feasible methods such as measuring anthropometric indices can potentially be useful to identify high-risk individuals. This presentation aims to first assess sex-specific discriminatory power of anthropometric indices (i.e. body mass index (BMI), waist circumference (WC), waist to height ratio (WHtR), and waist to hip ratio (WHR)) in predicting T2D incidence and delineate their optimal cut-points; second, to evaluate the performance of the derived cut-points in identifying individuals with high risk of T2D in a cohort study of an Iranian population.

Materials and methods: Overall, 2419 men and 3319 women aged 20-60 years and free of T2D at baseline were selected. To compare the discriminatory power of anthropometric indices in each sex, area under the receiver operating characteristics curves (AUC) for all the indices were calculated and the equality of AUCs were assessed. Sex-specific cut-point for each index was derived at the sensitivity of 75%. Sensitivity, specificity, positive predictive value (PPV), and negative predictive values (NPV) were calculated for every cut-point.

Results: During a median follow-up (Interquartile range) of 11.91 (4.57) years, 354 new cases of T2D in men and 490 ones in women were detected. AUCs for BMI, WC, WHtR, and WHR were 0.68 (95% Confidence interval: 0.65-0.71), 0.67 (0.64-0.70), 0.69 (0.66-0.72), and 0.68 (0.65-0.71) in men and 0.72 (0.69-0.74), 0.74 (0.72-0.76), 0.75 (0.73-0.77), and 0.71 (0.69-0.73) in women, respectively. WHtR had the highest AUC in both sexes compared to other indices; however, this difference was statistically significant only in women. Derived cut-points for BMI, WC, WHtR, and WHR were 25.56 kg/m^2, 89 cm, 0.52, and 0.91 in men and 27.12 kg/m^2, 87 cm, 0.56, and 0.83 in women, respectively. Table 1 reports the performance of derived cut-points. In the sensitivity of 75%, all of the anthropometrics had acceptable specificity ranging from 52% to 63%, leading to the PPVs ranging from 21% to 26% and NPVs ranging from 92% to 94%.

Conclusion: In the Iranian population, optimal cut-points for anthropometric indices generally differ from those of other ethnicities**.** All anthropometric indices had acceptable discriminatory power and performance in both sexes. Out of every 4 to 5 individuals with high anthropometric measures, one developed T2D, while more than 90% of individuals with normal anthropometric measures did not develop T2D. Therefore, as a non-invasive, inexpensive, feasible, and easy-to-measure tool, anthropometric indices can be used to identify individuals with high-risk of T2D in the Iranian population.

Table 1. Sex-specific Predictive Accuracy [a] of the Anthropometric Indices for Incident Type 2 Diabetes, TLGS study (1999-2015)

	Cut-point	TP	FP	TN	FN	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Men (N=2419)									
BMI	≥25.56 kg/m^2	269	989	1076	85	75.9	52.1	21.3	92.6
WC	≥89.00 cm	265	989	1076	89	74.8	52.1	21.1	92.3
WHtR	≥0.52	262	967	1098	92	74.0	53.1	21.3	92.2
WHR	≥0.91	267	980	1085	87	75.4	52.5	21.4	92.5
Women (N=3319)									
BMI	≥27.12 kg/m^2	373	1246	1583	117	76.1	55.9	23.0	93.1
WC	≥87.00 cm	376	1181	1648	114	76.7	58.2	24.1	93.5
WHtR	≥0.56	373	1041	1788	117	76.1	63.2	26.3	93.8
WHR	≥0.83	371	1218	1611	119	75.7	56.9	23.3	93.1

[a] Cut-points were derived by fixing the sensitivity at 75%, using receiver operating characteristics curves.
TP, true positive; FP, false positive; TN, true negative; FN, false negative; PPV, positive predictive value; BMI, body mass index; WC, waist circumference; WHtR, waist to height ratio; WHR, waist to hip ratio; HC, hip circumference.

Supported by: Grant No.121 from the NRC of IR Iran
Disclosure: **N. Zafari:** None.

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Visceral adiposity is a significantly stronger predictor of diabetes incidence in men than in women

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Background and aims: Visceral adiposity is a paramount risk factor for the development of type 2 diabetes. Whether it equally increases diabetes risk in men and in women is not known and is addressed in the present study.

Materials and methods: We prospectively recorded diabetes incidence in a large high-risk cohort of 1142 nondiabetic patients, including 755 men and 387 women who were undergoing coronary angiography for the evaluation of established or suspected coronary artery disease. Visceral adiposity was measured with the validated visceral adiposity index using waist circumference, serum triglycerides, age and gender to diagnose this metabolic abnormality; diabetes was diagnosed according to ADA criteria.

Results: At baseline, visceral adiposity did not differ significantly between men and women (p=0.247). Prospectively, 133 (10.4 %) of our patients newly developed diabetes during a follow-up period of 3.7±0.9 years. Visceral adiposity significantly predicted the incidence of diabetes in men but not in women both univariately (ORs 1.71 [1.40-2.10]; p<0.001 and 1.09 [0.81-1.49]; p=0.565, respectively) and after multivariate adjustment including fasting plasma glucose (ORs 1.56 [1.24-1.97]; p<0.001 and 1.05 [0.75-1.46]; p=0.790, respectively). Interaction terms visceral adiposity x gender were significant both for univariate and for multivariate analyses (p<0.001 and p=0.017, respectively), indicating that visceral adiposity was a significantly stronger predictor of diabetes among men than among women.

Conclusion: We conclude that in angiographied coronary patients visceral adiposity is a significantly stronger predictor of diabetes incidence in men than in women.

Disclosure: **H. Drexel:** None.

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Fatty liver index predicts the development of diabetes among the Japanese general population with and without impaired fasting glucose

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Background and aims: Fatty liver is considered to be the hepatic manifestation of the metabolic syndrome related to insulin resistance, and associated with the development of type 2 diabetes. Fatty liver index (FLI) has been recently introduced as a useful indicator of hepatic steatosis, which makes it possible to diagnose fatty liver without performing imaging tests. We aimed to examine whether FLI predicts the development of diabetes in the general population, especially, in those with normal fasting glucose (NFG).

Materials and methods: We followed population who received Specific Health Checkups conducted from April 1st, 2008 to March 31st, 2009 in Habikino city, Osaka, Japan for five years. 4,445 participants (1,501 men and 2,944 women) without diabetes at baseline were selected in this study. All participants were divided into six groups according to tertile of FLI (low, moderate and high) and the presence or absence of impaired fasting glucose (IFG), by gender. The development of diabetes was defined by fasting blood glucose ≥126mg/dL (7.0 mmol/L), HbA1c (NGSP) ≥6.5%, non-fasting blood glucose ≥200mg/dL (11.1mmol/l), or receiving any medication for diabetes. Hazard ratios (HRs) for incident diabetes according to each group were calculated using a Cox proportional hazard model after adjusting for age, systolic blood pressure, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, smoking habits, and alcohol drinking status.

Results: Mean follow-up period was 3.0 years. During follow-up, 496 incident diabetes cases (176 men and 320 women) were identified. Compared to low FLI with NFG group, high FLI with NFG group was significantly associated with incident diabetes in men (HR: 1.90, 95% confidence interval (CI): 1.08-3.36) and women (HR: 1.73, 95%CI: 1.19-2.52). In addition, IFG groups were significantly associated with incident diabetes regardless of FLI in men (low; HR: 3.94, 95%CI: 2.10-7.30, moderate; HR: 5.70, 95%CI: 3.27-9.93, high; HR: 6.80, 95%CI: 4.01-11.51) and women (low; HR: 4.25, 95%CI: 2.46-7.34, moderate; HR: 6.17, 95%CI: 3.97-9.59, high; HR: 6.08, 95%CI: 4.04-9.15).

Conclusion: Our results showed that FLI was associated with the development of diabetes regardless of sex and the presence or absence of IFG, and it

might be a useful predictor to identify the future risk for incident diabetes even in individuals with NFG.

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Modelling the dynamics of glycated haemoglobin in persons with type 2 diabetes to predict all-cause mortality

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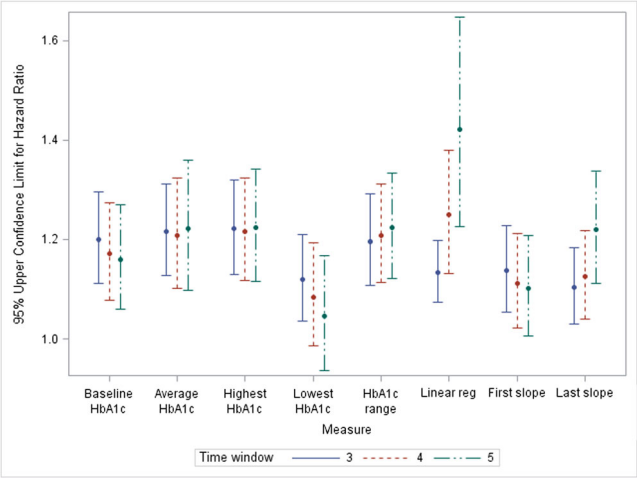
Background and aims: Glycated hemoglobin (HbA1c) measurements for people with type 2 diabetes (T2D) are used in prediction models in various ways. The dynamics of HbA1c measurements over time and its relation to complications is poorly understood while it may be very important to clinical decision making. We aim to determine which measure, designed to capture the dynamics of HbA1c over time, best predicts all-cause mortality in people with T2D.

Materials and methods: 5903 persons with T2D from the Hoorn Diabetes Care System (DCS) cohort were included in this study. In the DCS cohort patients were included from 1998 onward and followed annually on both clinical parameters and complications. Eight measures to express HbA1c dynamics were investigated. The baseline, highest, lowest and average HbA1c were used as absolute values. The slope between the first two measurements, the slope between the last two measurements, the linear regression over the measurements and the range between the lowest and highest HbA1c values were used as relative values. The measures were calculated after either 3, 4 or 5 years of follow-up from baseline while the model predicted from that point onward until either death or censoring. The measures were standardized and compared for these time windows. A multivariable analysis was performed using a Cox regression model adjusted for the baseline variables sex, age, diabetes duration, HbA1c, BMI, triglycerides and total cholesterol. Hazard ratios (HRs) with 95% confidence intervals (CIs) were reported.

Results: Of the 5903 participants of the cohort, 480 died. Mean follow up was 7.3 years, mean age at baseline was 61.1 years and mean HbA1c at baseline was 55.6 mmol/mol. In the analysis hazard ratios (HR) ranged from 1.05 (CI 95% 0.94-1.17) for lowest HbA1c to 1.42 (CI 95% 1.23-1.65) for linear regression, both for time window 5. The results are presented graphically in figure 1.

Conclusion: The results for the linear regression measure clearly stand out and suggest that the overall HbA1c trajectory of a person over time should play a role in clinical decision making with regards to all-cause mortality. Further investigation is needed to determine the role of the measures of HbA1c dynamics with regards to other complications and whether they can be combined to increase prediction accuracy.



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Disclosure: **R.P.T. van Wijngaarden:** Grants; Dutch Diabetes Research Foundation (Grant no. 2014.00.1753).

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Incidence and predictors of metabolic syndrome in an urban, adult Sri Lankan population: a community cohort 7-year follow-up study

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Background and aims: In 2007, we reported a 38.9% prevalence of metabolic syndrome (MetS) in an urban, adult population. Published data on incident MetS from South Asia is lacking. This study investigated the incidence and risk factors for MetS after a 7-year follow-up of the initial cohort.

Materials and methods: The study population (selected by age-stratified random sampling from the Ragama MOH area) was screened in 2007 (aged 35-64 years) and re-evaluated in 2014 (aged 42-71 years). On both occasions, structured interview, anthropometric measurements, liver ultrasound, biochemical and serological tests were performed. MetS was diagnosed on established International Diabetes Federation (IDF 2012) criteria. Total body fat (TBF) and visceral fat percentage (VFP) were measured in 2014, using impedance. Abnormal TBF was defined as >32% for females and >25% for males. Abnormal VFP was defined as >10% for both sexes. Non-alcoholic fatty liver disease (NAFLD) was diagnosed on established ultrasound criteria, safe alcohol consumption (Asian standards: <14 units/week for men, <7 units/week for women) and absence of hepatitis B and C markers.

Results: 2137/2967 (72.0%) of the initial cohort attended follow-up [1229 (57.5%) women; mean-age 52.4 (SD-7.7) years]. 1000/2137 [548 (54.8%) women; mean age 57.5 years (SD-7.74)] had MetS (prevalence-46.8%). Out of 1246 individuals who initially did not have MetS in 2007, 318 [225 (70.8%) women; mean age 57.5 (SD 7.7) years] had developed incident MetS after 7 years (annual incidence-2.13%). Comparison of incident MetS with those with no MetS in 2014 is shown in Table 1. On logistic regression, female sex (OR 3.6, p<0.001), central obesity [OR 4.58, p<0.001], BMI >23kg/m2 [OR 4.84, p<0.001], increase in weight 2%-5% [OR 2.02, p<0.001], increase in weight >5% [OR 5.3, p<0.001), increase in waist circumference (WC) 5-10-cm [OR 3.68, p<0.001], increase in WC >10cm [OR 10.34, p<0.001] and NAFLD (OR 2.44, p<0.001) in 2007 were independently predictive of incident MetS in 2014. Abnormal VFP [OR 4.23, p<0.001] and abnormal TBF [OR 5.25, p<0.001] were also associated with incident MetS.

Conclusion: In this prospective community study, the annual incidence of MetS was 2.13%. Female gender, increase in weight and WC from baseline and the presence of NAFLD predicted the development of incident MetS.

Obesity at baseline was the only defining individual component of MetS that predicted future MetS.

Table 1 – Comparison of participants with Incident MetS and no MetS (2014)

	Incident MetS (n=318)	No MetS (n=928)	P value*
Males (%)	93 (29.25)	567 (61.43)	<0.001
Mean age (SD)	57.5 (7.7)	58.8 (7.9)	0.037
BMI >23 kg/m² (%)	213 (67)	274 (29.5)	<0.001
Increase in weight	84 (26.4) /	203 (21.9) /	<0.001 /
2-5% (%) / >5% (%)	104 (32.7)	96 (10.3)	<0.001
Central obesity (%)	146 (45.9)	145 (15.6)	<0.001
Increase in waist >5 cm (%)	156 (49.1)	148 (15.9)	<0.001
Abnormal TBF (%)	288 (90.6)	600 (64.7)	<0.001
Abnormal VFP (%)	172 (54.1)	202 (21.8)	<0.001
Diabetes / Hypertension	74 (23.3) /	213 (23) /	0.908 /
- known or on Rx (%)	143 (45)	390 (42)	0.360
Elevated TG / Low HDL	76 (23.9) /	211 (22.7) /	0.671 /
- known or on treatment (%)	70 (22)	167 (18)	0.115
NAFLD (%)	224 (70.4)	812 (87.5)	<0.001

*chi-square method

Disclosure: **S.T. De Silva:** None.

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Implementation and outcomes of a Diabetes Prevention Programme for people at high risk of developing diabetes in the workplace

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Background and aims: In people with pre-diabetes, lifestyle intervention programmes directed at weight loss are effective in preventing the progression to type 2 diabetes. The Madrid City Council has developed and implemented a Diabetes Prevention Program (ALAS, High Risk Strategy), among its employees. Aims: 1 -To achieve 5%-7% weight reduction; 2- To improve glycemic regulation.

Materials and methods: During a Health examination, workers are encouraged to complete the Findrisc test. Those with positive results that means, punctuation >14 are asked to undergo Oral glucose tolerance test (OGTT).When they were Findrisc (+) and BMI more than 30 Kg/m^2 or fulfilled criteria for pre-diabetes, they were invited to participate in the Prevention programm. It is delivered over 6 months and it is made up of 10 group sessions focused on modest (5 - 7 %) weight loss through diet, physical activity and behaviour modification. From February 2014 up to now 147 employees were recruited and have finished the workshops.

Results: 147 participants have completed the Program; 99 were Prediabetic (67%) and 48 Normoglycemic at high risk (33%). Participant's ages ranged from 37 to 71 (an average age of 53.6 SD=7, 6), 59 males (40,13%) and 88 females (59,87%). After six months, participants lost an average of 5,65 Kg or 7% of body weight and 28,05% had normalized the glycemic control; 50,34 % achieved 5% or more weight loss; 77, 85 % people attended 5 or more sessions. Weight loss was negatively associated with age, and positively with attendance.

Conclusion: Working people spend a lot of time at their work place which is an ideal setting for health programs targeting weight loss and disease prevention. The work site intervention caused a big improvement in glycemic status and revert pre-diabetes to normal glucose regulation. The Findrisc Test should be included in health tests in the workplace. In addition, the implementation of a group-based, Diabetes Prevention workshop could be introduced as part of the health screening examination that companies provide.

Disclosure: **C. Martin Ridaura:** None.

PS 008 Microvascular complications and diabetic ketoacidosis

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Decreasing incidence of blindness in people with and without diabetes in southern Germany, 2008-2012

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Background and aims: The St Vincent Declaration in Europe (1989) aimed to improve diabetes treatment and thus reduce the risk of diabetes related blindness by one third in 5 years. However, surveillance for blindness among persons with diabetes has not been conducted nationally and population-based data on incidence rate (IR) of blindness in in the diabetic population compared with the non-diabetic population are scarce worldwide. In a previous study in 2008 in a large region in Southern Germany IR of blindness was found to be 2.4-fold higher in the diabetic compared to the non-diabetic population. The aim of the study was to estimate the time trend of IR of blindness in the diabetic and the non-diabetic populations in Germany and the corresponding relative risk over a 5-year period.

Materials and methods: The data were based on administrative files of the welfare administration (35 rural and 10 urban districts in the federal state of Baden-Württemberg, Southern Germany) to assess all individuals who were newly registered as blindness allowance recipients between 1.1.2008 and 31.12.2012. We could use data of 22 out of these 45 districts (total population: n=4,823,570), where the written medical statement was available to define the diabetes status. We estimated age-sex standardised IR of blindness in people with and without diabetes. Furthermore, we estimated relative risks comparing diabetic and non-diabetic population from the standardized IR. We examined age and sex adjusted time trends of IR using Poisson regression models for individuals with and without diabetes. In order to investigate if the relative risk changed over time, we additionally computed Poisson models including a variable presence of diabetes (yes vs. no) and an interaction term for diabetes and years since 2008.

Results: In our study region, we identified 1,897 new cases of registered blindness allowance recipients in the years 2008-2012 (62.5% female, 54.8% ≥ 80 years, diabetes prevalence 2008: 26.1%, 2012: 20.7%). We observed a strong decrease of IR in the population with diabetes (2008: 17.3, 95% CI: 13.6-21.1 per 100,000 person years; 2012: 8.9, 6.3-11.6). Likewise, we found a somewhat weaker but still considerable decrease of IR in the population without diabetes (2008: 9.3, 8.3-10.3; 2012: 6.6, 5.8-7.4). The relative risk fell slightly from 1.9, 1.5-2.4 in 2008 to 1.4, 1.0-1.9 in 2012. With regard to time trend, we found during the observation period a significant decline of IR in the population with diabetes by 16% per year. When considering the population without diabetes, we observed a weaker albeit significant decrease of IR by 9% per year. The interaction diabetes*calendar year was non-significant, indicating that the relative risk between diabetic and non-diabetic populations did not materially alter. All results were similar in both sexes.

Conclusion: We found a significant reduction in risk of blindness in both the population with and without diabetes. Therefore the relative risk did not materially change. These findings may be explained by effective secondary prevention therapies and a better ophthalmologic care beyond diabetic retinopathy, in particular with regard to macular degeneration, which means earlier detection of the diseases.

Supported by: German Ministry of Health and the German Diabetes Foundation

Disclosure: **H. Claessen:** Grants; German Ministry of Health and the German Diabetes Foundation.

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Impact of ideal cardiovascular health in childhood on the retinal microvasculature in mid adulthood: the cardiovascular risk in young Finns study

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Background and aims: Cardiometabolic risk factors in childhood and adulthood are associated with changes in the retinal microvasculature. These changes are closely linked with an increased future risk of diabetes as well as its two major microvascular complications; diabetic retinopathy and nephropathy. We examined the association between ideal cardiovascular health (CVH) and the retinal microvasculature in mid adulthood, using a life course approach that takes account of the contribution of CVH in both childhood and adulthood.

Materials and methods: The Cardiovascular Risk in Young Finns Study included children, from five Finnish University cities, with participants chosen randomly from the national population register. Of these, 418 underwent retinal imaging and provided complete ideal CVH data. The age of participants included in the current analyses in childhood (1986) ranged from 12 to 18 years and in mid-adulthood (2011) ranged from 37 to 43 years. Ideal CVH was defined according to the American Heart Association criteria. Retinal microvascular measures included diameters, lengths, length/diameter (L/D) ratio and tortuosity.

Results: There was a positive association between improved ideal CVH from childhood to adulthood and adult arteriolar diameter ($\beta = 0.122$; 95% CI: 0.01 to 0.24; $p = 0.033$), with no association evident for childhood ideal CVH, after adjustment for confounding factors (age and sex). A negative association was evident between improved ideal CVH from childhood to adulthood and adult L/D ratio ($\beta = -0.666$; 95% CI: -1.25 to -0.08; $p = 0.026$), with no association shown for childhood ideal CVH. Improved ideal CVH from childhood to adulthood showed no association with adult arteriolar tortuosity, but childhood ideal CVH was negatively association with adult arteriolar tortuosity ($\beta = -0.008$; 95% CI: -0.01 to -0.003; $p = 0.001$). When stratified by glucose metabolism, among those with diabetes and impaired fasting glucose (IFG) there was a negative association between childhood ideal CVH and adult venular diameter (diabetes $\beta = -2.75$; 95% CI: -5.46 to -0.04; $p = 0.047$ and IFG $\beta = -2.13$; 95% CI: -4.18 to -0.08; $p = 0.042$).

Conclusion: Cardiovascular health and ideal CVH from childhood to adulthood appear to have a protective effect on the retinal microvasculature.

Disclosure: **M.D. Campbell:** None.

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Mortality rate in patients with type 1 diabetes with multiple micravascular complications

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Background and aims: It is known that the presence of microvascular complications in type 1 diabetes is associated with higher mortality. However, it is unclear whether the different combinations of complications and different sequences in which they occur carry a different mortality risk. We set out to describe the effect of an individual's microvascular complication status on their risk of death in a Danish patient cohort.

Materials and methods: We used a cohort of type 1 diabetes patients treated between 2001 and 2013 at the Steno Diabetes Center Copenhagen, a specialized tertiary care centre. Neuropathy (biothesiometry ≥ 25 mV on either limb), retinopathy (≥ grade two in either eye) and diabetic kidney disease (micro- or

macro-albuminuria) were assessed at regular structured screening examinations (2-4 times a year), creating a dataset with dates of onset of all microvascular complications as well as date of death. We included all patients attending the clinical center in the outlined time period regardless of the complication status at first assessment. Poisson regression models were used to calculate mortality rate ratios between groups with different combinations of microvascular complications in comparison to the reference group without any complications. Models were adjusted for sex, baseline duration of diabetes and HbA1c.

Results: The 4221 patients (54.3% men) fulfilling the inclusion criteria were followed for a median of 8 years (IQR: 3;11). At inclusion the median age was 43 years (32;56), median HbA1c was 70 (mmol/mol) (61;83) and median duration of diabetes was 17.0 years (7;19). During the 29.000 person years of follow-up, 526 deaths occurred (crude mortality rate in patients without complications was 5.2 per 1000 person years (95%CI: 3.9;6.9)). The mortality rate for a woman with diabetes duration of 15 years and HbA1c of 60 mmol/mol was 4.9 /1000 PY (3.6;6.7). Mortality rate was not statistically significantly higher for men (RR: 1.11(0.9;1.3). Table 1 shows mortality rate ratios between groups with different level of complications and the reference group with no complications adjusted for sex, baseline diabetes duration and HbA1c.

Conclusion: Patients affected by all three complications have a 6 times higher mortality rate than patients free of complications. Patients with diabetic kidney disease alone had higher mortality rate ratio than patients with neuropathy, which was higher than both retinopathy alone and with concurrent nephropathy. This observation may be due to the limited time patients remain in the intermediate compliation status groups on their course from 0 to 3 complications.

Complication level	Number of deaths	Mortality Rate Ratio (95%CI)
None	46	-
Diabetic kidney disease	25	4.8 (2.9;7.8)
Neuropathy	48	3.9 (2.5;5.9)
Retinopathy	52	1.2 (0.8;1.8)
Diabetic kidney disease + Retinopathy	34	1.3 (0.8;2.1)
Diabetic kidney disease + Neuropathy	0	-
Neuropathy+ Retinopathy	0	-
Diabetic kidney disease + Retinopathy + Neuropathy	321	6.0 (4.3;8.6)

Number of deaths at different complication level and Mortality Rate Ratios between different complication level and patients with out complications.
Adjusted for sex, duration of diabetes and HbA1c at baseline.

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Disclosure: **L. Bjerg:** None.

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Narrower retinal microvascular calibre is not associated with reduced estimated glomerular filtration rate in type 2 diabetes

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Background and aims: The retinal blood vessels provide a non-invasive "window" to study changes in the microcirculation, with similarities between the microvascular physiology in the eye and kidney. Previous studies have suggested change in retinal vessel calibre may be a useful predictor of estimated glomerular filtration rate (eGFR) in patients with diabetes but the reported direction of this association has not always been consistent. Our objective was to evaluate longitudinal changes in retinal vascular morphometry and eGFR in a population with type 2 diabetes mellitus (T2D).

Materials and methods: GoDARTS is a bioresource with comprehensive longitudinal electronic medical records including hospital admissions, mortality, clinical biochemistry and medicine prescription records linked by patient-specific identifiers for a population of individuals with T2D. Through routine diabetic retinopathy (DR) screening, the earliest available fundus image of 1072 individuals were assessed with a follow-up (FuP) image captured 2 to 4 years later. Biochemical and blood pressure (BP) assessments during a period of 6 months either side of the date of each retinal photograph were obtained and the median value used to reflect the burden of these risk factors. Median serum creatinine was used to estimate GFR with the CKD-EPI equation. Retinal vascular measurements were captured using VAMPIRE 3.0 software to determine calibre-related coefficients, tortuosity and fractal dimension.

Results: The mean time between baseline and follow-up was 3.01 years. At follow-up, eGFR and diastolic BP were significantly lower but HbA1c was higher, compared with baseline measures. At follow-up retinal arterioles and venules were significantly narrowed with associated diminished fractal dimension and increased venular tortuosity, compared with baseline (P<0.001). Following linear regression to adjust for potential confounders, retinal parameters were no longer associated with percentage change in eGFR. Using logistic regression, no retinal parameters were found to be associated with DR at baseline.

Conclusion: While we were able to demonstrate significant temporal changes in retinal vascular calibre during the study period we were unable to identify any changes in retinal parameters significantly associated with declining eGFR in individuals with T2D over a 3-year time period. This study has used existing clinical data to evaluate potential early prognostic indicators. Failure to detect significant associations may be due to several reasons. (1) Variation in retinal parameters may not reflect ongoing changes in eGFR. (2) Our sample has insufficient power to detect associations of this effect size. (3) The 3-year time period between sampling is of insufficient duration to detect significant change in eGFR beyond normal age-related decline. (4) A specific eGFR threshold may be more relevant to detectable morphological changes. (5) It is possible that the progressive changes observed in the retina simply track temporal changes in the kidney.

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Disclosure: **G.J. McKay:** None.

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The different clinical phenotypes of type 1 diabetes due to the age of diabetes onset

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Background and aims: The aim of the study was to conduct a comparative analysis of clinical data (HbA1c, BMI, insulin doses, diabetic complications frequency) in type 1 diabetic patients with different age of onset.

Materials and methods: We included in the study the patients from the database of Federal diabetes registry of Russian Federation with newly diagnosed type 1 diabetes (T1D) with marked date of onset from 1st January to 31st December, 2016, duration less 1 year (n=9130). All patients stratified into three groups by the age of T1D onset: 0-20 years (group 1, n=3815), 21-40 years (group 2, n=3661) and >40 (group 3, n=1654). Statistics performed by SPSS 22.0 (IBM) and tables Microsoft Excel, 2013. Intergroup differences examined for statistical significance (p<0.05) using Mann-Whithey U-test.

Results: The groups differed significantly by the average age [10,82±0,09/ 31,98±0,09/ 52,66±0,24], age at onset [9,82±,09/ 30,98±0,09/ 51,66±0,24], gender distribution: men [54,7%/63.7%/58.1%], BMI [17,57±0,07/ 23,36 ±0,09/ 25,30±0,17] and HbA1c [9,10±0,05/ 8,63±0,06/ 8,91a±0,10], while parameters of lipids were similar: total cholesterol [4,63±0,03/ 4,60±0,03/ 4,62±0,04], LDL [2,56±,04/ 2,57±,04/ 2,53±,06]. The mean insulin dose [0.83±0.01/ 0.65±0.01/ 0.61±0.01] and proportion of basal-bolus treatment [90.2%/ 88.7%/ 75.9%] were highest in group 1, as well as rate of acute complications: coma [18(0.5%)/ 4(0.1%)/ 3(0.2%)], ketoacidosis without coma [169(4.4%)/ 105(2.9%)/ 34(2.1%)] and severe hypoglycemia [3(0.1%)/ 4(0.1%)/0], p<0.05 while the frequency of chronic vascular complications prevailed in group 3 (table data).

Conclusion: Clinical phenotypes of T1D differs depend on the age of onset: classic T1D with highest insulin dose, worst DM control and high frequency of

acute complications in the youngest group, intermediate phenotype in the middle age group and features of type 2 diabetes with high frequency of micro and macrovascular complications in patients with onset age >40, that might reflect the late diagnostics in cases of late autoimmune diabetes in adults.

	Age at T1D onset			P
	Group 1 0-20 yr	Group 2 21-40 yr.	Group 3 >40 yr.	
Arterial hypertension	7_a (0.2%)	113_b (3.1%)	223_c (13.5%)	0.0001
Atherosclerosis	1_a (0.0%)	8_b (0.2%)	17_c (1.0%)	0.0001
Diabetic cataracts	4_a (0.1%)	6_a (0.2%)	23_b (1.4%)	0.0001
Diabetic neuropathy	118_a (3.1%)	317_b (8.7%)	201_c (12.2%)	0.0001
Diabetic retinopathy	44_a (1.2%)	154_b (4.2%)	104_c (6.3%)	0.0001
Diabetic nephropathy	23_a (0.6%)	206b (5.6%)	110b (6.7%)	0.000
Myocardial infarction	0^5 (0.0%)	3_a (0.1%)	14_b (0.8%)	0.0001
Ischemic heart disease	0^5 (0.0%)	5_a (0.1%)	31_b (1.9%)	0.0001
Diabetic foot syndrome	0^5 (0.0%)	7_a (0.2%)	11_b(0.7%)	0.0001
Amputation	0^5 (0.0%)	1_a(0%)	8_b(0.5%)	0.0001
Chronic heart failure	1 (0.0%)	5 (0.1%)	4 (0.2%)	0.070
Cerebrovascular disease	0^5 (0.0%)	4_a (0.1%)	16_b (1.0%)	0.000

Supported by: Aston Consulting Group for technical support for the registry
Disclosure: **M. Isakov:** None.

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Type 2 diabetes in general practice in Norway, status and time trends

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Background and aims: Improved risk factor control reduces the risk of cardiovascular disease among type 2 diabetes patients. Systematic screening for diabetes complications and prompt intervention may prevent or delay the development of target organ disease. Guidelines recommend that general practitioners (GPs) should perform an annual structured screening program. There is no up-to-date study assessing GPs implementation of guidelines in Norway. The objectives of this study are to assess changes in quality of care between 2005 and 2014 and identify areas of diabetes care that need improvement.

Materials and methods: Two cross-sectional surveys in selected areas in three of four health regions were performed that identified all patients (n=5463 attending 59 practices in 2005, n=9464 attending 77 different practices in 2014) with type 2 diabetes. Quality of care was assessed based on key recommendations in National guidelines. Data was extracted electronically with manual verification. Differences in clinical performance between 2005 and 2014 were assessed and tested for statistical significance in regression models adjusting for age, sex, county and clustering within GP practices.

Results: Recommended treatment targets were achieved in a higher proportion of patients in 2014 compared with 2005; HbA1c ≤ 7.0% (53 mmol/mol) in 62.5% vs 54.6% , blood pressure ≤135/80 treated/ ≤140/85 untreated in 50.0% vs. 42.8%, and total cholesterol ≤4.5 mmol/l in 49.5% vs. 34.1% (all, p<0.001). Screening for important microvascular complications was poor in both surveys. Screening for all three of the complications albuminuria, retinopathy and neuropathy was only recorded in the case notes of 13.4% in 2014, and 9.6% in 2005. There was no record of screening for any of the complications in 28.0% of patients in 2014 compared with 22.8% in 2005.

Conclusion: Between 2005 and 2014 there has been statistically significant improvements in risk factor control for patients with type 2 diabetes in general practice, accompanied by an intensification in glucose lowering-, antihypertensive- and lipid lowering therapy. However screening for microvascular complications remains disturbingly low and we suggest that additional incentives to encourage practitioners to adhere to national guidelines are required.

Supported by: Extrastiftelsen Norwegian Diabetes Association 6 pharmaceutical companies
Disclosure: **Å. Bakke:** None.

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Event rates and risk factors for diabetic ketoacidosis in adult type 1 diabetes: analysis from the German/Austrian DPV registry based on 48,067 patients

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Background and aims: Prevention of diabetic ketoacidosis (DKA) in patients with established type-1 diabetes (T1DM) is a major goal in the long-term care for patients. Recent data indicate that in children and adolescents, the rates of DKA during the course of diabetes are not declining. For adult T1DM patients, only few data on DKA event rates, and risk factors for the development of DKA, are available to date.

Materials and methods: Between 1995 and 2017 the DPV registry longitudinally collected standardized data on 491654 patients with diabetes in Austria and Germany. Data are documented in a dedicated electronic health record at participating institutions (n=455) and anonymized, aggregated data are exported for central analysis every 6 months. This report is based on 48067 adult T1DM patients (52.3 % males, median age 38.6 [Q1-Q3: 21.2 - 55.1] years, median duration of diabetes 13.6 [7.0-24.2] years). For the most recent year of observation in each patient, DKA event rate was analysed based on a Poisson regression model accounting for overdispersion (SAS 9.4).

Results: On average, 2.54 hospital admissions due to diabetic ketoacidosis per 100 patient years (py) were observed (95% confidence interval: 2.10-3.05). Event rate was highest in patients aged 18-30 years (3.98/100 py) compared to 1.9/100 py (age 31-45 years), 1.5/100 py (age 46-65 years), and 1.3/100 py (age > 65 years), respectively (adjusted p < 0.0001). For the entire group, no significant difference between males (2.44/100 py) and females (2.64/100 py) was found. Insulin pump therapy was not associated with a higher rate of DKA (2.43/100 py) compared to multiple daily insulin injections (MDI; 2.83/100 py; p=0.11).

Conclusion: These data from a large, multicentre, binational registry report low rates of hospital admissions due to DKA in patients with type-1 diabetes during the course of the disease. Interestingly, pump therapy is not associated with a higher DKA rates. However, event rates are significantly higher in younger adults, both males and females and decline with age.

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Disclosure: **S.M. Schmid:** None.

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Mortality due to diabetic ketoacidosis: population-based findings from the Yorkshire register of type 1 diabetes in children and young people

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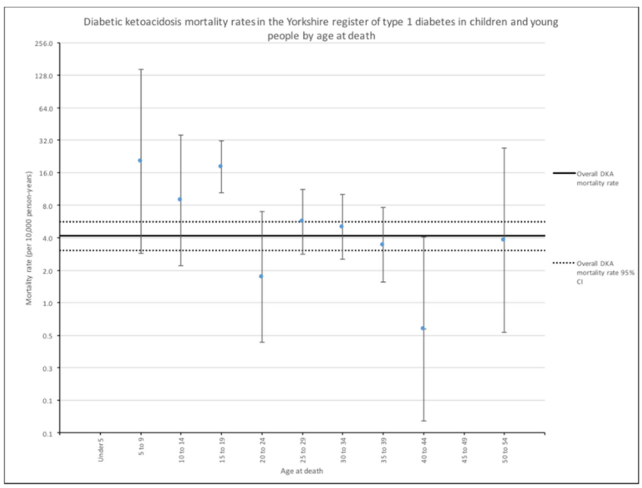
Background and aims: Data from the population-based Yorkshire register of type 1 diabetes in children and young people (YRDCYP) previously showed a four-fold excess in mortality compared to the general population. Deaths due to diabetic ketoacidosis (DKA) by age at death, time since diagnosis and place of death was assessed.

Materials and methods: The YRDCYP includes children under 15 years diagnosed with type 1 diabetes within Yorkshire between 1978 to 2013, and young people aged 15 to 29 diagnosed within West Yorkshire between 1991 to 2013. The YRDCYP was linked to death certification data. Underlying cause of death was verified by a specialist clinician. Death due to DKA was identified by ICD10 code E101. Mortality rates per 10,000 person-years for DKA were calculated for the overall cohort by age at death and time since diagnosis. Place at death was also examined.

Results: The cohort total was 5,498 individuals, contributing 100,959 person-years of follow-up. Previous research found 229 deaths. Median age at death was 29.7 years (range 6.5 to 52.5). Deaths due to type 1 diabetes accounted for 52% of deaths. Of all type 1 diabetes deaths, 47.1% (n=56) were due to acute complications, 36.1% (n=43) were due to chronic complications and 16.8% (n=20) had no recorded complications. Of deaths due to acute complications, three times more deaths were due to DKA (n=42) compared with severe hypoglycaemia (n=14). More than two thirds (64.3%) of DKA death occurred at the deceased's home. Remaining deaths occurred in hospital or elsewhere. The largest number of deaths at home occurred in the 15 to 19, 25 to 29 and 30 to 34 year age groups (n=7 for all groups). The largest number of deaths in hospital occurred in the 15 to 19 year age group (n=5). Overall mortality rate for DKA was 4.2 per 10,000 person-years (95% CI 3.5 - 5.6 per 10,000 person-years). Death at 15 to 19 years of age had the largest number of fatalities (n=13). This was the only age group where the mortality rate (18.2 per 10,000 person-years (95%CI: 10.6 - 31.3 per 10,000 person-years)) was significantly higher than the overall DKA mortality rate. This was also significantly higher than all older age groups, apart from 25 to 29 year olds. Analysis by time since diagnosis showed higher DKA mortality rates for shorter diabetes duration. The highest number of deaths appear in the 5 to 9 years duration group (n=12), with a mortality rate of 17.5 per 10,000 person-years (95%CI: 9.9 - 30.7 per 10,000 person-years). Of the 13 deaths in the 15 to 19 age group, 8 occurred within 10 years since diagnosis.

Conclusion: The majority of people who die from DKA die at home, suggesting that these individuals did not receive hospitalised treatment which could have prevented their death. Deaths due to DKA peaked at younger ages, particularly during adolescence and before 10 years of diabetes duration. This suggests that surveillance and DKA awareness education should be targeted within this particular group of individuals to avoid premature DKA death.



Disclosure: **T.C. Evans-Cheung:** None.

PS 009 Genetics and novel risk factors for type 1 diabetes

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AGER gene polymorphisms in various HLA-DR/DQ haplotypes and their effects on the risk of type 1 diabetes: a transmission analysis

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Background and aims: The AGER gene located within the HLA class II region close to the DQ locus is encoding the receptor for advanced glycation end-products (RAGE). High quantities of advanced glycation end-products derived from the Western diet have been implicated to contribute to the increasing incidence of type 1 diabetes (T1D) and the effect to be transmitted through the RAGE receptor. Polymorphisms of the AGER gene have also been shown to be associated with the risk of T1D but this finding may also be explained by the strong linkage disequilibrium with HLA gene alleles affecting T1D risk. In the current study we compared various HLA-DR/DQ haplotypes for the presence of AGER gene polymorphisms observed earlier to be associated with T1D risk and further analyzed the possible effects of such SNPs on the transmission of these HLA haplotypes to a diabetic child in a family series.

Materials and methods: We genotyped rs17493811, rs9469089 and rs2070600 SNPs using Sequenom platform in 1838 trio families from the Finnish Paediatric Diabetes Register where information on HLA-DR/DQ haplotypes and their transmission was also available. Allele and transmission frequencies were compared using the Chi-square test.

Results: In all SNPs a clearly dominant allele was found with frequencies varying between 81.2% and 96.5%. Minor alleles were strongly associated with particular HLA-DR/DQ haplotypes, rs9469089 minor allele was actually found in the majority of the (DR1/10)-DQB1*0501 haplotypes and rs2070600 minor allele was present in most (DR4)-DQA1*03-DQB1*0301 and (DR9)-DQA1*03-DQB1*0303 haplotypes and was common also in T1D associated DRB1*0401-DQA1*03-DQA1*0302 haplotypes. This strongly T1D associated haplotype also carried more often the minor allele of rs17493811 than other haplotypes (151/1147=13.2% vs. 69/5052=1.4%). Transmission analysis revealed that this haplotype was significantly less often transmitted to the affected child when the minor allele of rs9469089 was present (69.7% vs. 87.0%. P=0.00005). A less significant deviation was also observed in the case of the minor allele of rs17493811 (79.5% vs. 85.9%, P=0.04) and rs2070600 where the minor allele was associated with an increased transmission (89.4% vs. 84.1%, P=0.03). The minor allele of rs9469089 also decreased the transmission of the HLA-(DR8)-DQB1*04 haplotype (9.1% vs. 52.5%, P=0.004).

Conclusion: The results suggest that polymorphisms of the AGER gene or other genetic factors in linkage disequilibrium with it affect the T1D risk conferred by various HLA DR/DQ haplotypes.

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Disclosure: **J. Ilonen:** Grants; The Academy of Finland, Sigrid Juselius Foundation, Juvenile Diabetes Research Foundation, Helmsey Foundation, Turku University Hospital.

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Pancreatic islet regulatory variants broadly influence type 1 diabetes risk

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Background and aims: Genetic studies of T1D have identified 57 risk loci, and previous studies reported enrichment of T1D loci at immune-related regulatory sites. However, the mechanisms of how causal variants at most loci influence T1D pathology are unknown. We aimed to more comprehensively define cellular mechanisms of T1D risk by integrating genetic data with epigenome, expression QTL, and transcription factor binding data.

Materials and methods: We used a Bayesian model to determine the effects of >200 annotations from the Epigenome Roadmap and ENCODE on variants at 57 loci using T1D GWAS data. We used the model effects as priors on the evidence for variants at each locus, and calculated a causal probability (PPA) at each variant. We grouped T1D loci based on the PPA of variants in each annotation, and determined the direction of effect of T1D risk alleles in each group on GWAS data for 26 complex traits. We used accessible chromatin profiled in four islet samples with ATAC-seq, mapped reads with BWA and identified sites using MACS2. Genomic sequence around alleles of candidate islet chromatin variants were cloned in both directions into pGL4 minimal promoter vectors and transfected into MIN6 cells in triplicate. We tested candidate variants for shared eQTLs using published data from 118 islet RNA-seq samples using Bayesian co-localization. We collected >2k transcription factor motifs and predicted motifs using allelic sequence at each variant with fimo. We used a Bayesian model to determine the effect of motif variants across 1MB windows genome-wide using T1D GWAS data.

Results: In a joint model of variants at 57 T1D loci we identified enrichment of variants in coding exons and UTRs (enrich=3.2,2.4), active regulatory sites in immune-related cells including primary, helper and regulatory T cells, natural killer cells, and fetal thymus (enrich=3.1,2.7,4.8,1.5,2.5), and active enhancers in pancreatic islets (enrich=1.7). We identified distinct groups of T1D loci based on annotation patterns at likely causal variants, one of which was specific to islet enhancers and included 9p24, 2p23 and 16q23 among other loci. T1D risk alleles at islet enhancer loci had no apparent effects on other autoimmune disease, and had both risk and protective effects on T2D. A single candidate variant at each locus mapped within islet accessible chromatin, and several demonstrated allelic differences (P<.05) in islet regulatory activity in gene reporter assays in MIN6 cells. Candidate T1D variants also had evidence (P_{shared}>.9) for islet eQTLs at 2p23 and 16q23, and both variants were also predicted *in silico* to disrupt CREB3L1/2 binding. We tested whether variants disrupting CREB3L1/2 binding broadly influence T1D risk. We identified 3k variants genome-wide in islet enhancers that disrupt *in silico* CREB3L1/2 binding, and collectively these variants had strong enrichment for T1D risk (enrich=5.1). Among these variants were 7 outside of established loci (PPA>.01) that may represent novel T1D risk factors.

Conclusion: These results demonstrate a role for both immune and pancreatic islet regulatory variants in the mechanistic basis of T1D, suggest a complex relationship of islet variants on T1D and T2D risk, and implicate CREB3L1/2 as a broad regulator of T1D-relevant islet regulatory processes.

Disclosure: **K.J. Gaulton:** None.

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HLA-B supertype frequencies in Finnish families with type 1 diabetic offspring and in control families

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Background and aims: Human leukocyte antigen (HLA) gene region associates with the risk of several autoimmune diseases including type 1 diabetes. The difference in HLA-B allele/ supertype frequencies between families with type 1 diabetic patients and control families has not been extensively studied. We evaluated HLA-B supertype frequencies in Finnish families with type 1 diabetic patient and in control families.

Materials and methods: HLA-B alleles were genotyped in type 1 diabetic patients (n=246), mothers of the patients (n=234), non-diabetic control children (n=193) and mothers of the control children (n=191). Due to high polymorphism of the HLA-B locus, the alleles were divided into supertypes that share similar peptide binding specificity as follows: Supertype B07 (B*07, B*35, B*51, B*55, B*56), B08 (B*8), B27 (B*14, B*27, B*38, B*39, B*48), B44 (B*18, B*37, B*40, B*41, B*44, B*45), B58 (B*57, B*58), B62 (B*15). B*13, B*47 and B*49 do not belong to any of the HLA-B supertypes. The supertype frequencies between type 1 diabetic patients and control children, and between mothers of the patients and control mothers, were compared.

Results: The frequency of HLA-B7 and HLA-B8 supertypes differed between type 1 diabetic patients and non-diabetic control children (p=0.001 and p<0.001, respectively). The frequency of HLA-B8 supertype differed between mothers of the patients and mothers of the control children (p=0.004).

Conclusion: In this study we found that certain HLA-B supertype frequencies differ not only between the type 1 diabetic patients and controls, but also between mothers of the patients and mothers of the non-diabetic control children.

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Polymorphisms in ST3GAL4, ST6GAL1, PHIP, and FUT8 are associated with variation within the serum N-glycome of Scottish type 1 diabetes patients

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Background and aims: We conducted a genome wide association study (GWAS) of N-glycans in people with type 1 diabetes (T1DM). N-glycans are of interest in T1DM, as altered flux through the hexosamine pathway, hypothesised as a pivotal pathway in diabetic complications, would be expected to yield increased UDP-N acetylglucosamine, the substrate for N-linked glycosylation. We previously identified associations of serum N-glycan profile with HbA_{1c}, albumin:creatinine ratio (ACR) and estimated glomerular filtration rate (eGFR) slope in T1DM. Ascertaining genetic effects on these N-glycans will enable assessment of causality of such associations through a Mendelian randomisation approach.

Materials and methods: Ultra-performance liquid chromatography-hydrophilic interaction chromatography profiling was used to measure total and IgG serum N-glycans in 767 patients from the Scottish Diabetes Research Network Type 1 Bioresource (SDRNT1BIO). We tested for association between 8,443,390 directly genotyped and imputed SNPs with N-glycans in these participants. We further used MultiABEL to conduct multivariate GWAS testing.

Results: SNPs in four loci were associated (p<5E-8) with N-glycans that varied with HbA_{1c}, ACR or eGFR slope. Two of these are novel associations unreported in GWAS of N-glycans in non-T1DM individuals and are in regions containing strong biologically plausible candidate genes: ST3GAL4 (β=-0.73[SE=0.11], p=1E-10) which catalyses the transfer of sialic acids to N-glycans, and PHIP (β=-0.64[SE=0.11], p=1E-8) a probable regulator of the insulin signalling pathway. The other significant associations in ST6GAL1 (β=0.21[SE=0.102], p=4E-24) and FUT8 (β=-0.38[SE=0.05], p=2E-12) were reported previously in people without T1DM.

Conclusion: The results from this and existing GWAS will now be used to generate polygenic risk scores for all patients in SDRNT1BIO resource (N = 6,127), that can be tested for prediction of incident diabetic kidney disease.

Supported by: JDRF

Disclosure: **M.L. Bermingham:** None.

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Gut microbiome dynamics during progressive beta cell destruction in LEW.1AR1-iddm rats: a model of autoimmune diabetes

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Background and aims: The composition of the gut microbiome plays an important pathogenic role in autoimmune diseases including type 1 diabetes mellitus. It is a major challenge to investigate the changes of the gut microbiome communities within the network of genetic determinants, nutrients and viral infections as potential trigger for activation of autoaggressive T cells. The LEW.1AR1-iddm rat model has several advantages to monitor the role of the gut microbiome: (1) a homozygous genetic background, (2) islet infiltration around day 40 and (3) diabetes manifestation after complete beta cell loss between day 60 and 70 after birth. In this study we analyzed changes of the gut microbiome during development of autoimmune diabetes in the LEW.1AR1-iddm and diabetes-resistant LEW.1AR1 control rats.

Materials and methods: Stool samples were collected at the age of 40, 50, 60 days and the timepoint of diabetes manifestation from LEW.1AR1-iddm (n= 68) and LEW.1AR1 (n= 20) rats. Total chromosomal DNA was isolated from stool samples using a Qiagen Kit^{TM} extraction kit. The 16S rRNA genes were amplified on the V3-V4 regions and sequenced on using the paired end Illumina MiSeq platfrom. The resulting sequences were analyzed and quality controlled using QIIME and SILVA_NGS.

Results: Multidimensional scaling based on Bray-Curtis dissimilarities showed clear differences of the gut microbiome of LEW.1AR1 and LEW.1AR1-iddm rats. The differences in the bacterial community in the early phase of islet infiltration were higher at the age of 40 and 50 days but absent at day 60 after progressive beta cell loss. LEW.1AR1 rats revealed an age-dependent decrease of Bacteroidetes/Firmicutes ratio (2.0/0.6) between day 40 and 50 while the ratio in LEW.1AR1-iddm rats remained constant at a level of 1.3. Furthermore we observed a low abundances of Coprococcus and Blautia in LEW.1AR1-iddm rats in the phase of progressive beta cell destruction. On the other hand LEW.1AR1 rats showed a significantly higher abundance of Akkermansia ($p < 0.05$) in the trigger phase (day 40). Akkermansia was correlated with protective effects in human autoimmune diseases. In the time course from day 40 to day 60 the abundance of the genus Prevotella decreased in the LEW.1AR1 rats whereby an increase could be documented for the LEW.1AR1-iddm. The genus Prevotella comprises several species which are linked to inflammatory and autoimmune diseases in humans.

Conclusion: Our study has several implications for microbiome studies in humans: (1) Strong dissimilarities of the microbiome occur during the triggering phase of autoimmunity but not after onset of diabetes. (2) A high Bacteroidetes/Firmicutes ratio apparently provides a proinflammatory milieu, which favors activation of autoaggressive cells. (3) Microbiome signatures significantly change during aging. (4) Minor genomic differences from diabetes susceptibility loci confer significant changes of the microbiome signature. Thus, the dynamic changes of the microbiome may be more relevant than identification of a specific "diabetic microbiome signature".

Disclosure: **T. Schoeppe:** None.

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Immunogenetic factors influencing the rate of pancreatic beta cell destruction: five-year follow up study of residual beta cell function

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Background and aims: Type 1 diabetes usually leads to absolute insulin deficiency due to destruction of pancreatic β-cells by cellular-mediated autoimmunity. The disease is defined by the presence of autoimmune markers containing autoantibodies to GAD (GADA), tyrosine phosphatase IA-2 (IA-2A), and ZnT8 (ZnT8A). Type 1 diabetes also has strong HLA association, especially with linkage to the DQA and DQB genes. However, the pathogenesis of the disease is heterogeneous and the rate of β-cell destruction is variable, especially in the adult-onset type. The aim of the study is to evaluate the influence of factors such as islet-related autoantibodies and HLA-DR/DQ alleles on long-term insulin secretion.

Materials and methods: The subjects were 69 patients (20 males and 49 females) diagnosed as autoimmune diabetes with elevated GADA. Their age at diagnosis was 45±15 years. The patients were continuously investigated IA-2A, ZnT8A and HLA DRB1-DQB1 haplotypes. Data for serum C-peptide and C-peptide index were examined in three periods after diagnosis: 0 to 1 year (0-1 y), 2-3 y, and 4-5 y.

Results: Onset was rapid in 35 patients, presenting as metabolic disorder such as ketosis or ketoacidosis as the first manifestation of the disease, and was relatively slow in 34 patients. The residual C-peptide level at 4-5 y was significantly lower in the rapid onset group (0.18±0.30 vs. 1.38±1.04 ng/mL, $p<0.0001$). The rate of reduction in insulin secretion compared to that at 0-1 y was significantly accelerated at 4-5 y in patients with rapid onset (81.8±22.7 vs. 29.6±55.7%, $p<0.0001$). Thirty-three patients were positive for GADA only and 36 patients were positive for two or three autoantibodies. Insulin secretion during the study period was markedly decreased in patients with plural autoantibodies. The rate of reduction of insulin secretion at 4-5 y was significantly higher in patients with plural autoantibodies compared to those with GADA only (75.8±34.6 vs. 34.8±54.5%, $p<0.0005$). Reduction in insulin secretion at 4-5 y was significantly lower in patients with HLA haplotypes resistant to the disease compared to patients without these haplotypes (24.3 ±54.3 vs. 65.4±44.4%, $p<0.005$). Residual insulin secretion was reduced by approximately seven times in patients with plural autoantibodies and non-resistant or sensitive haplotypes for the disease, compared with patients with GADA only and resistant haplotypes. This suggests that the combination of autoantibodies with HLA-DR/DQ alleles may influence pancreatic β-cell destruction.

Conclusion: Our findings strongly suggest that at least three factors, metabolic disorder at onset, plural islet-related autoantibodies, and HLA-DR/DQ alleles resistant for the disease, are important for predicting future residual β-cell function in patients with type 1 diabetes.

Disclosure: **H. Suda:** None.

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Progression of albuminuria among patients with type 1 diabetes: a 30-year follow-up study

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Background and aims: In type 1 diabetes mellitus (DM1) good glycemic control and early specific intervention may delay kidney disease. The purpose of the present study was to determine whether patients with DM1 have shown improvement, stabilization or deterioration of their urine albumin excretion (UAE) levels during a close follow-up.

Materials and methods: A cohort of 256 patients with DM1, 18 to 79 years of age, a median duration of diabetes of 20 years and a mean follow-up duration of 13 years were included in the study. All patients used intensive insulin management and at baseline they were educated for titration of basal insulin & for counting carbohydrates and correction boluses of rapid insulin. Patients were treated with RAAS inhibition and statins when indicated. Age, sex, duration of diabetes, duration of follow-up, smoking habits, BMI, data regarding diabetic retinopathy, ischemic heart disease, UAE levels and values of various biochemical parameters were recorded too. Participants were divided into three groups according to the UAE levels normoalbuminuric, microalbuminuric and macroalbuminuric, if UAE levels were <30mg/24h, 30-300mg/24h and > 300mg/24h, respectively. The non-parametrical Kruskal-Wallis criterion was used for all analyses.

Results: Mean glycosylated hemoglobin was statistically significantly decreased during the follow-up period, from 8.2% to 7.8% (p=0.004). Normoalbuminuria was present in 66 patients and remained so in 56 patients until the end of the follow-up, while 9 patients progressed to microalbuminuria and one patient to macroalbuminuria by the end of the study. Microalbuminuria was present in 15 patients: regression was observed in 8 patients, and progression in one patient. Regression of macroalbuminuria to microalbuminuria was noted in one patient and to normoalbuminuria was noted in one participant, too. Treatment with ACE or AT2 inhibitors as well as treatment with statins resulted in statistically significant improvement when compared with deterioration of the UAE levels (p=0.009 and p=0.023 respectively). Mean decrease in annual eGFR was less than expected, ie. 0,66ml/min/1.73m^2, using the CKD-EPI equation. Thirty-seven participants had diabetic retinopathy at the beginning of the study and 51 patients had diabetic retinopathy at the end of the study. One hundred and twelve patients had no retinopathy at the beginning of the study among whom 82 had no retinopathy at the end of the study, too.

Conclusion: Improvement of glycemic control with close monitoring of DM1 patients together with the appropriate use ACE or AT2 inhibitors and statins, where indicated, seems to exert nephron-protective potential and to delay or even reverse the presence of micro/macroalbuminuria.

Disclosure: **T. Stratigou:** None.

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Chronic fatigue is more prevalent in patients with type 1 diabetes than in the background population: a cross-sectional study

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Background and aims: Fatigue is a common symptom in chronic diseases, but in a recent systematic review, few studies were identified focusing primarily on fatigue in patients with type 1 diabetes mellitus (T1DM). Even though one study reported significantly more chronic fatigue (CF) in T1DM patients compared to matched controls (40% versus 7%; 95% CI, 34- 47%), data comparing T1DM to background population (BP) data is lacking. Moreover, there is limited knowledge about factors associated with fatigue and T1DM. The primary aim of the present study was to determine the prevalence of CF in T1DM compared to BP. Secondary aims were to identify socio-demographic and clinical factors associated with CF.

Materials and methods: In the period of April 2015 to December 2016, patients with established T1DM who attended diabetes outpatient clinics for routine follow-up at three Norwegian hospitals were invited to participate in the study. Patients were included after providing written informed consent. Socio-demographic data were collected during clinical examination, while clinical data were collected through clinical examination, medical records and laboratory tests. Fatigue was measured using the Fatigue Questionnaire (FQ), while symptoms of anxiety and depression were measured using the Hospitality Anxiety and Depression scale (HADS). In addition, sleep problems were measured using the Basic Nordic Sleep Questionnaire (BNSQ) item on sleep quality in the last three months. BP comparison was based on FQ data from 2287 Norwegian citizens. The FQ consists of 11 questions, each asking if there is less of a problem than usual (0), no more than usual (1), more than usual (2) or much more than usual (3), total score range is 0-33. Higher FQ scores indicate greater levels of fatigue. The same questions can be dichotomized (0 or 1), a score of 4 or above combined with a symptom duration of 6 months or more defines that the patient has CF. The HADS is divided into two scales: (A) anxiety and (D) depression. Each scale has seven items that are scored on a four-point Likert scale (0-3); the higher the score, the greater the level of anxiety and depression. Sum scores of 8 or above for each dimension indicates borderline anxiety or depression. The BNSQ item on sleep quality was dichotomized into, "no sleep problems" and "sleep problems". Potential associations between clinical and socio-demographic variables and CF, was calculated using multivariate logistic regression analysis.

Results: In total, 288 patients were included, (F:M = 152/136), mean age F:M= 44,6/45,0 years (NS). Mean duration of T1DM was F: M = 23.4/22.4 years (NS). CF was reported in 26.4% (76/288) of the T1DM patients compared to 11% (260/2287) in the BP ($p < 0.001$), with no significant gender differences. Multivariate logistic regression analysis revealed that HADS-D ≥ 8 (OR 4.78, p<0.001), HADS-A ≥8 (OR 2.76, p=0.003), time since diagnosis (OR 1.04, p=0.003) and sleep problems (OR 4.47, p<0.001) to be factors significantly associated with CF in T1DM.

Conclusion: Chronic fatigue was significantly more prevalent in T1DM patients compared to the Norwegian background population. Symptoms indicating borderline anxiety or depression, time since diagnosis and sleep problems were factors significantly associated with CF.

Disclosure: **Ø. Jensen:** None.

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High prevalence of IA-2ic- and ZnT8-R-antibody positivity in people with adult-onset diabetes from Singapore

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Background and aims: Autoantibodies against islet-cell antigen 2 (IA-2icA), Zinc transporter 8 (ZnT8-R) as well as GAD2 can occur in adult-onset subjects considered on clinical grounds as Type 2 diabetes patients. The aim of this study was to determine the prevalence and pattern of islet-cell antibodies in subjects with adult-onset diabetes from Singaporean and Europe.

Materials and methods: Subjects with adult-onset diabetes mellitus were recruited during the first year of disease onset; 1,020 Europeans (age at disease onset: 42.0 ± 1.2 years, age range 20 to 60 years; males 61.1%) and 1,652 Singaporeans (age at disease onset: 45.0 ± 1.4 years, range 20-60 years; males 63.4%). Inclusion criteria: Diabetes diagnosis according to WHO criteria. Exclusion criteria: Prior insulin therapy, ketosis at diagnosis, pregnancy, presence of any other severe disease. The responsible ethics committees approved the study. GAD-, IA-2- and ZnT8R-antibodies were measured according to IASP protocols. The phenotype was assessed by age of disease onset, BMI and subsequent insulin therapy.

Results: Prevalence of GADA-positivity was 13.9% (95%-CI: 12.1-16.0; p<0.001) in the European cohort as compared to 7.7% (95%-CI: 5.2-11.2; p<0.001) in Asians. Among Asians, GADA-positivity was highest in Indians with 11.4% (95%-CI: 7.7 -16.6) and lowest in both Malay and Chinese (Malay: 6.03% [95%-CI: 3.6-9.9]; Chinese: 5.75% [95%-CI: 4.3-7.7]; p<0.001). In Europeans IA-2icA-positivity was 7.8% (95%-CI: 6.4-9.4) as compared to 16.5% (95%-CI: 12.8-17.0; p<0.001) in Asians. Among Asians, IA-2icA-positivity was highest in Malays (16.8% [95%-CI: 12.6-22.2]) and Chinese people (15.7% [95%-CI: 13.2-18.6]). ZnT8-RA-positivity in Europeans was 4.09% (95%-CI: 3.0-5.4) compared to a significantly higher rate 19.8% (95%-CI: 17.9-21.8; p<0.001) in Asians. Among Singaporeans, ZnT8-RA-positivity was lowest in Indians with 6.7% (95%-CI: 4.2-10.5) and highest in Malays (38.9% [95%-CI: 34.1-44.0]) and intermediate in Chinese with 15.6% (95%-CI: 13.6-18.0). The mean GAD-, IA-2ic-, and ZnT8-R-ab-titres for Europeans were 35.1 DK, 2.9 DK and 0.9 DK units/ml, respectively. Singaporeans revealed mean GAD-, IA-2ic- and ZnT8-R-titres of 12.2 DK, 3.7 DK, and 1.2 DK units/ml. The rates of double autoantibody-positivity were also different between the Asian and the European cohorts; Asians: GADA and IA2A: 1.3%; GADA and ZnT8A: 0.3%; IA2A and ZnT8A: 0.5%; Europeans: GADA and IA2A: 5.7%; GADA and ZnT8A: 2.3%; IA2A and ZnT8A 0.4%. Islet-cell antibody-positive Europeans revealed a lower BMI (Mean BMI 23.9 kg/m^2 [95%-C.I. 19.9-27.9; p<0.001)] as compared to antibody-negatives (27.2 [25.2-29.2]), whereas this association is absent in Asians (ab-positives mean BMI 27.9 kg/m^2 (27.5-28.3) vs. antibody-negatives (27.5 [26.7-28.3]).

Conclusion: A significant involvement of islet-cell autoimmunity was seen in Asian and European adult-onset diabetes patients. IA-2iCA- and ZnT8A-positivity was highest in the three ethnic groups in Singapore, whereas GADA-positivity was the key feature of the European cohort. Our data suggest

significant ethnic-specific differences both related to antibody profiles as well as clinical features.

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Fetuin-A and fetuin-A/palmitic acid indices in newly diagnosed diabetes

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Background and aims: Free fatty acids play a role in the pathogenesis of diabetes, but the mechanism of an inflammatory response in beta-cells is still unclear. Fetuin-A is a proinflammatory cytokine which plays a role in the pathogenesis of insulin resistance. There have been some reports suggesting that fetuin-A/free palmitic acid (PA) index may be used as a new biomarker of insulin resistance in diabetic patients. The aim of the study was to compare fetuin-A/free PA, fetuin-A/ceramide contained PA and fetuin-A/sphingomyelin contained PA indices in patients with newly diagnosed diabetes and healthy controls.

Materials and methods: 60 subjects with newly diagnosed diabetes were recruited, including 40 subjects with autoimmune diabetes (AD): 20 with type 1 diabetes (T1D), 20 with LADA, and 20 patients with type 2 diabetes (T2D). The control group consisted of 20 volunteers (CG). Fetuin-A concentration was measured by ELISA method. Ceramide and sphingomyelin contained palmitic acid concentrations and also free PA concentration were analyzed by gas-liquid chromatography. Insulin resistance was calculated using eGDR (estimated glucose disposal rate).

Results: Serum fetuin-A concentration and all three indices of fetuin-A/PA were significantly higher in LADA, T1D and T2D groups in comparison to the control group ($p<0.001$). eGDR was significantly lower in LADA, T1D and T2D as compared to CG ($p<0.001$). Negative correlations were found between eGDR and fetuin-A/free PA ($r=-0.228$, $p=0.04$), fetuin-A/ceramide contained PA ($r=-0.330$, $p=0.003$) and fetuin-A/sphingomyelin contained PA ($r=-0.332$, $p=0.004$) in AD. In the patients with LADA, eGDR negatively correlated with fetuin-A/fPA and fetuin-A/sphingomyelin contained PA indices ($r=-0.575$, $p=0.004$ and $r=-0.446$, $p=0.04$). In T2D group a negative correlation between eGDR and fetuin-A/ceramide contained PA ($r=-0.594$, $p=0.03$) was found.

Conclusion: Significantly higher fetuin-A concentrations in newly diagnosed AD and T2D may confirm the role of this cytokine in the pathogenesis of both types of diabetes. Moreover, our preliminary results suggest that not only fetuin-A/fPA but also fetuin-A/ceramide contained PA and fetuin-A/sphingomyelin contained PA indices probably might be used as a biomarkers of insulin resistance in diabetes, in particular in AD. The study on greater number of patients is ongoing.

Disclosure: **K. Siewko:** None.

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Association between plasma phospholipid saturated fatty acids and metabolic markers in eight European countries: a cross-sectional analysis in the EPIC-InterAct study

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Background and aims: Accumulating evidence suggests that individual circulating saturated fatty acids (SFAs) are heterogeneous in their associations with cardio-metabolic diseases, but evidence about associations of SFAs with metabolic markers of different pathogenic pathways is limited. We aimed to examine associations of plasma phospholipid SFAs with metabolic markers of lipid, hepatic, glycaemic and inflammation pathways.

Materials and methods: We measured nine individual plasma phospholipid SFAs and derived three SFA groups (odd-chain: C15:0+C17:0, even-chain: C14:0+C16:0+C18:0 and very-long-chain: C20:0+C22:0+C23:0+C24:0) in individuals from the European Prospective Investigation into Cancer and Nutrition (EPIC)-InterAct case-cohort study across 8 European countries. Using linear regression in 15919 members of the subcohort, adjusted for potential confounders and corrected for multiple testing, we examined cross-sectional associations of SFAs with 13 metabolic markers. Multiplicative interactions of the 3 SFA groups with prespecified factors, including body mass index and alcohol consumption, were tested.

Results: Higher odd-chain SFA group levels were associated with lower levels of major lipids (total cholesterol [TC], triglycerides, apolipoprotein A-1 [ApoA1], apolipoprotein B [ApoB]) and hepatic markers (alanine transaminase [ALT], aspartate transaminase [AST], gamma-glutamyl transferase [GGT]). Higher even-chain SFA group levels were associated with higher levels of low-density-lipoprotein-cholesterol (LDL-C), ratios of TC to high-density-lipoprotein cholesterol (HDL-C), triglycerides, ApoB, ApoB/A1 ratio, ALT, AST, GGT and CRP, and lower levels of HDL-C and ApoA1. The very-long-chain SFA group levels showed inverse associations with triglycerides, ApoA1 and GGT, and positive associations with TC, LDL-C, TC/HDL-C, ApoB and ApoB/A1. Associations were generally stronger at higher levels of BMI or alcohol consumption.

Conclusion: Sub-types of SFAs are associated with metabolic markers of lipid metabolism, liver function and chronic inflammation differentially, suggesting that odd-chain SFAs are associated with lower metabolic risk, even-chain SFAs with adverse metabolic risk, and with mixed findings for very-long-chain SFAs. Clinical and biochemical implications of these findings may vary by dietary exposure and adiposity.

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Disclosure: **J. Zheng:** None.

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Insulin secretion is inversely associated with high-molecular-weight adiponectin levels in a Japanese population-based study

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Background and aims: Loss of beta-cell function is progressive over time, and decreased insulin secretion is far advanced by the time diabetes is diagnosed clinically. Recently, it has been reported that increases in adiponectin concentration were related to worsening of insulin secretion in people with newly diagnosed diabetes. In the current study, we investigated the association between insulin secretion and high-molecular-weight (HMW) adiponectin levels in a Japanese population-based study.

Materials and methods: We analyzed data from the Dynamics of Lifestyle and Neighborhood Community on Health Study (DOSANCO Health Study), which was a Japanese population-based cohort study. From these data, subjects whose fasting blood was collected were included in this analysis. Insulin secretion was estimated by homeostasis model assessment of beta-cell function (HOMA-%B). Simple linear regression analysis was performed to examine the association between HOMA-%B ($\log_e$-transformed) and serum HMW adiponectin levels ($\log_e$-transformed). Multiple linear regression analysis was used to calculate age-, sex-, and body mass index (BMI)-adjusted coefficients.

Results: A total of 546 subjects (245 males and 301 females) were included. The mean age and BMI were 58.8 ± 12.5 years and 23.9 ± 3.7 kg/m^2, respectively. Serum HMW adiponectin levels were 4.4 ± 3.1 μg/ml. HOMA-%B was significantly associated with age, BMI, and HMW adiponectin levels in simple linear regression analysis. Additionally, a multiple linear regression analysis revealed that HOMA-%B was inversely associated with HMW adiponectin levels adjusted for age, sex, and BMI (standard partial regression coefficient=−0.17, P<0.01). When the subjects were classified into non-elderly (below 65 years of age) and elderly groups (above 65 years of age), HOMA-%B was inversely associated with HMW adiponectin levels adjusted for age, sex, and BMI in both groups (non-elderly group: standard partial regression coefficient=−0.11, P<0.05; elderly group: standard partial regression coefficient=−0.26, P<0.01). Furthermore, in subjects without diabetes, HOMA-%B was inversely associated with HMW adiponectin levels adjusted for age, sex, and BMI (standard partial regression coefficient=−0.26, P<0.01). When insulin secretion was estimated by fasting insulin or C-peptide levels, similar results were observed.

Conclusion: Our results demonstrated that insulin secretion was inversely associated with HMW adiponectin levels in a Japanese population-based study. This association was applicable to elderly subjects as well as subjects without diabetes. Further prospective observational studies are needed to elucidate the causal association.

Disclosure: **A. Nakamura:** None.

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Elevated alpha-hydroxybutyrate and branched-chain amino acids during an OGTT characterise insulin resistance and predict deteriorations of glycaemic control in youth

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Background and aims: Traditional risk factors for type 2 diabetes mellitus (T2DM) are weak predictors of changes in glucose tolerance and insulin sensitivity in youth. The oral glucose tolerance test (OGTT) provides a dynamic view of biochemical changes produced by glucose-induced hyperinsulinemia; therefore, it offers higher sensitivity than fasting metabolite analysis in identifying subtle early alterations of insulin-induced fuel processing that mark incipient insulin resistance. By measuring the time-course during an OGTT of six metabolites that reflect different axes of insulin action (i.e. proteolysis, ketogenesis, and glycolysis), we aimed at identifying early metabolic features of insulin resistance in youth and whether they can predict longitudinal deterioration of glucose homeostasis.

Materials and methods: Seventy-eight non-diabetic children and adolescents aged 8-18 years were recruited. Plasma concentrations of α-hydroxybutyrate (αHB), branched-chain amino acids (BCAAs; valine, leucine, and isoleucine), β-hydroxybutyrate, and lactate were measured at fasting and every 30 min during an OGTT by proton magnetic resonance spectroscopy. Associations between newly-identified baseline metabolic alterations and longitudinal changes in glucose tolerance and β-cell function relative to insulin sensitivity were tested after 2.3±0.6 years follow-up.

Results: Elevated fasting αHB levels were observed in adolescents with reduced insulin sensitivity, as indicated by a lower OGTT-derived whole-body insulin sensitivity index (WBISI), after adjusting for age, gender, ethnicity, Tanner stage and BMI *z*-score (p=0.01). In the same group, plasma αHB and BCAA levels were increased throughout the course of the OGTT (p<0.03). Notably, a gradual decline in αHB levels during the OGTT identified adolescents with borderline insulin resistance (p=0.02), in whom plasma αHB shifted from elevated baseline concentrations characteristic of subjects with marked insulin resistance to low post-load concentrations typical of insulin sensitive adolescents. Increased baseline αHB concentrations during the OGTT were associated with longitudinal worsening of glucose tolerance, as indicated by higher 2h glucose (beta 79.8, SE 25.0, p=0.01), and of the disposition index (beta -7.89, SE 3.34, p=0.04) after adjustment for confounding factors.

Conclusion: Fasting concentrations of αHB and the time-courses of αHB and BCAAs in response to a glucose challenge characterise insulin resistance in youth. Furthermore, alterations of αHB metabolism predict incipient deterioration of β-cell function relative to insulin sensitivity and longitudinal worsening of glycemic tolerance. These findings provide new insights into the pathogenesis of insulin resistance and novel strategies for early risk stratification of adolescents with a high likelihood of developing impaired glucose metabolism.

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Disclosure: **D. Tricò:** None.

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Serum uromodulin is significantly associated with both type 2 diabetes and prediabetes

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Background and aims: Serum uromodulin has recently been described to be associated with chronic kidney disease and type 2 diabetes. Whether it is also associated with prediabetes is unknown and is addressed in this study.
Materials and methods: We measured serum uromodulin in 494 subjects who prospectively were investigated over a follow-up period of 4 years.
Results: At baseline, uromodulin was significantly and inversely correlated with fasting plasma glucose (r=-0.165; p<0.001), plasma glucose two hours after an oral 75g glucose challenge (r=-0.164; p=0.001), and HbA1c (r=-0.106; p=0.018). It was positively correlated with insulin sensitivity (r=0.199; p<0.001) as well as with beta cell function (r=0.102; p=0.025). In subjects with prediabetes (n= 267; FPG 100-125 mg/dl; 2h post challenge glucose 140-199 mg/dl, or HbA1c 5.7-6.4%) uromodulin (168±81 ng/ml) was significantly lower than in subjects with normal glucose metabolism (n=81; 184±72ng/ml, p=0.012) but significantly higher compared to patients with diabetes (n=146; 148±70 ng/ml, p=0.014). Taking into account parameters of glucose metabolism after 4 years of follow-up, serum uromodulin significantly decreased from subjects with normal glucose metabolism both at baseline and after 4 years, over patients with normal glucose metabolism at baseline who developed abnormal glucose metabolism during follow-up to those who already had abnormal glucose metabolism at baseline (188±74, 173±66, and 161±78 ng/ml, respectively; p_{trend}=0.001).
Conclusion: We conclude that serum uromodulin is significantly associated with abnormal glucose metabolism including prediabetes.
Disclosure: **S. Waeger:** None.

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Localisation of Advanced Glycation End products (AGEs) and fluorescence in skin

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Background and aims: Skin autofluorescence (SAF) is a non-invasive method to assess dermal accumulation of AGEs, which is considered to reflect systemic AGE burden. SAF is used for assessment of cardiovascular risk and detection of diabetes mellitus (DM). Although SAF has been validated against AGEs measured in dermis homogenates of skin biopsies in Caucasian skin, it has not been validated in subjects with high skin pigmentation. SAF measurement is hampered by absorption of excitation and emission fluorescent light by melanin. This limits its performance in subjects with dark skin types. Furthermore, little is known about the location of AGEs in different layers of the skin and their relative contribution to non-invasively assessed SAF. This is of relevance for interpretation of SAF in dark skin types, as well as for its reflection of the systemic AGE burden. Therefore, aim of our present study is to assess the histological distribution, and fluorescence signals of AGEs in the dermis and epidermis in dark skinned subjects with and without DM in relation to non-invasively assessed SAF.
Materials and methods: In 6 healthy young subjects (median age 22 (20-41) years, 4 male) and 6 older DM (type 1 and 2) patients (median age 65 (45-71) years, 5 male) with skin pigmentation Fitzpatrick scale IV (Mediterranean) to VI (African), volar forearm measurements and subsequently two skin biopsies from the same site were obtained. SAF, using the AGE Reader (excitation 370, emission 420-600 nm), and pigmentation measurements, using the Mexameter, were performed. Confocal fluorescence microscopy was used to measure intrinsic fluorescence (Excitation = 375, 405 and 440 nm, emission spectra between 414 and 690 nm) and light microscopy to measure depth distribution of specific AGEs (Alkaline phosphatase chromogenic reporter for carboxymethyl lysine (CML), pentosidine and the hydroimidazolone MG-H1) from these skin biopsies. Immunohistochemical (IHC) staining was performed with anti-AGE-antibodies (anti-CML, anti-pentosidine and anti-MG-H1).
Results: CML, pentosidine and MG-H1 were preferentially visible around blood vessels, colocalized with collagen, elastin, and near fibroblasts in the dermis, and were also present in the stratum corneum and suprabasal layer. Intrinsic fluorescence was most prominent in the dermis, but also present in the stratum corneum and suprabasal layer. Immunohistochemically CML and MG-H1 were more visible in the dermis of patients, as was fluorescence intensity with confocal microscopy. A clear change in pentosidine levels with age could not be observed. In healthy subjects MG-H1 levels in the epidermis appeared more prominent than in patients. SAF levels increased with age, and were concordant with the IHC and fluorescence data.
Conclusion: Although skin AGEs accumulation and intrinsic fluorescence occur in different skin layers, in older diabetic patients they are most prominent in the dermis. This pattern was more pronounced for CML and MG-H1, underscoring the accumulation of AGEs with age and diabetes. Concordance often existed with non-invasively measured SAF, although more research is needed to allow reliable SAF measurements in very dark skinned subjects.
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Skin autofluorescence improves the Finnish diabetes risk score in the detection of diabetes in a large population cohort

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Background and aims: In type 2 diabetes mellitus, a long, clinically latent period often exists in which diabetes is not yet detected, but in which silent development of micro- and macrovascular complications frequently occurs. These complications may be prevented or delayed by early detection and treatment of diabetes in high risk individuals. Several diabetes detection approaches exist of which the Finnish diabetes risk score (FINDRISC) is one of the most accepted. The aim of the present study was to investigate whether skin autofluorescence (SAF) further improves the FINDRISC in detecting undiagnosed diabetes in a large population based cohort.
Materials and methods: Subjects included were participants of Lifelines, a large population cohort study in the northern region of the Netherlands. SAF, as a reflection of advanced glycation endproduct accumulation in the skin, was assessed in an unselected subset of the participants using the AGE reader. After exclusion of participants with previously diagnosed diabetes (n=1635), pregnant women (n=58), and participants using corticosteroids (n=345), 79,248 subjects remained for analysis. Diabetes was defined by fasting blood glucose ≥7.0 mmol/l, non-fasting blood glucose ≥11.1 mmol/l, or HbA1c ≥6.5%. Discrimination of the risk models was estimated using the area under the

receiver operating characteristic curve (AUROC), calibration was assessed by Hosmer-Lemeshow (HL) chi^2 test, and clinical relevance was addressed by reclassification analysis.

Results: Diabetes was detected in 1042 participants (aged 55 ± 12 years, 54% male). Participants without diabetes were 44 ± 12 years old and comprised 42% men. SAF improved the AUROC of the FINDRISC from 0.802 to 0.811 (p<0.001). The FINDRISC + SAF model showed good calibration (HL chi^2 = 11.7, p=0.113). Risk reclassification showed that 8 -15% of all participants were reclassified into more accurate risk categories due to the addition of SAF to the FINDRISC (NRI = 0.080, 95% CI 0.052 - 0.110). The FINDRISC + SAF model had a score range between 0 and 21. At a score of 8, the sum of sensitivity and specificity was maximised (sensitivity 76%, specificity 72%). When SAF was added to a simplified model (using age and BMI categories only), the discriminative performance of this model was similar to the full model + SAF (AUROC=0.806, p=0.062).

Conclusion: In the Lifelines cohort study, discrimination, calibration, and reclassification criteria showed significant and clinically relevant additional value of SAF in diabetes detection screening. The new model was especially useful in reclassifying participants in intermediate risk categories in which further blood glucose or HbA1c testing should confirm the presence of diabetes. Furthermore, a simplified model (using age, BMI, and SAF only) was found that did not affect the discriminative value of the full model.

Diabetes risk reclassification comparing the FINDRISC model and the FINDRISC model + SAF.

Prevalence of diabetes **FINDRISC** model	Prevalence of diabetes **FINDRISC + SAF** model				total reclassified
	0-1%	1-5%	5-10%	>10%	
0-1%					
Number (%) of participants	48707 (91.1)	4779 (8.9)	0	0	4779 (8.9)
Observed diabetes prevalence	0.3	1.3	-	-	
1-5%					
Number of participants	5537 (24.7)	15673 (70.0)	1015 (4.5)	175 (0.8)	6727 (30.0)
Observed diabetes prevalence	1.1	2.7	5.9	9.7	
5-10%					
Number of participants	0	555 (20.0)	1794 (64.7)	422 (15.2)	977 (35.3)
Observed diabetes prevalence	-	5.0	6.5	9.5	
≥10%					
Number of participants	0	0	49 (8.3)	541 (91.7)	49 (8.3)
Observed diabetes prevalence	-	-	6.1	12.6	

Disclosure: **B.T. Fokkens:** Stock/Shareholding; Andries J. Smit is founder and shareholder of Diagnoptics Technologies, which developed the AGE reader™.

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The association between serum testosterone and insulin resistance: Is it really bidirectional?

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Background and aims: Low concentrations of testosterone have been associated with increased risk for type 2 diabetes and the risk is presumed to act through the increase in insulin resistance. It has been suggested that the association between insulin resistance and testosterone concentrations is bidirectional. The aim was to investigate whether testosterone concentrations predict insulin resistance and whether the levels of insulin resistance predict the concentrations of testosterone in a prospective study.

Materials and methods: Longitudinal observational study. Between 2002-2005 a sample of 2816 subjects (M=1400) were randomly selected for a cohort study with the goal to detect risk factors for cardiovascular disease at an early stage. The cohort was followed up 2012-2014. The mean follow-up time was 9.9±1.1 years and the protocol was completed in a subset of 1327 (M=657) subjects. Measurements of all hormones and plasma glucose were performed at fasting at both visits. Insulin resistance was defined using homeostatic model assessment of insulin resistance (HOMA-Ir). Immunoassay techniques were used for measurements of sex hormones. Analyses were stratified for sex. Linear regressions were computed to investigate the association between sex hormones and insulin resistance. General linear models were also performed to compare the variance of testosterone concentrations in different quartiles of logHOMA-Ir and the variance of logHOMA-Ir in different quartiles of testosterone.

Results: In men, concentrations of testosterone were strongly associated with insulin resistance both at baseline (β=-0.112 p=0.003), and at follow-up (β= -0.208 p<0.001), in multivariable analysis including age and waist hip ratio. In the longitudinal analysis in men, low concentrations of testosterone at baseline were associated with high levels of HOMA-Ir at the follow-up in a multivariate model including age, waist hip ratio, follow-up time and HOMA-Ir at baseline as covariates (β=-0.105 p=0.002). Men within the lowest quartile of testosterone at baseline had significantly higher logHOMA-Ir at follow-up than other quartiles (Q1 vs Q2 p=0.006, Q1 vs Q3 p=0.001, Q1 vs Q4 p=0.004). No differences were observed between quartiles 2, 3 and 4 in this matter. In women, there were no significant associations between testosterone levels and insulin resistance at baseline (β=0.055 p=0.141) or at follow-up (β=0.021 p=0.554). In the longitudinal analyses investigating the impact of insulin resistance on testosterone concentrations in men and women at follow up no significant associations were found in a multivariable analysis including baseline concentrations of testosterone (Men β=-0.012 p=0.738, Women β=0.051 p=0.231). The variance of testosterone levels at follow up was also investigated in different quartiles log HOMA-Ir at baseline in the multivariable model but no significant differences between quartiles were observed.

Conclusion: In this longitudinal observational study testosterone concentrations at baseline predicted high insulin resistance at follow-up in men, while high insulin resistance at baseline did not predict low testosterone. The results suggest a strong effect of testosterone on the development of insulin resistance and on the incidence of diabetes in men.

Supported by: Research and Development Council in primary care in Gothenburg

Disclosure: **K. Ottarsdottir:** Grants; Research and Development Council in primary care in Gothenburg, Research and Development Council of the Region Västra Götaland.

PS 011 Gene-diet interactions and environmental factors

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Gene-diet interaction analysis, fine mapping and genomic annotation of the FADS1-2-3 gene cluster reveals regulatory potential in diabetes

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Background and aims: Polymorphisms at the fatty acid desaturase gene cluster (FADS1-FADS2-FADS3) have been associated with multiple metabolic and anthropometric traits in Greenlandic Inuit. We systematically assessed whether loci in the FADS region modify the association between dietary fat intake and cardiometabolic traits and functionally annotated top variants to estimate causal loci.

Materials and methods: Data analyses consisted of: 1) interaction analyses between the six candidate genetic variants; 2) gene-centric joint analyses to detect interaction signals in the FADS region; 3) haplotype-centric joint tests across 30 haplotype blocks in the FADS1-3 region to refine interaction signals: 4) functional annotation of top loci. These analyses were undertaken in Swedish adults from the GLACIER Study (N=5,160); data on gene variation (Metabochip array) and height, body weight, fasting and 2hr-glucose, triglycerides, and HDL-, LDL- and total cholesterol were available. Dietary intakes of n-3, n-6 and total polyunsaturated fatty acids (PUFA) were calculated from food-frequency questionnaires. Results were adjusted for multiple testing.

Results: SNP-level multiplicative interactions were observed between rs174570 and n-6 PUFA intake on fasting glucose ($P_{interaction}$=0.007) and between rs174602 and n-3 PUFA intake on total cholesterol ($P_{interaction}$=0.015). Gene-centric analyses demonstrated evidence for joint main and interaction effects for FADS on body weight ($P_{n\text{-}3.joint}$ = 0.018, $P_{n\text{-}6.joint}$ = 0.021, $P_{PUFA.joint}$ = 0.024) and on BMI ($P_{n\text{-}3.joint}$ = 0.031, $P_{n\text{-}6.joint}$ = 0.029, $P_{PUFA.joint}$ = 0.033) irrespective of types of fatty acid intake. An interaction was detected for FADS1-3 and n-3 PUFA on triglycerides (P_{int}=0.005). The haplotype analyses revealed three blocks ($P_{int} \leq 0.011$) that drive the interaction between FADS1-3 and n-3 PUFA on triglycerides. Genomic annotation showed that the rs5792235 variant demonstrated the highest functionality score (Figure).

Conclusion: The association between FADS1-3 variants and triglycerides may be modified by PUFA intake. The intronic rs5792235 variant is a potential causal variant in the region. It is likely that the region harbours multiple causal loci.

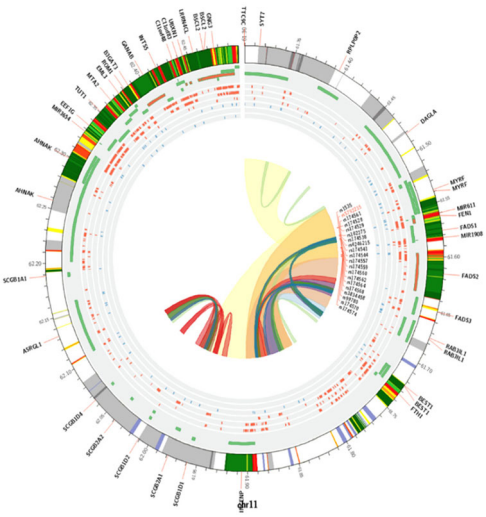

Figure. Circos plot based on rs5792235 (shown in red text). The plot shows rs5792235 and its proxies (shown in black text around rs5792235). From outer to inner, the circles represent ChromHMM chromatin states, annotated genes (green), histone modification set (red), transcription factor set (blue), current SNP (rs5792235) and associated SNPs, and 3D chromatin interactions, respectively. The three circles in the histone modification set are H3K4me1, H3K4me3, H3K27ac, and the three circles in the transcription factor set are CTCF, CEBPB and CEBPD. Color schemes for the ChromHMM chromatin states and the 3D interaction loops are found at http://biotech.bmi.ac.cn/3dsnp/documentation/tutorials/ .

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Disclosure: **P.W. Franks:** None.

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Diabetes protection is associated to a C3H-specific locus on chromosome 15

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Background and aims: The genetic factors underlying the susceptibility for type 2 diabetes are not fully understood. To identify new diabetes-associated genes we investigated an outcross population generated by crossbreeding of diabetes-susceptible New Zealand Obese (NZO) mice with the diabetes-resistant C3HeB/FeJ mouse strain.

Materials and methods: We measured diabetes-related traits in a (NZOxC3H)NZO backcross population of 300 males. Animals were fed a high-fat-diet (45% fat/cal.) throughout the experiment. Body weight, body composition, plasma glucose and insulin were measured at different time points. At 21 weeks mice were sacrificed and tissues were harvested. The N2 population was genotyped using a genome-wide high-density SNP panel. Linkage analysis was performed by *in silico* calculation of phenotype-genotype associations. Differential gene expression between the parental strains in liver, skeletal muscle, white adipose tissue, brown adipose tissue and pancreatic islets was analyzed by microarray and qPCR.

Results: A major QTL (Quantitative Trait Locus) for blood glucose was identified on chromosome 15 (LOD score 7.5). A locus located at 13 cM was associated to a lowering of 100 mg/dl in blood glucose of the protective C3H allele carriers at 15 weeks of age. A significant lowering ($p<0.05$) in glycemia could be associated to identified locus from 8 weeks of age on, demonstrating an early diabetes protective effect linked to C3H allele carriers. Moreover, diabetes prevalence in Chr.15/C3H was 57% compared to 81% in Chr15/NZO at 21 weeks. This effect has shown to be independent of the QTL locus found on body weight. Similarly, mortality rate was strongly reduced in C3H alleles carriers, 6,5% compared to 26,4% at 21 weeks of age. Microarray expression profiling revealed 44 genes ($p<0.05$) differentially expressed in

liver, brown adipose tissue, white adipose tissue, muscle and/or pancreatic islets, and mapping to the critical regions of the identified QTL. Comparisons using the SNP database (SANGER) identified 16 genes with C3H-specific variants. As revealed by a Variant effect Predictor analysis (Ensembl), protein function could be affected by those SNP.

Conclusion: A major QTL was found on Chr. 15 triggering diet-induced effects on diabetes development already at early stages of life. We hypothesized that the C3H allele confers diabetes protection by improving islet function and survival in NZO mice background. Future analysis aimed to investigate the pathophysiological effect of the current candidate genes associate to the presented diabetes locus on chromosome 15.

Supported by: Deutsches Zentrum für Diabetesforschung DZD

Disclosure: **D. Altenhofen:** None.

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The changes in serum metabolites after meal challenge are affected by genetic variants of TCF7L2

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Background and aims: The single nucleotide polymorphisms (SNPs) of TCF7L2 gene were found to be one of the most predictive genetic factors of type 2 diabetes mellitus (T2DM) development and have been implicated in the pathogenesis of T2DM primarily due to its influence on pancreatic β-cell function and insulin secretion. It has been shown that a low carbohydrate diet of TCF7L2 risk carriers may decrease the risk of T2DM development. To study the potential mechanism of this effect, we reached for a metabolomics approach. LC-QTOF-MS was used to evaluate changes in serum metabolites after high-carbohydrate (HCarb) and norm-carbohydrate (NCarb) meal intake, performed in men carrying 2 or 0 risk alleles for investigated SNP of TCF7L2 (rs7901695).

Materials and methods: The study group consisted of 21 men without T2DM. We genotyped previously identified SNP TCF7L2 rs7901695. The study population was divided into 2 groups: individuals carrying 2 risk alleles (homozygous genotype CC, n=8, 31±6 y, BMI=29±8) and subjects with 0 risk alleles (homozygous genotype TT, n=13, 35±10 y, BMI=28 ±6). Fasting, 0.5hr, 1hr, 2hr and 3hr, serum samples were taken during HCarb (89% of energy from carbohydrate) and NCarb (45% of energy from carbohydrate) meal challenges. Samples were fingerprinted using LC-QTOF-MS. Data was collected in both ESI (+/-) modes (50-1,000 m/z). Based on the relation between time points and metabolite's intensity, AUC was calculated for each metabolite. Obtained AUCs were forwarded for partial least square discriminant analysis (PLS-DA) modelling. Based on PLS-DA model volcano plots were built to select significant metabolites according to multivariate statistics. Additionally, for each significant metabolite, p-value was calculated by Welch's t-test.

Results: Based on AUCs obtained for metabolites after HCarb meal PLS-DA models were built showing a perfect separation between men with and without risk alleles (CC vs. TT). The parameters of the models were as follow: R2=0.992, Q2=0.818 in ESI+ and R2=0.999, Q2=0.729 in ESI- ion modes. Individuals with 2 risk alleles showed significantly lower AUCs for acylcarnitines (- 61-84%, p=0.001-0.05), lysophospholipids (- 30-85%, p=0.001-0.09), phospholipids (- 20-49%, p=0.009-0.1), diacylglycerols (- 27-80%, p=0.009-0.08), oxidized fatty acids (- 36-83%, p=0.01-0.2), and oxoproline (- 65%, 0.03). After NCarb meal no significant differences were observed. In fasting state participants carrying 2 risk alleles had lower levels of hydroxyarachidonic (- 67%, p=0.1) and hydroxylinoleic (- 91%, p=0.03) acids and higher level of phosphatidylethanolamine, PE 40:7 (+ 66%, p=0.1).

Conclusion: The carriers of 2 risk alleles of TCF7L2 rs7901695 responded differentially to HCarb, but not to NCarb meals, than individuals without risk alleles. HCarb meals influenced lipid metabolism, which was manifested by decreased AUCs of metabolites involved in energy metabolism (acylcarnitines) and oxidative stress (oxidized fatty acids, oxoproline). The potential impact of observed changes in lipid metabolism after HCarb meals on T2DM development in TCF7L2 risk carriers requires further investigations.

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Disclosure: **E. Adamska:** None.

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The changes in serum metabolites after meal challenge are affected by genetic variations in rs340874 PROX1

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Background and aims: The single-nucleotide polymorphism (SNP) of PROX1 gene rs340874 was found to be associated with glucose metabolism, fasting glucose concentration and type 2 diabetes mellitus (T2DM). Moreover, previously we have observed that it is associated with alterations in postprandial glucose and lipid metabolism. The mechanism by which PROX1 gene affects the susceptibility to T2DM seems to be more complex and it was not well established yet. To study the potential mechanisms we reached for a metabolomics approach. LC-QTOF-MS was used to evaluate changes in serum metabolites after high-carbohydrate (HCarb) and norm-carbohydrate (NCarb) meal challenge tests performed in men dependently on genotypes of PROX1 rs340874.

Materials and methods: The study participants were divided into 2 groups dependently on PROX1 rs340874 genotype: the carriers of CC (n=12, 35±9 years old, BMI=29±8) and carriers of CT or TT (n=16, 35±6 years old, BMI=27±4). Fasting, 0.5h, 1h, 2h and 3h serum samples were taken from participants during HCarb (89% of energy from carbohydrate) and NCarb (45% of energy from carbohydrate) meal challenge. Samples were fingerprinted by LC-QTOF-MS. Data were collected in both ESI (+/-) modes (50-1,000 m/z). Based on the relation between time points and metabolite's intensity for each metabolite AUC was calculated. Obtained AUCs were forwarded for partial least square discriminant analysis (PLS-DA) modelling. Based on PLS-DA models volcano plots were built to select significant metabolites according to multivariate statistics. Additionally for each significant metabolite p-value was calculated by Welch's t-test.

Results: PLS-DA models built based on AUCs obtained from HCarb and NCarb data showed a clear separation of studied groups and good models parameters (e.g. for ESI+ data: R2=0.992, Q2=0.753 for HCarb and R2=0.998, Q2=0.43 for NCarb). After both meals AUC for acetylcarnitine was increased in CC carriers (+92%, p=0.003 and +55% p=0.07 for HCarb and NCarb meal, respectively). In case of NCarb meal individuals with risk alleles showed significantly lower AUCs for oxidized fatty acids (- 32-81%, p=0.02-0.1) and higher for linoleic acid (+80%, p=0.01). After HCarb meal in the CC carriers group decreased AUC for ornithine (-45%, p=0.05), ketosphingosine (- 48%, p=0.01), linoleamide (- 45%, p=0.1), and several lysophospholipids (- 40-56%, p=0.01-0.05) was observed. After both meals differences in AUCs for bile acids between studied groups were noticed. In the CC carriers group higher AUC for sulfodeoxycholate (+42%, p=0.08) and lower for taurocholate (-59%, p=0.07) after HCarb meal, while after NCarb meal higher AUC for glycodeoxycholate (+70%, p=0.1) were observed.

Conclusion: The carriers of 2 risk alleles of PROX1 rs340874 responded differentially to HCarb and NCarb meals. The obtained results indicate for differences in fatty acid metabolism (after NCarb meal), phospholipid metabolism (after HCarb meal) likewise differences in bile acids signalling between CC and CT/TT carriers. Further investigations are needed for deeper exploration of mechanisms by which CC phenotype promotes T2DM.

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Disclosure: **P. Samczuk:** None.

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Built environmental characteristics and diabetes risk: a systematic review and meta-analysis

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Background and aims: Physical inactivity, poor dietary habits and lack of sleep are established lifestyle-related risk factors for type 2 diabetes mellitus (T2DM). Built environmental characteristics may influence these risk factors, and in turn T2DM development. We aimed to systematically review the evidence on built environmental characteristics related to these lifestyle factors and the prevalence and incidence of T2DM as well as glycaemic markers.

Materials and methods: A literature search was performed in PubMed, Embase and Web of Science. We included studies reporting: 1) populations ≥18 years old, 2) T2DM prevalence, T2DM incidence, or glycaemic markers as outcomes, and 3) built environmental characteristics related to physical activity, dietary behaviour and sleep. After screening by two independent reviewers, data were extracted by one reviewer according to a standardized protocol and quality of the studies was assessed by two reviewers according to the Quality Assessment Tool for Quantitative Studies. When ≥3 studies investigated the same outcome and exposure, adjusted for at least age and gender and received a strong or moderate quality rating, we included these for meta-analyses using a random effects model.

Results: After screening 9,089 studies, 86 studies were eligible for data extraction. In total, 46 studies compared T2DM risk or prevalence in urban built environments versus rural built environments. Thirty-two of these studies were not meta-analysed; 14 due to a weak quality rating and 19 studies for not correcting for confounders. Pooled risk ratios of 13 included studies indicated a significant association such that more urbanized areas were associated with a higher T2DM prevalence (1.50 (95%-CI: 1.24 - 1.81; I^2 72.8%)). These findings mainly originated from studies in lower middle income countries (n=5) and higher middle income countries (n=6). A pooled risk ratio of four studies indicated that higher neighborhood walkability was associated with lower T2DM risk (0.87 (95%-CI: 0.81 - 0.93; I^2 0%)). Three green space studies indicated a non-significant inverse association (0.84 (95%-CI: 0.61 - 1.15; I^2 96.6%)). Higher availability of fast-food outlets/convenience stores (n=4) and perceived healthiness of food environment (n=2) were relatively consistently associated with increased and decreased T2DM risk, respectively. Associations between supermarkets/grocery stores (n=4), combined index scores of food environment (n = 8), residential noise (n=3), availability of recreational facilities (n=4), infrastructure (n=4), safety (n=3) and T2DM were more heterogeneous.

Conclusion: A large body of literature is available linking built environmental characteristics with T2DM. Most studies investigated urbanisation, where T2DM risk/prevalence in urban areas is generally higher compared to rural areas, especially in middle-income countries. Physical activity environment was relatively consistently associated with T2DM risk, whereas associations were more heterogeneous for food environment.

Disclosure: **N.R. den Braver:** None.

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Changes in objective physical activity in a randomised lifestyle intervention among diabetes prone Middle-Eastern immigrants and its association with insulin sensitivity

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[1]Lund University, Malmo, Sweden, [2]Gothenburg University, Gothenburg, Sweden.

Background and aims: Middle-Eastern immigrants in Sweden exhibit high levels of physical inactivity, obesity and type 2 diabetes. The aim of this study is to examine changes in objectively assessed physical activity levels following a culturally adapted lifestyle intervention among Middle-Eastern immigrants. The secondary aim is to examine the association between changes in insulin sensitivity and objectively assessed physical activity levels.

Materials and methods: A randomized controlled trial of four-months duration, addressing Iraqi immigrants exhibiting one or more risk factors for type 2 diabetes. The intervention group (n=50) was offered a culturally adapted lifestyle intervention comprising of seven group sessions including self-empowerment and cooking sessions. The control group (n=46) received "usual care", comprising of written information on healthy lifestyle habits. All participants underwent health examinations and wore accelerometers for 10 days at the start, mid and end of the study. A novel approach was used to process accelerometer data which summarized average physical activity intensity as vector magnitude (VM) in units of acceleration (mG's).

Results: At baseline, participants spent approximately 80 percent of the time in sedentary activities. The intervention and the control group didn't differ in terms of change over time in average physical activity intensity (VM) (β=0.011 95% CI -0.011 to 0.033, *P*=0.32). However, there was a significant increase in number of hours per day spent in light intensity physical activity in the intervention group compared to the control group (β=0.023 95% CI 0.001 to 0.045, *P*=0.046). This was accompanied by a trend towards decrease in number of hours per day spent in sedentary activities. Improvement in insulin sensitivity index was significantly associated with objectively measured physical activity (β= 0.01 95% CI 0.004 to 0.025, *P*=0.006).

Conclusion: Increase in the time spent in light intensity physical activity combined with a trend toward decrease in sedentary time, in a sedentary immigrant population, will potentially have beneficial effects for diabetes prevention in this group at high-risk of type 2 diabetes.

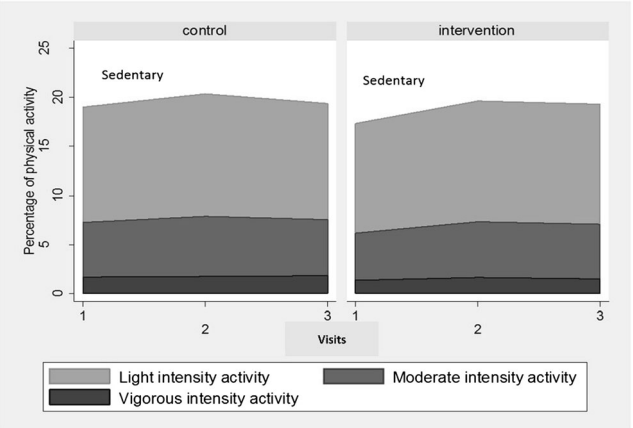

Figure 1: Change over time in percentage of time spent in light, moderate and vigorous intensity activities in the two groups.

Clinical Trial Registration Number: NCT01420198

Supported by: ALF, Region Skåne, Malmo Stad, Crafoord foundation, SLS, NovoNordisk

Disclosure: **F. Siddiqui:** None.

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Differential effects of bisphenol S and bisphenol F on ion channel activity and expression in pancreatic beta cells

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Background and aims: Many dairy products found in plastics, cosmetics and food packaging material among others, have been proved to be harmful to human health due to its actions as endocrine-disrupting chemicals (EDCs).

Low doses of bisphenol A (BPA), one of the most widespread EDCs, alter pancreatic beta-cell function, induces insulin resistance and other metabolic alterations in cellular and animal models. The increasing concern about BPA has led to its substitution with other compounds chemically similar. Recent findings, however, suggest that these "alternatives to BPA" might elicit similar physiological alterations. In the present work we study the effects of bisphenol S (BPS) and bisphenol F (BPF) on ion channels activity and gene expression in pancreatic beta-cells.

Materials and methods: Whole islets or dispersed islets from C57BL/6 or beta estrogen receptor knockout (BERKO) mice treated either, acutely or during 48 h with BPA (as a positive control), BPS or BPF. We used the patch clamp techniques in its single channels recording form or in the whole-cell configuration to measure K_{ATP} channel activity and calcium currents, respectively. Gene expression of ion channels was analyzed by quantitative RT-PCR.

Results: Both BPS and BPF reproduced the inhibitory effect of BPA on beta-cell K_{ATP} single channel. Nevertheless, a higher BPF concentration was needed to reach K_{ATP} inhibition similar to that observed with BPA or BPS. In beta-cells from WT mice, K_{ATP} activity was reduced by 65 ± 20% (n=9, p=0.04) and 40 ± 15% (n=7, p=0.01) after addition of BPS (1 nM) or BPF (10 nM), respectively. This reduction was not observed in BERKO mice under the same conditions (97 ± 51% and 80 ± 34%, respectively; n=7). Upon 48 h of treatment with BPS or BPF, calcium channel activity was measured in isolated beta-cells. Low concentration (1 nM) of BPS, but not BPF, reduced peak calcium currents compared with vehicle (vehicle: 29.2 ± 1.9; BPS: 24.1 ± 1.6; BPF: 27.2 ± 2.6 pA/pC; n=11, n=15 and n=21, respectively). A higher BPF concentration (1 μM) was needed to reduce calcium currents (vehicle: 36.1 ± 3.0 pA/pC at 0 mV (n=13) *vs.* 1 μM BPF: 23.3 ± 2.2 pA/pC (n=17, p=0.001)) in isolated beta-cells. However, this reduction was not observed in beta-cells from BERKO mice, supporting the hypothesis that these effects are mainly produced through estrogen receptor beta. These effects on calcium currents were associated with lower Cav2.3 channel mRNA levels compared to beta-cells treated with vehicle.

Conclusion: Our results show that, as BPA, BPS can act as a xenoestrogen, modulating K_{ATP} activity, calcium currents and gene expression at low concentrations (1 nM). On the other hand, concentrations 10-fold to 1000-fold higher were necessary to reach the same effects with BPF. Therefore, BPS fully imitate BPA and should not be considered a safe alternative. More experiments are needed to identify BPF as a safer substitute of BPA.

Supported by: Ministerio de Economía y Competitividad

Disclosure: **L. Marroqui:** None.

PS 012 Infection, inflammation and diabetes

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TNFα antagonism and insulin resistance in non-diabetic patients suffering from psoriasis: secondary analysis of a randomised active-controlled trial

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Background and aims: Patients suffering from psoriasis exhibit an increased cardio-metabolic burden due to the systemic inflammatory nature of the disease. According to numerous scientific reports the pro-inflammatory cytokine TNFα plays a major role in the pathogenesis of psoriatic disease and it also has been demonstrated to increase insulin resistance including diminished peripheral glucose uptake. TNFα blockers have been shown not only to mitigate the course of psoriasis, but also to improve metabolic parameters including insulin resistance. In this secondary analysis of a RCT we hypothesized that the TNFα blocker Adalimumab (ADA) is superior to fumaric acid esters (FAE), a well established conventional, immuno-modulatory systemic treatment for psoriasis, in improving insulin resistance in non-diabetic patients with chronic moderate to severe plaque type psoriasis.

Materials and methods: Between 2009 and 2012, 65 patients were randomized to receive either ADA or FAE as active control at a tertiary university hospital. Five patients were excluded because of pre-existing treated diabetes resulting in 32 receiving FAE and 28 receiving ADA in our final analysis. The duration of treatment was 24 weeks with visits at baseline, 12 weeks and 24 weeks. At each visit disease severity was assessed, blood was drawn and a standard 2h OGTT (75g of glucose) was performed with measurements at 0 and 120 min. The main outcome parameters were the HOMA-IR index and the composite insulin sensitivity index (ISI) by Matsuda which were log-transformed for further analysis and reported by their geometric means. We used Student's t-test for the comparison of baseline values and linear mixed models to estimate treatment differences at follow-up adjusting for baseline values.

Results: The study population consisted of 80% men and were 44±11 years of age. The patients displayed a median baseline disease severity score (PASI) of 14(IQR: 12 -20) and a median affected body surface area of 15(12 - 28)%. Before treatment, HOMA-IR was 1.54±2.57 in FAE-treated and 1.19±2.21 in ADA-treated patients (p=0.302). ISI values were 6.37±2.78 in FAE patients compared to 7.80±2.07 in ADA patients(p=0.424). After the initiation of treatment, there was a significantly higher HOMA-IR of 2.17 (95%CI: 1.67 - 2.67) in the FAE group compared to 1.51 (1.22 - 1.88) in the ADA group (p=0.021) with a trend towards an increase by 18(-2 - 43) % between the two follow-up visits(p=0.081). At follow-up, ISI was significantly lower in FAE-treated (4.65 (3.76 - 5.74)) compared to ADA-treated patients (7.33 (5.92 - 9.08), p=0.004) without a significant temporal effect. Disease severity scores and CRP decreased comparably in both groups (p<0.05).

Conclusion: In our population of non-diabetic psoriasis patients, ADA might be superior to FAE in maintaining insulin sensitivity. Contrary to previous reports, ADA did not improve insulin sensitivity, which could be due to the low baseline degree of insulin resistance in our sample. Since ADA was able to slow the progression of insulin resistance, while FAE was not, ADA might be a better choice for psoriasis patients at a high risk for developing type 2 diabetes.

Clinical Trial Registration Number: NCT01088165

Disclosure: **C.T. Herz:** None.

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Diabetes and impaired glucose metabolism is associated with more cold-related cardiorespiratory symptoms

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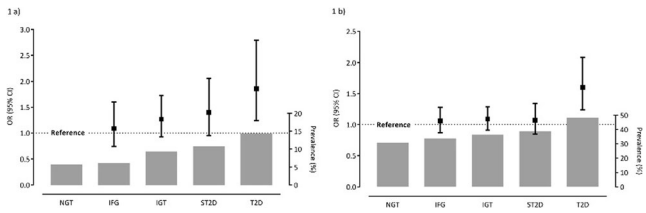
Background and aims: Diabetes and impaired glucose metabolism cause metabolic, neural and circulatory disturbances that may predispose to adverse cooling, elicit symptoms and increase health risks during the cold season. This cross-sectional study assessed the prevalence of cold-related cardiorespiratory symptoms in the general population according to glycaemic status.

Materials and methods: The study population consisted of 2436 men and 2708 women aged 45-74 years from the National FINRISK cold sub-studies in 2002 and 2007. A questionnaire assessed cold-related symptoms (respiratory, cardiac, peripheral circulation). Glycaemic status was determined based on fasting blood glucose, oral glucose tolerance tests or reported diagnosis of diabetes and categorized into normal glucose metabolism, impaired fasting blood glucose, impaired glucose tolerance, screen-detected type 2 diabetes and type 2 diabetes.

Results: Type 2 diabetes was associated with increased odds for cold-related dyspnoea [Adjusted OR 1.72 (95% CI, 1.28-2.30)], chest pain [2.10 (1.32-3.34)] and respiratory symptoms [1.85 (1.44-2.38)] compared with normal glucose metabolism. Screened type 2 diabetes showed increased OR for cold-related dyspnoea [1.36 (1.04-1.77)], cough [1.41 (1.06-1.87)] and cardiac symptoms [1.51 (1.04-2.20)]. Worsening of glycaemic status was associated with increased odds for both cardiac (from 1.11 in impaired fasting glucose to 1.99 in type 2 diabetes, p=0.000, Fig. 1 a) and respiratory (1.14 to 1.84, p=0.000, Fig. 1 b) symptoms. Also, individual cardiorespiratory symptoms were reported more often with worsening glycaemic status. These were dyspnoea (from 1.16 in impaired fasting glucose to 1.72 in type 2 diabetes, p=0.000), cough (1.02 to1.27, p=0.032), chest pain (1.28 to 2.10, p=0.006) and arrhythmias (0.87 to 1.74, p=0.020).

Conclusion: Diabetes is related to higher occurrence of cardiorespiratory symptoms in the cold. These are reported increasingly with worsening glycaemic status and may predict either an aggravated diabetic condition in cold, or reveal individuals with impaired glucose metabolism or diabetes. Increasing awareness assist individuals with impaired glucose metabolism to protect themselves from cold weather. Health care personnel may utilize this information for providing appropriate advice for health risk management in cold conditions.



Supported by: The Finnish Work Environment Fund is acknowledged for the support.

Disclosure: **T.M. Ikäheimo:** Grants; Finnish Work Environment Fund.

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Enteroviruses in the pancreas of live adult patients with newly diagnosed type 1 diabetes. Results from the DiViD study

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Background and aims: One possible environmental factor contributing to the development of type 1 diabetes (T1D) is enterovirus (EV) infection of pancreatic endocrine cells. The lack of well-preserved pancreatic tissue taken at the time of the clinical onset is a restriction for virus investigations. One of the aims of the Diabetes Virus Detection study (DiViD) is to search for virus in pancreatic tissue of optimal quality collected from live adult patients newly-diagnosed with T1D. We have reported the presence of EV genome by PCR and of EV proteins by IHC. Here we further analyze the presence of viruses in the DiViD cases using new methods.

Materials and methods: Six recent onset T1D patients (age 24-35) were included in the DiViD study. Minimal pancreatic tail resection was processed under sterile conditions. 11 live cases of pancreatic carcinoma without diabetes served as controls. In the present study, we used EV detection methods that combine virus growth in cell culture, gene amplification, and immunofluorescence (IF) for viral proteins. Pancreas homogenates were produced in cell culture medium. Cell lines (AV3, RD, HEp-2, and LLC-MK2) were incubated with homogenates and serially passaged 3-6 times. DNA and RNA were extracted from both pancreas and cell cultures. Real time PCR was used for detecting >20 viruses other than EVs (6 herpesviruses, parvovirus B19, HBV, JCV, BKV, HCV, GBV-C, rubella, influenza A/B, parainfluenza 1-4, RSV, astrovirus, norovirus, rotavirus, HAV). EVs and polioviruses were searched for using end-point PCR with primer pairs targeting the 5'UTR region of the A, B, C, D species. Amplicons were directly sequenced. In cultured cells exposed to pancreas homogenates, the expression of EV capsid proteins was evaluated by IF with a panel of EV antibodies.

Results: 6/6 T1D cases and 2/11 non-diabetic control cases contained EV genomes ($p<0.05$). In contrast, genomes of over 20 human viruses other than EVs could be detected in only 2/17 cases (one EBV, one Parvovirus B19). EV detection was confirmed by IF of cell lines incubated with pancreatic extracts. EV proteins were expressed in the cytoplasm of approx. 1% of cells. Infection could be transmitted from EV-positive cell cultures to uninfected cell lines using cell culture supernatant that had been filtered through 100 nm membranes. This shows that an infectious agent of less than 100 nm in diameter is present in pancreata of diabetic subjects. Due to slow progression of infection in EV-carrying cell cultures, cytopathic effects could only be perceived using time-lapse microscopy. Sequences of 5'UTR amplicons were compatible with EVs of the A and B species. Compared to control cell cultures exposed to EV-negative pancreatic extracts, EV-carrying cell cultures produced high levels of IL6, IL8 and MCP1.

Conclusion: The results confirm with new, more sensitive assays that EVs in pancreas are consistently present in subjects newly diagnosed with T1D and rarely present in non-diabetic control patients. The data are consistent with an ongoing low grade enteroviral infection in the pancreas of newly-diagnosed diabetics.

Disclosure: **L. Krogvold:** None.

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Pandemic influenza diagnosis and subsequent risk of type 1 diabetes

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Background and aims: The 2009 pandemic influenza A H1N1 (swine flu) has been associated with development of several autoimmune diseases. In this register-based study, we aimed to test whether pandemic influenza diagnosis was associated with increased risk for type 1 diabetes (T1D).

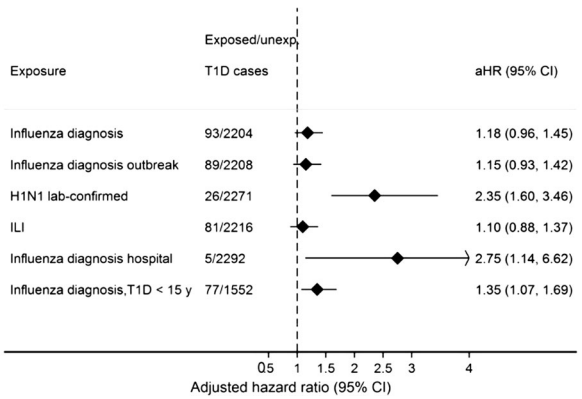
Materials and methods: In this nationwide study, the Norwegian population under age 30 years was followed from October 2009 through June 2014 by linking prospectively collected data from national health registries with patient level information. We analysed data from the Norwegian Patient Register (inpatient and outpatient clinic data), the Primary Care Database, diabetes medication from the Norwegian

Prescription Database and laboratory confirmed influenza A (H1N1) infection (reverse-transcriptase polymerase chain reaction result) with influenza A (H1N1) from the Surveillance System for Communicable Diseases. We defined pandemic influenza as a clinical influenza or influenza like illness (ILI) diagnosis during the pandemic outbreak in Norway, or a laboratory-confirmed influenza A (H1N1). Incident cases of type 1 diabetes were defined as the first registration of at least one T1D diagnosis in primary or specialist health care and initial insulin treatment continued for at least 6 months. We used Cox regression to estimate hazard ratio (aHR) for associations between influenza and subsequent risk of T1D, adjusted for year of birth, sex, place of birth, education, and pandemic influenza vaccination.

Results: We identified 2297 individuals diagnosed with T1D after the pandemic, incidence rate: 26 per 100,000 person-years. Individuals registered with ILI during the 2009-2010 pandemic had an aHR for type 1 diabetes of 1.18 (95% CI: 0.96 - 1.45). When restricted to the age-group under 15 years, ILI was associated with a significantly higher risk of T1D (aHR=1.34, 95% CI: 1.06-1.68). A laboratory confirmed infection with influenza A (H1N1)pdm9, or hospitalization with an influenza diagnosis were both associated with a significant two- fold higher risk of T1D, while those diagnosed with ILI in the Primary Care Database during the pandemic season did not have a significantly increased risk of T1D (Figure 1).

Conclusion: ILI diagnosis was associated with increased incidence of new-onset T1D in children under 15 years. Patients with a lab-confirmed influenza A (H1N1) or who were hospitalized with influenza during the 2009-2010 pandemic had two-fold higher risk of developing T1D.

Figure 1. Association between pandemic influenza diagnosis in primary or specialist care, and risk of type 1 diabetes in up to 2.26 million Norwegians. Hazard ratios were adjusted for year of birth, sex, place of birth, education and pandemic influenza vaccination. ILI, Influenza Like Illness. Pandemic influenza outbreak from 1st October 2009 to 31st December 2009



Disclosure: **P.L.D. Ruiz:** None.

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TNFSF14: a potential contributor to hyperinsulinaemia in childhood obesity

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Background and aims: Chronic low-grade inflammation is a characteristic of obesity. Obesity is also associated with hyperinsulinemia and the risk for developing type 2 diabetes. Yet the role of inflammation in the early pathogenesis of hyperinsulinemia, impaired glucose tolerance and T2D has not been defined. We aimed to identify novel markers of inflammation associated with early onset obesity and to study their association with impaired glucose tolerance and hyperinsulinemia in obese children and adolescents.

Materials and methods: Children and adolescents with obesity (n=137) and lean controls (n=34) were recruited. Of the children and adolescents with obesity 64 had normal glucose tolerance (NGT), 65 had impaired glucose tolerance (IGT) and 8 had type 2 diabetes (T2D). Relative plasma levels of 92 proteins related to inflammation were measured by proximity extension assay (Olink Proteomics, Uppsala, Sweden). The method uses two antibodies for each protein. The two antibodies carry complementary single DNA-strands which, when bound in proximity on the target protein, hybridize and can be extended and amplified by qPCR to obtain relative protein levels. In this way 92 proteins are simultaneously quantified in 1 ul plasma. Plasma levels of the proteins were compared between the obesity and lean group and also between normal and impaired glucose tolerance groups within the obesity group. Human islets and human beta-cell line EndoC-betaH1 were cultured in the presence of 0, 10, 100 or 1000 ng/ml TNFSF14 and accumulated insulin secretion to the culture medium over 24 and 48 h was measured by ELISA.

Results: Plasma levels of 21 of the 92 proteins differed between children with obesity and lean controls, among these were 8 pro-inflammatory cytokines. None of the proteins differed between obese subjects with NGT and those with IGT or T2D. Eleven proteins correlated with fasting insulin concentrations, among these was TNFSF14. The addition of recombinant TNFSF14 to the culture medium increased insulin secretion from human islets by 31% during 48 hours culture with 100 ng/ml ($p<0.01$ vs control) and from human EndoC-betaH1 cells by 33% during 24 hour culture with 10 ng/ml ($p<0.05$ vs control).

Conclusion: Several pro-inflammatory cytokines are elevated in childhood obesity and also related to fasting plasma insulin concentrations but not to IGT. TNFSF14 is a potential contributor to fasting hyperinsulinemia through its direct effect on insulin secretion from human beta-cells.

Supported by: VINNOVA - Sweden's Innovation Agency

Disclosure: **H. Manell:** None.

PS 013 Trends in diabetes and obesity

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Time-trends in incidence and prevalence of type 2 diabetes patients requiring glucose-lowering treatment from 2007-2015: nationwide data from Norway and Sweden

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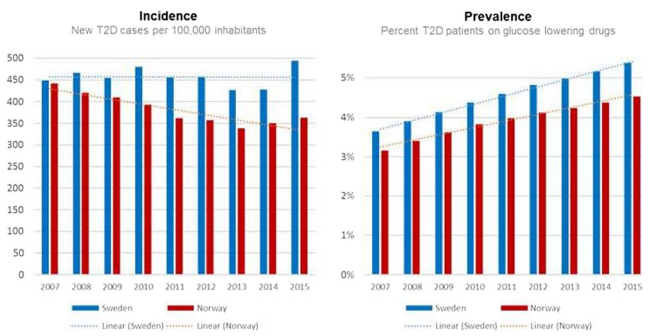
Background and aims: Type 2 diabetes (T2D) is recognized as a growing concern, reaching epidemic proportions worldwide. Information on incidence and prevalence are important to monitor for health care providers to assess treatment strategies and plan for future health care resourcing. The aim was to examine time-trends in T2D incidence and prevalence, and to compare the development between two neighbouring countries with similar health care structure.

Materials and methods: All patients dispensed with glucose lowering drugs (GLDs) during 2007—2015 were identified in nationwide registries in Norway and Sweden. Patients aged <18 years, with polycystic ovarian syndrome, type 1- or gestational diabetes were excluded. Dispensed drugs were calculated annually. Annual incidence was calculated by comparing incident cases with the general T2D free population. Prevalence was assessed annually by dividing the total number of T2D patients by the total general population.

Results: In 2015, T2D patients in Norway were younger, 64.5 vs 68.4 years, and less often women, 46.5% vs 43.3%, compared to Sweden. Patients in Sweden were to higher extent treated with CV drugs (antihypertensives [ACEi and ARBs] and statins) vs Norway; 84.5% vs 73.9%. These proportions have remained relatively stable in both countries during the observation time. Metformin, sulphonylurea, insulin, DPP-4i and SGLT-2i proportions were in Norway vs Sweden, respectively, 59.9% vs 67.3%; 18.6% vs 14.0%; 18.7% vs 32.8%; 10.4% vs 9.2% and 5.1% vs 1.9%. The most prominent changes between 2010-2015 were decrease in sulphonylurea (>35%) and increase in DPP-4i (>130%) in both countries. T2D incidence decreased in Norway (-28%) but remained higher and stable in Sweden. In 2015 the incidence was 363 and 494 cases per 100,000 inhabitants respectively (figure, left panel). During the observation period the prevalence increased 58.3% in total in Norway and Sweden and was in 2015 182,923 (4.5%) and 422,013 (5.4%) patients, respectively (figure, right panel).

Conclusion: In a modern time-era, a marked GLD treated T2D prevalence increase of approximately 60% was observed in total in Norway and Sweden over the last decade. While incidence is declining in Norway it seems to persist at a high level in Sweden. These recent data confirm that occurrence of T2D is a growing problem, despite declining or stabilized incidence, and that the disease will continue to pose a challenge to the health care system.



Disclosure: **D. Nathanson:** Employment/Consultancy; Astra Zeneca. Lecture/other fees; Eli Lilly, Novo Nordisk, Boehringer Ingelheim.

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Predicting total burden of diabetes in Denmark till 2030

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Background and aims: In some countries (Scandinavia, Scotland) the total number of diabetes patients is largely known from health care registers. Predictions of the number of diabetes patients in the future has not been made based on a firm modeling basis however. In Denmark the regular update of the National Diabetes Register has been discontinued since 2012, and reliable figures are therefore not available.

Materials and methods: We used the National Patient Register, the Medicines Products Statistics Register ("Prescription register"), the Danish Adult Diabetes Register, the Health Services register and the Eye Screening Database to reconstruct the Danish National Diabetes Register. We used the person register of the entire Danish population to obtain information on deaths, immigration and emigration. Based on this we could estimate 1) incidence rates of diabetes and mortality rates of persons 2) with and 3) without diabetes for the period 1996–2015 in ages 0-99 years. These rates were modeled separately for men and women by age-period-cohort models with smoothing spline effects. The rates were predicted for the period 2016-2030 using the fitted models, and also under a scenario assuming a 2.5 % annual increase in diabetes incidence rates over the period. We used the rates and population predictions from Statistics Denmark to predict the number of prevalent diabetes patients in the population in the period 2016-2030 in all ages.

Results: Incidence rates of diabetes were declining about 3% per year since 2011; a tendency we found unlikely to persist, so apart from the model extrapolating this decline we also used a model where we assumed the diabetes incidence rate to increase 2.5% per year. In all models we assumed mortality to decrease at as slightly attenuated rates compared to the period 1996-2016. The simple prediction assuming a decline would predict a fall in the prevalent number of diabetes patients from 280,000 (2016) to 230,000 in 2030; whereas the scenario with increasing diabetes incidence rates resulted in a total number of diabetes patients in 2030 of 382,000.

Conclusion: The diabetes incidence rates showed a decline after 2011, a decline we were reluctant to extrapolate, as this may be a consequence of changing diagnostic criteria, so an alternative model to the simple extrapolation was chosen. The most realistic number of diabetes patients in Denmark in 2030 is 380,000, a 65% increase since 2016. This is somewhat less than previous extrapolations have predicted, but our prediction is based on a carefully developed statistical model and a critical appraisal of possible future scenarios of incidence rate development. It should also be borne in mind that the large birth post-war cohorts from the 1940s will begin do die out in the late 2020s, and this has an influence on the number of cases.

Disclosure: **B. Carstensen:** Stock/Shareholding; Novo Nordisk.

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Trends of obesity prevalence among Spanish adults with diabetes, 1987-2012

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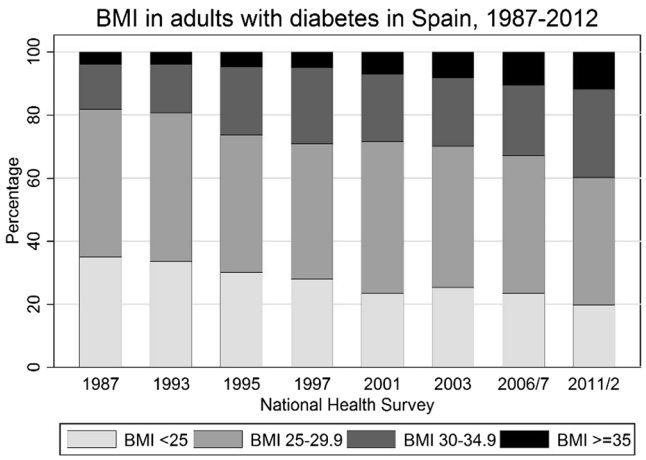
Background and aims: Obesity is directly associated with mortality in patients with diabetes. Our objective was to examine the trend of obesity in Spanish adults with diabetes from 1987 to 2012.

Materials and methods: Data were collected from 8 waves of the national health surveys (from 1987 to 2012) that are cross-sectional studies conducted

in representative samples of the Spanish adult population. Data of 7378 adults (>= 16 years) who reported having been diagnosed of diabetes were analyzed. Previously validated self-reported weight and height were used to estimate body mass index (BMI). Following recommendations, overweight was defined as a BMI of 25.0 to 29.9 kg/m2 and obesity as a BMI of 30.0 kg/m2 or higher. Age-adjusted prevalences were calculated by the direct standardization method. The 2003 survey population with diabetes was chosen as standard population.

Results: From 1987 to 2012 age-adjusted prevalence of obesity in participants with diabetes increased from 18.2% (95% Confidence interval [CI]: 14.2%-22.2%) to 39.8% (95% CI: 36.8%-42.8%). Age-adjusted prevalence of obesity in males with diabetes increased from 13.2% (95% CI: 7.3%-19.1%) to 38.0% (95% CI: 33.8%-42.1%) and in females with diabetes increased from 23.0% (95% CI: 17.6%-28.4%) to 42.3% (95% CI: 38.0%-46.6%). Obesity prevalence increased in participants with diabetes of both sexes across all age groups (16-39, 40-59 and >=60 years). Between 1987 and 2012 the proportion of participants with a high BMI increased (figure).

Conclusion: Between 1987 and 2012 the prevalence of obesity markedly increased in Spain among adults with diabetes.



Disclosure: **F. Basterra-Gortari:** None.

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Estimates of pre-diabetes and undiagnosed diabetes in Denmark

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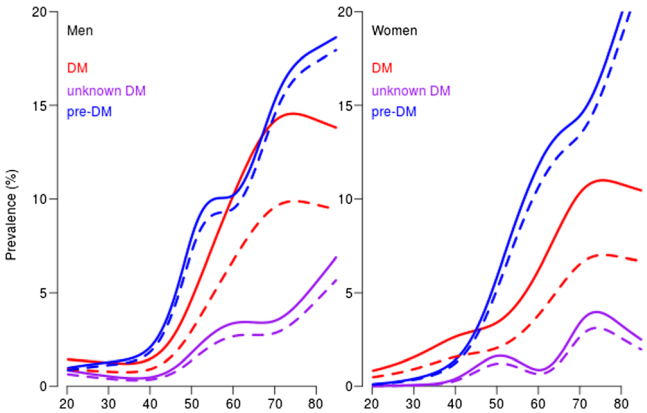
Clinical Epidemiology, Steno Diabetes Center Copenhagen, Gentofte, Denmark.

Background and aims: Up-to-date information on unknown diabetes (DM) and pre-diabetes based on current diagnostic criteria as well as estimates for the entire adult age span are lacking. The aim of the study was to model the number of individuals with unknown diabetes and pre-diabetes in Denmark based on existing population based cohorts, correcting for differential response rates between person with and without disease.

Materials and methods: Four population-based Danish studies after 2000 were identified where information on HbA1c, date of examination, gender, age (date of birth), and known diabetes was available. It is known that response rates in surveys are smaller from person with disease than for persons without, so prevalences from the surveys will underestimate the prevalence of DM and possibly also unkown DM and pre-diabetes. Within each study we estimated the age-specific prevalences of DM, unknown DM and pre-diabetes (survey prevalences), and from a National Diabetes Register we estimated the population level age-specific prevalences of known DM. We could then estimate the survey participation rate among DM patients, and when combining this with the known overall response rates from the studies we could correct the survey prevalences to credible population level figures.

Results: The prevalence of known-, previously unknown- and pre-diabetes was highest among men and increased with age with a peak at age 70 (see figure). The prevalence of undiagnosed diabetes is about half of that of diagnosed diabetes, and the prevalence of pre-diabetes is close to the prevalence of diabetes among men, but substantially higher among women.

Conclusion: The estimated numbers with undiagnosed diabetes and prediabetes are markedly lower than suggested by previous studies, but it is not clear whether this reflects a true fall in incidence or the change to HbA1c-based diagnostic criteria in 2011. Correction for non-differential survey participation is essential in studies of diabetes and precursors.



Supported by: Danish Diabetes Association

Disclosure: **M.E. Jørgensen:** Stock/Shareholding; Novo Nordisk.

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The dramatically high prevalence of diabetes and pre-diabetes in the adult Kuwaiti population

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Background and aims: Diabetes mellitus is one of the most important health burdens for the primary-health care system in Kuwait. The objective of this study was to determine the prevalence of diabetes and pre-diabetes in Kuwaiti adults in 2014.

Materials and methods: A total of 3918 randomly selected Kuwaiti men and women aged 18-69 years were surveyed in 2014 using the WHO Instrument for Chronic Disease Risk Factor Surveillance comprising demographics, medical and family history, physical measurements and blood biochemistry. Diabetes was defined as fasting plasma glucose ≥7 mmol/L, HbA1c ≥6.5% or being treated with glucose-lowering drugs. People with fasting plasma glucose between 6.1 and 6.9 mmol/L or HbA1c between 5.7-6.4% were considered as having pre-diabetes.

Results: In men, the prevalence of screen-detected diabetes was 9.4% and known diabetes 16.5%, giving a total diabetes prevalence of 26.0%. In women, the corresponding prevalence were 6.4%, 14.5% and 20.9%, respectively. The prevalence of pre-diabetes was 18.9% in men and 19.4% in women. The prevalence of diabetes increased with increasing age, BMI and waist circumference. Half of the adults in their fifties and almost 70% of adults in their sixties had diabetes. Almost 20% of overweight Kuwaiti men had diabetes and 23% had pre-diabetes. Over 51% of morbidly obese Kuwait men had diabetes. In overweight Kuwaiti women the prevalence of diabetes was 14.2% and pre-diabetes 17.6%, and almost half of morbidly obese Kuwaiti women had diabetes.

Conclusion: Our findings revealed that the prevalence of diabetes in Kuwait is higher than previously estimated. Since an oral glucose tolerance test was not

included in the survey, these percentages are likely underestimates. These figures illustrate the stark challenge facing Kuwait and other countries in the region and call for urgent action for detection and primary prevention.

Disclosure: **A. Alkandari:** None.

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Impact of weight changes on the incidence of diabetes: a Korean nationwide cohort study

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Background and aims: Obesity is a well-known risk factor for type 2 diabetes (T2DM), but few data exist on the association between weight changes and future diabetes risk in nonobese subjects. This study was aimed to investigate the effect of weight changes on the incidence of T2DM using a Korean prospective nationwide data.

Materials and methods: A total of 51405 nondiabetic subjects who had received health examinations in 2002 and completed follow-up in 2006 were included.

Results: Individuals who developed incident T2DM were more likely to be older and men; have higher values of body mass index (BMI), blood pressure, fasting plasma glucose, and total cholesterol; have a greater prevalence of current smokers, higher alcohol consumption, HTN, and hyperlipidemia; and have more often family history of DM, as compared to those without T2DM. Compared with the continuously nonobese group, there were a higher hazard ratio for incident DM (95% confidence interval) in "becoming obese" [1.50 (1.27-1.78)], "slimming down" [1.89 (1.61-2.22)], and "still obese" subjects [2.60 (2.56-3.05)] during 4 -years after adjustment for confounding factors. In the analysis stratified by BMI categories, risks for incident DM were significantly decreased according to the reduced BMI amounts and the trends were more evident in nonobese group than obese group. Regarding increased BMI, however, there was no significant association with incident DM.

Conclusion: Weight loss was significantly associated with decreased DM risk both in nonobese and obese Korean population. Further long-term studies are needed to establish weight reduction as preventive strategy for T2DM.

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Disclosure: **S. Moon:** None.

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Mortality and incidence of diabetes: retrospective cohort of hospitalised patients with stress hyperglycaemia

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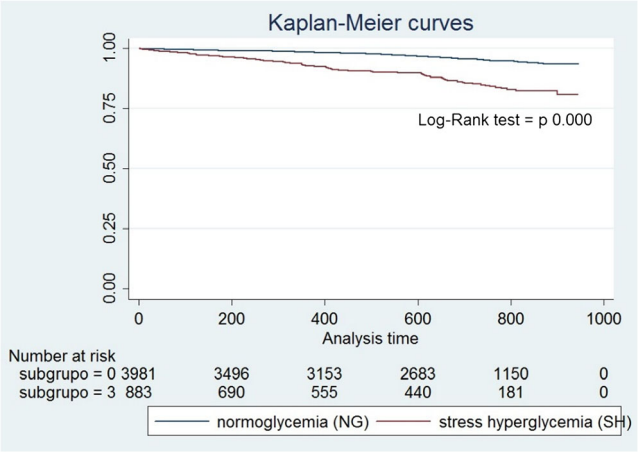
Background and aims: The aim of this study was to estimate mortality and calculate the incidence of diabetes in hospitalized patients with stress hyperglycemia (SH) versus normoglycemia (NG), during the follow up after discharge.

Materials and methods: A retrospective cohort was conducted with adult non-diabetic inpatients admitted from 2014 to 2015 in an argentine university hospital. We included patients with SH (>=140 mg/dl and HbA1c<6.5) or NG (all glycemia values <140 mg/dl) during hospitalization. Patients were followed up from discharge to the occurrence of diabetes, death, lost (disaffiliation) or administrative censored data. Diabetes was defined with at least one of the following criteria (the first date): new problem in ambulatory medical history, or new insulin/hypoglycaemic consumption from pharmacy registry, or HbA1c>=6.5%.We calculated the frequency, proportion, and incidence rates of new diagnosis of diabetes and mortality. Incidence rates are reported as crude cumulative incidence at one year with their respective 95% confidence intervals (CI). Kaplan Meier curves for both groups were performed and compared with log rank test. We used a Cox proportional hazard model to calculate hazard ratios (HR) and 95%CI.

Results: We identified 3981 patients with NG and 884 with SH. During the observation period, there were 84 cases of diabetes and 831 deaths. The median observation period was 669 days.Diabetes incidence was 10.97% (95%CI 8.98-13.22) in SH and 3.97% (95%CI 3.38-4.62) in NG, with p<0.001. The one year diabetes incidence was 7.39% (CI95% 5.7-9.56) in SH and 1.59% (95%CI 1.23-2.06) in NG. SH was significantly associated with diabetes incidence (crude HR 1.33, 95%CI1.03-1.73, p 0.025), even after adjustment for age and gender (adjusted HR 1.38; 95%CI 1.06-1.78; p 0.014).Mortality rate occurred 20.36% (95%CI 17.75-23.16) in SH and 16.35% (95%CI 15.21-17.53) in NG, with p 0.004.

Conclusion: Cumulative incidence of diabetes and mortality were higher in SH group. This information may help to patient-physician decision-making, and could be used to determine prospective strategies of follow up after discharge. Mortality could be a competitive event for presenting new diabetes. Is still pending treating death as a competing risk analysis.



Disclosure: **M.P. Russo:** None.

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New onset diabetes after kidney transplantation: incidence and associated factors

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Background and aims: New onset diabetes after transplantation (NODAT) is a frequent metabolic complication of kidney transplantation, associated with increased morbidity and mortality. Prior studies reported that approximately 15-30% of non-diabetic kidney transplant recipients develop NODAT in the first year after transplant. Multiple risk factors have been identified. These include older age, race, family history of diabetes, pre-transplant impaired fasting glucose (IFG) and impaired glucose tolerance, obesity, hepatitis C and cytomegalovirus infection, genetic factors, deceased donor, autosomal dominant polycystic kidney disease, acute rejection, as well as the type of immunosuppressive agents used to prevent or treat rejection. The aim of this study was to evaluate the incidence of NODAT and associated factors among kidney transplant recipients, in a central hospital and transplant center.

Materials and methods: Retrospective study of non-diabetic transplant recipients, who underwent kidney transplant between January of 2012 and March of 2016, with a minimum follow-up of 12 months. Patients were divided into two groups: with and without NODAT, for comparison.

Results: In total, 125 patients were eligible for the analysis. NODAT was identified in 27.2% of the patients (n=34; 53% female; mean age 49.6 ± 10.8 years). In the group that did not develop NODAT (n=91), 47% were female and the mean age was 46.0 ± 13.6 years**.** The mean time to diagnosis was 3.68 ± 5.7 months after transplantation and 76.5% of the patients developed NODAT in the first 3 months. All patients received immunosuppression

with corticosteroids, tacrolimus and mycophenolate mofetil. As inducing treatment, 6 patients received antithymocyte globuline (ATG) and 28 received basiliximab in the NODAT group, whereas 24 patients received ATG and 67 received basiliximab in the group without NODAT. In the NODAT group, the pre-transplant fasting plasma glucose levels were significantly higher ($p<0.05$) and the pre-transplant IFG was significantly more frequent (51.5% *versus* 27.7%, $p<0.05$). No differences were found between the two groups for other potential associated factors: age, race, body mass index, hepatitis C or cytomegalovirus infection, acute rejection and type of transplant (live or deceased donor).

Conclusion: The high incidence of NODAT observed was similar to that reported by others. NODAT was particularly frequent in the first 3 months after transplant. Pre-transplant IFG was significantly associated with NODAT, raising awareness to the need of periodical blood glucose screening. Identification of patients with IFG will allow early implementation of lifestyle modifications including weight control, diet and exercise in order to minimize future development of NODAT.

Disclosure: **V. Gomes:** None.

PS 014 Genetics of type 2 diabetes

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Parent-of-origin effects on gene expression in trios with type 2 diabetic offspring

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Background and aims: Type 2 diabetes (T2D) is seen more often in offspring of T2D mothers rather than of T2D fathers. Further, parental sex - specific effects on insulin concentrations have been reported, with lowest values seen in sons of diabetic mothers. Parent-of-origin effects (POE), wherein the phenotypic effect of an allele depends on whether it is inherited from the mother or the father, could explain these observations.

Materials and methods: We performed RNA sequencing of 80 independent trios enriched for type 2 diabetic offspring (77 offspring with T2D) and assessed for differences in correlation of expression between each parent-offspring pair on a background of parental expression correlations. Expression patterns of genes showing parent-of-origin effects in trios were studied in adult and fetal pancreas. Knockdown studies are ongoing to assess their influence on beta cell mass (proliferation) and function (insulin secretion).

Results: 4662 autosomal genes expressed in blood were assessed for parental biases in gene expression. 24 protein-coding genes showed parental biases in gene expression of which 6 genes showed the lowest paternal-maternal correlations including *BMP8A*, *CAMK2G*, *CLCF1*, *PA2G4*, *PSD4*, and *RPS23*. The Bone Morphogenic Protein 8A coding *BMP8A* showed significantly higher gene expression correlation between father-offspring (ρ_{BMP8A}=0.62, P_{BMP8A}=1.81x10^{-09}) than that of mother-offspring (ρ_{BMP8A}=0.11, P_{BMP8A} =3.42x10^{-01}, $Pdiff_{BMP8A}$=1.97x10^{-05}). These data were consistent even when assessed separately for sons and daughters. Differential expression analysis between genders was not significant either in blood or in pancreas ($p > 0.05$), showing clearly that these differences in correlations were not driven by gender. *BMP8A* showed significantly higher expression in the fetal pancreas whereas almost no expression was observed in adult pancreatic islets. The Beta-Actin encoding *ACTB* showed significantly higher father-offspring (ρ_{ACTB}=0.86, P_{ACTB}=3.42x10^{-23}) compared to mother-offspring (ρ_{ACTB}=0.60, P_{ACTB}=6.88x10^{-09}, $Pdiff_{ACTB}$=7.66x10^{-07}) . The only gene showing higher mother-offspring correlations was the cardiotrophin-like cytokine factor 1 coding *CLCF1* (ρ_{CLCF1}=0.23, P_{CLCF1}=4.24x10^{-02}, $Pdiff_{CLCF1}$ = 2.06x10^{-04}).

Conclusion: This study demonstrates that parental biases in gene expression exist beyond imprinted genes and can have strong spatial and temporal effects independent of gender. Some of these genes could have significant roles in fetal development.

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Disclosure: **R.B. Prasad:** None.

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Evaluation of the association of single nucleotide polymorphisms in the sodium glucose co-transporter 2 gene with glucose homeostasis and type 2 diabetes

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Background and aims: Sodium glucose co-transporter 2 (SGLT2) is the major transporter involved in glucose reabsorption in the kidney and mutations in the SGLT2 encoding gene SLC5A2 cause renal glucosuria. However, the

association between genetic variation in SLC5A2 and T2DM is still unclear and is addressed in the present study.

Materials and methods: We genotyped the SLC5A2 tagging SNPs rs9934336, rs3813008, and rs3116150 (completely capturing all common genetic variations in the gene locus of SLC5A2 according to HapMap SNP database, release 27) in 1684 coronary patients, including 400 patients with T2DM.

Results: The rare allele of rs9934336 was significantly associated with decreased fasting glucose and 2-hour glucose in oral glucose tolerance tests (p=0.041 and 0.035, respectively) as well as decreased HbA1c and estimated average fasting glucose (p=0.033 and 0.026, respectively). Further, variant rs9934336 was significantly associated with the presence of T2DM, in univariate (OR=0.82 [0.68-0.99]; p=0.037) as well in multivariate regression analysis adjusting for age, sex, body mass index, metabolic syndrome, hypertension, and estimated glomerular filtration rate (OR=0.79 [0.64-0.95]; p=0.019). However, rs9934336 was not associated with serum insulin, or with the homeostatic model assessment indices of insulin resistance and beta cell function, respectively. Polymorphisms rs3813008 and rs3116150 were neither associated with fasting or postchallenge glucose nor with T2DM.

Conclusion: We conclude that genetic variation within the SLC5A2 gene locus contributes to fasting as well as postchallenge plasma glucose and to the manifestation of T2DM.

Disclosure: **A. Muendlein:** None.

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PPARG Pro12Ala variant in relation to adipose tissue metabolism and differentiation: a small genotype-based recall study

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Background and aims: The minor (Ala) allele of the common Pro12Ala polymorphism in the peroxisome proliferator-activated receptor (PPARG) gene affects transcriptional activity of isoform 2 of the protein and has been associated with reduced risk of type 2 diabetes. Protective effects of this polymorphism have been considered to be mediated by effects on adipose tissue development and metabolism. We aimed to study the effect of the Pro12Ala variant on adipogenesis and metabolic activities and expression of PPARG target genes in adipose tissue.

Materials and methods: Using the genotype-based recall approach we recruited Pro12Pro (4M/8F, mean age 64 ±SD 9y), Pro12Ala (6M/9F, 63±9y) and Ala12Ala (4M/9F, 64±8y) carriers without diabetes to participate in a detailed clinical investigation. It included BMI, body fat %, waist circumference; fasting and post-oral glucose tolerance test (OGTT) glucose, insulin, non-esterified fatty acids and glycerol concentrations and HbA1c, C-peptide, HOMA-IR, and lipids. In addition, subcutaneous adipose tissue was obtained by a needle biopsy before and after OGTT to study the expression of PPARG target genes, glucose uptake and lipolysis regulation in isolated adipocytes. Furthermore, the stromal vascular fraction was collected to study preadipocyte differentiation (interim data, n=4+4 for Pro12Pro and Ala12Ala).

Results: No major differences were seen in any of the anthropometric or metabolic variables assessed between three groups Pro12Pro, Pro12Ala and Ala12Ala, respectively, for BMI (kg/m2), 26.8±3.3, 24.3±3.2 and 26.6±3.6, nor HbA1c (mmol/mol), 35.3±3, 34.7±3.2 and 35.0±3.6. Upon fasting overnight and post-OGTT, only a trend of an increased basal and insulin-stimulated glucose uptake in isolated adipocytes was observed in the Ala12Ala participants compared to other two groups (p=NS). Similarly, neither basal nor isoproterenol-stimulated or antilipolytic effect of insulin was altered in adipocytes on fasting state between the three groups. Post-OGTT, the antilipolytic effect of insulin was higher in the Pro12Pro participants compared to Pro12Ala (p<0.05), but not with Ala12Ala. The expression of PPARG target genes involved in adipogenesis (CD36, CEBPα, PPARG, FABP4, and ADIPOQ), glucose (GLUT4, IRS2) and lipid metabolism (LPL and FAS) remained unchanged in adipose tissue obtained before as well as after OGTT. The mean adipocyte diameter was also similar between all groups. Lastly, the preliminary analyses of preadipocyte differentiation rate assessed by percent of differentiated cells and expression of adipogenic marker genes (PPARG, CEBPα, ADIPOQ) did show any difference between Pro12Pro and Ala12Ala groups.

Conclusion: We could not show any major differences in adipocyte development, metabolism, or gene expression in adipose tissue between carriers and non-carriers of the PPARG Pro12Ala allele. Potential explanations to our findings include lack of power to detect small effects of this variant, or that the variant acts via mechanisms other than those assessed, including effects in other tissues.

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Disclosure: **P.G. Kamble:** None.

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The influence of dopamine-beta-hydroxylase and catechol o-methyltransferase gene polymorphisms on the efficacy of insulin detemir in patients with type 2 diabetes

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Background and aims: Neurotransmitters, such as dopamine, gamma-aminobutyric acid, serotonin and norepinephrine, are involved in the central nervous system's regulation of energy and glucose homeostasis and in food intake regulation. Dopamine-beta-hydroxylase (DBH) is an enzyme that catalyzes the conversion of dopamine to norepinephrine within norepinephrine and epinephrine producing neurons and neurosecretory cells. Plasma DBH activity is under the genetic control of a single-nucleotide polymorphism (SNP), DBH -1021C/T, in the 5' flanking region of the DBH gene. Catechol O-methyltransferase (COMT) is one of the major enzymes involved in catecholamine and estrogen degradation. Changes in the COMT activity, associated with genetic variants in the COMT gene have consequences in various mechanisms related to development of obesity, personality changes and behavior disturbances. The aim of the study was to investigate the association of COMT Val108/158Met and DBH-1021C/T polymorphisms with effectiveness of insulin detemir in achieving glucose control and body weight control.

Materials and methods: Observational study included 341 subjects, 185 T2DM patients inadequately controlled with premix insulin analogues, which were replaced with three doses of insulin aspart at mealtime and insulin detemir at bedtime that were followed for 52 weeks, and 156 healthy controls. After DNA isolation from blood samples, genotyping of DBH-1021C/T polymorphism (rs1611115) and COMT Val108/158Met polymorphism (rs4680) was performed.

Results: The mean age of T2DM patients was 67.1±8.01 years (mean age of healthy controls was 44.1±11.6 years), mean duration of T2DM was 16.1±5.9 years. HbA1c and fasting plasma glucose were significantly decreased after 52 weeks (8.58% vs. 7.78%, 11.7mmol/l vs. 8.7mmol/l, respectively, p< 0.001). Insulin detemir had a significant weight sparing effect in overweight patients. COMT A carriers (the combined AG and AA genotype) achieved significantly better HbA1c values after the 52-week treatment compared to patients carrying the GG genotype (7,7% vs. 8,0%, p=0.029, Mann Whitney test). This difference was not gender dependent. The group of patients who lost at least 1 kg of weight showed the most prominent difference in achieved HbA1c values between the COMT genotypes (A carriers: 7.55% vs. GG genotype: 8.1%, p=0.022, Mann Whitney test). Among the patients who had HbA1c decrease over 1% and achieved HbA1c<7%, the GG genotype of the COMT was less frequently present when compared to the patients with higher levels of HbA1c (p<0.005, Chi square). DBH-1021C/T genotypes were not significantly associated with any of the measured variables.

Conclusion: Presence of one or two A allele of the COMT Val108/158Met was associated with improved glycemic response, and with a better response to insulin detemir therapy in T2DM patients.

Clinical Trial Registration Number: 9386

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Disclosure: **T. Bozek:** None.

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Clinical phenotyping of carriers of the R138X mutation in the SLC30A8 gene

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Background and aims: A Loss-of-Function mutation (R138X, rs200185429) in the *SLC30A8* gene encoding the Zinc Transporter 8 (ZnT8) was shown to be associated with a 53% reduction in risk of Type 2 Diabetes (T2D) by Flannick in 2015, but the mechanisms have remained unclear. We, therefore, performed clinical and metabolic phenotyping of additional family members with the mutation by taking advantage of the enrichment of this mutation (0.22%) in a small Botnia region, as compared with the rest of Europe (0.01%).

Materials and methods: Altogether 53 (25 women) R138X carriers (LoF) and 47 (23 women) non-carriers (Contr) from the same families underwent a 190 min mixed meal test (75g glucose, 17g protein, 15g fat) with blood samples drawn at 0, 20, 40, 70, 100, 130, 160 and 190 min for the determination of glucose, insulin, c-peptide, glucagon and zinc (Zn) concentrations.

Results: There were no differences in mean age (LoF: 50.5 ± 2.1vs. Contr: 53.4 ± 2.2 years, p 0.34), BMI (27.4 ± 0.6 vs. 26.1 ± 0.4 kg·m^{-}2, p 0.09) or waist circumference (men: 91.8 ± 1.7 vs. 93.7 ± 1.3 cm, p 0.39 and women: 91.6 ± 2.6 vs. 87.3 ± 1.9 cm, p 0.20); neither were there differences in fasting glucose, HbA1c, insulin, c-peptide and glucagon concentrations, respectively. No differences were observed in stimulated glucose, insulin, c-peptide, glucagon/insulin AUC nor in early or late phase insulin secretion. Given the suggestion by Tamaki in 2013, we also estimated hepatic insulin clearance during test meal, but observed no differences between LoF and Control. Interestingly, among LoF carriers, Zn was higher both at fasting (14.7 ± 0.3 vs. 13.3 ± 0.3 μmol·L^{-1}, p 0.004) and during test meal (2500 ± 59 vs. 2333 ± 56 μmol·min·L^{-1}, p 0.044, respectively), the difference being more pronounced among men. We also explored the distribution of genotypes of the T2D-associated common arg325trp -variant (rs13266634) in the SLC30A8 gene and observed that LoF carriers lacked the TT genotype (CC 70% vs. 36%, CT 30% vs. 30% and TT 0% vs. 34%).

Conclusion: Comprehensive phenotyping of carriers with LoF mutation in the *SLC30A8* gene (R138X) did not provide any clear explanation for the protective effect of this genotype from T2D. However, mutation carriers had higher plasma zinc concentrations. Carriers also lacked the homozygous protective common variant genotype TT.

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Disclosure: **M. Lehtovirta:** None.

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Estimation of heritability for diabetic complications based on genome-wide association study in Chinese population

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Background and aims: Genome-wide association studies (GWASs) have successfully identified a number of common single-nucleotide polymorphisms (SNPs) associated with diabetic complications or associated traits. However, genetic variants identified by GWAS explain only a small proportion of the expected narrow-sense heritability (h^2) defined as the ratio of additive effects of genotyped SNPs to phenotypic variance. In this study, we aim to estimate the narrow-sense heritability for diabetes complications and associated traits utilizing GWAS data from the Hong Kong Diabetes Registry (HKDR).

Materials and methods: The Hong Kong Diabetes Registry includes prospective follow-up of more than 8,000 patients with type 2 diabetes (T2D). Samples were genotyped using the Illumina Omni 2.5+ exome array. After standard quality control, ~1.2 million common SNPs were included in the final analysis. A recently developed method, genome-wide complex trait analysis (GCTA; v1.26.0), was used to estimate the heritability for different diabetes-related traits and diabetic complications based on the common SNPs. Phenotypes were adjusted for age, gender, top 20 principle components and use of medications at baseline. We also evaluated the genetic correlation between cardiometabolic traits and diabetic complications.

Results: After sample quality control, we included 5,742 unrelated Chinese subjects with T2D in this analysis (mean age of all subjects 57.5 ± 13 years, 45.5% male, median duration of diabetes 6 [IQR: 2-11] years at baseline). In the HKDR, the heritability h^2 accounted by common SNPs was 0.17 (SE=0.067) for eGFR, 0.07 (SE=0.07) for urine albumin/creatinine ratio (UACR), 0.36 (SE=0.072) for HDL cholesterol, 0.12 (SE=0.068) for LDL cholesterol, 0.24 (SE=0.07) for triglyceride, 0.28 (SE=0.071) for the ratio of total cholesterol and HDL. For binary outcomes, heritability on the liability threshold scale was 0.15 (SE=0.104) for chronic kidney disease (CKD), 0.23 (SE=0.149) for end-stage kidney disease (ESRD), 0.31 (SE=0.155) and 0.24 (SE=0.116) for neuropathy and retinopathy, respectively. The estimated genetic correlations between most pairs of traits were modest, except the correlation exhibited between HDL and UACR (ρ=-0.74, SE=0.456, P=0.009).

Conclusion: Our results provide novel insights on the genetic architecture of diabetic complications in Chinese. Our observed heritability is broadly similar to that recently reported for DKD in type 1 DM. Analysis are ongoing to identify the genetic overlap between the different complications in T2D.

Supported by: RGC Scheme, Focused Innovations Scheme, Faculty Postdoctoral Fellowship

Disclosure: **G. Jiang:** None.

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Identification of susceptibility loci for diabetes-related traits in a mouse model for the metabolic syndrome

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Background and aims: Inbred strains of mice can be used as model system for human metabolic diseases. New Zealand obese (NZO) mice present the metabolic syndrome with symptoms of obesity, hyperglycemia, hypercholesterolemia and hypertension and are considered as a polygenic model for obesity and type 2 diabetes (T2D). Until today, the genetic variants that predispose NZO mice for diabetes have not been elucidated. The aim of this study is to identify novel susceptibility loci for obesity and diabetes by combine gene-driven and phenotype- approaches. We crossbred obese diabetes-prone NZO with lean and diabetes-resistant 129P2 mice and performed QTL and expression analysis.

Materials and methods: Animals of the backcross population (300 females and 300 males) were phenotyped and genotyped by using a genome-wide high-density SNP panel. All mice received a high fat diet (45 % fat/calories) after weaning. After 21 weeks of age, mice were sacrificed and tissues as well as plasma were harvested. The calculation of phenotype-genotype associations was performed by QTL analysis. Expression levels were analysed using qRT-PCR and Microarray approaches.

Results: By linkage analysis four QTL for metabolic traits were identified on chromosome 4. A major QTL for blood glucose levels (Logarithm of the odds (LOD) 7.1) was detected at 35.4 cM. Heterozygous mice (NZO/129P2) showed higher levels of blood glucose, liver weight, final blood glucose and final plasma insulin compared to homozygous allele carriers (NZO/NZO). The T2D prevalence was nearly 20 % higher in NZO/129P2 compared to NZO/NZO mice. Expression analysis revealed candidate genes in adipose tissues

(brown adipose tissue and gonadal white adipose tissue) as well as skeletal muscle and liver tissue.

Conclusion: We identified novel susceptibility loci associated with T2D on mouse chromosome 4. Furthermore, we found several candidate genes carrying strain-specific variants and which were associated with the diabetogenic phenotype of the NZO/129P2 population. In the future, investigation of these candidates will be performed by functional studies in vitro and in vivo. Additionally, generation of recombinant congenic mice (RCS) is in process to narrow down the identified QTL region.

Supported by: German Center for Diabetes research (DZD)

Disclosure: **S. Lebek:** None.

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Deep re-sequencing of 9 type 2 diabetes GWAS loci by comparison of extremes of dynamic indices of insulin secrection

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Background and aims: The susceptibility genes for Type 2 Diabetes (T2D) identified so far are mainly involved in beta-cell function. To this point, Genome-Wide Association Studies (GWAS) have identified a large number of *loci.* Despite this large number, the major part of T2D inheritance is still uncovered. This missing heritability might be explained by multiple, low-frequency variants that are not captured by GWAS. A powerful approach to highlight causal variants is to deep re-sequence candidate genes. To enhance the probability to highlight causal or protective variants together with the appropriate statistical power, we aimed to identify by Next Generation Sequencing (NGS) low-frequency variants in GWAS *loci* for T2D, searching for primary defects in beta-cell insulin secretion. For this, we applied a two stage study design: **Stage 1**, deep re-sequencing of coding and flanking regions of 9 candidate genes that reach GWAS significance ($p<10^{-8}$), in individuals selected from the extremes of insulin secretion, adjusted for insulin resistance, i.e. Disposition Index (DI); **Stage 2**, confirmation of the association by genotyping the variants differently distributed between the two extremes of insulin secretion in larger and independent groups of Italian adults and children (N=3130).

Materials and methods: In a large population, very well characterized from OGTT, measures of insulin secretion and resistance have been calculated, including insulinogenic index (IGI30), ISI (insulin-sensitivity index), and DI (IGI30xISI). NGS was performed on MiSeq system (Illumina) with TruSeq Custom Amplicon approach. Variants are investigated by proper bioinformatics tools. Discovered variants will be genotyped by Real-Time PCR or by suitable methods such as SNP array and genotyping by sequencing.

Results: We sequenced 383 subjects from the discovery sample. Preliminary results show more than 1500 variants in this sample. Bioinformatics tools predict that at least 122 of them may have a functional effect on protein, being missense or nonsense. A small but relevant part of passing-filter variants seems to be newly discovered (no rs) or to presumptively affect protein function. We then searched for a different distribution of all the infrequent variants within the two extremes of DI. Variants in one of the genes, ADAMTS9, were significantly associated with the higher extreme (>80%) of DI distribution (OR= 1.30 p=0.03). A similar trend was observed for four of the 9 candidate genes. Multivariate analyses showed that carriers of one o more variants have an OR= 9.06 (1.73-47.42) p=0.009 and OR= 8.52 (1.32-55.01) p=0.024 respectively, to be in the >80% extreme of DI.

Conclusion: The next stage involves the in-depth analysis of all the variants discovered, evaluating distribution, frequency, and possible function, together with the replication studies in large cohorts to confirm the association with altered insulin secretion. We expect that this study will deliver several results: from confirmatory gene association to newer T2D associated polymorphisms, to possible new insights into potential biological mechanisms influencing T2D pathogenesis.

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Genetic variability in eIF2α gene can increase the risk for glucose disorder with aging or higher BMI in a Chinese Han population

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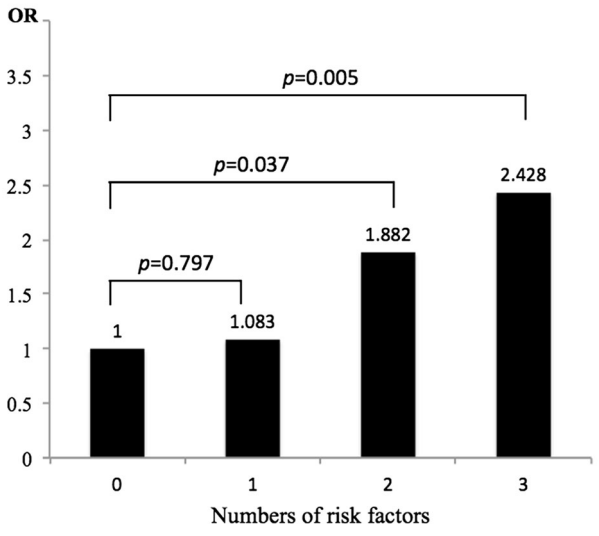
Background and aims: The increasing evidence suggested that unfolded protein response plays an important role in chronic metabolism disease such as type 2 diabetes. Accumulation of unfolded protein in the endoplasmic reticulum (ER) retards mRNA translation through activation of the protein kinase PERK and eukaryotic translation initiation factor 2 alpha (eIF2α). Some studies had demonstrated that genetic variation of the PKR- like ER kinase (PERK)/ eIF2α pathway leads to β-cell function failure. The aims of this article was to assess the possible association between the eIF2α gene and the risk of glucose metabolism abnormality.

Materials and methods: We selected 2 SNPs (rs9840992 T>C and rs13072593 A>G) at eIF2α locus according to CHB database from HapMap R#27 (r2<0.8 and MAF≥0.05) and genotyped 1,466 unrelated non-diabetic individuals screened by 75g-OGTT (845 prediabetic subjects and 621 normal controls) using mass spectrometry assay. Out of all subjects, 733 did insulin release test following 75g-OGTT. We evaluated various indicators of insulin resistance and islet β-cell function. All statistical analyses were assessed using the SPSS statistical package (version 13.0; SPSS, Chicago, IL) and Haploview 4.1(http:// www.broadinstitute.org/haploview/haploview).

Results: The carriers of genotype CC at rs9840992 had a higher insulin level at 120min after 75g glucose load compared to the non-carriers [49.86(30.38-79.61) vs 44.32(26.42-70.75) mU/L, p=0.038]. We also found that homozygotes of CC had higher ΔI30/ΔG30 [10.82(6.25-19.35) vs 8.97(5.35-16.49), p=0.008] and ΔI120/ΔG120 [23.02(13.47-43.21) vs 19.38(10.45-36.57), p=0.017] compared to non-carriers, and the differences were still significant after adjusted for insulin resistance (p'=0.004 and p'=0.006, respectively). Homozygotes of CC had greater AUCi measurements compared to non-carriers (p=0.023, p'=3.7×10^{-4} adjusted for insulin resistance). When age ≥ 65yr or BMI≥24kg/m^2, or carrying the risk allele T of SNP rs9840992 was taken as a risk factor of prediabetes respectively, the cumulative risk analysis showed that the more factors the individuals carried, the higher risk they were at for prediabetes (p=0.000). The subjects who carried all the 3 risk factors had a risk of about 2.5 folds as high as those who carried none of the given three factors (OR=2.428, p=0.005) for glucose metabolism abnormality(Figure 1).

Conclusion: Genetic variability at eIF2α locus may increase the risk for glucose disorder with aging or higher BMI in the Chinese population.

Figure 1. Numbers of risk factors and the relative risk of prediabetes



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Genetic control of glucose tolerance and insulin secretion by CD26/DPP4 is abrogated in prediabetes

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Background and aims: CD26/DPP4 enzymatic activity targets the gastrointestinal hormones, glucagon-like polypeptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) possibly counteracting their incretinic effect. Nevertheless, it is unclear the impact of CD26/DPP4 gene has a measurable action in glucose excursion and insulin secretion modulating thereby glucose metabolism. We have taken the approach of analysing the action of CD26/DPP4 on metabolic traits both in normoglycemic and prediabetes individuals using a human cohort and analysing a mutant mouse model.

Materials and methods: The PREVDIAB2 cohort was collected in 2014 and comprises 1,088 individuals from the Portuguese population that underwent OGTT analyzed at 0, 30 and 120min after the glucose challenge. We have genotyped this collection for 27 SNPs in the CD26 genomic region and preformed genetic association tests against glucose and C-peptide measurements taken in the course of the OGTT. We also analyzed glucose excursions and the C-peptide levels in the CD26 knockout mice upon OGTT as compared to wild-type mice.

Results: We found that in normoglycemic individuals, several CD26 SNPs were highly associated to measurements of glycemia excursion (highest association, $P=9.28X10^{-7}$) and C-peptide levels (highest association, $P=5.31X10^{-5}$) across the OGTT. This indicates that the CD26/DPP4 gene controls glucose metabolism efficiency in the post-prandial state. Strikingly, this control was absent in subjects with prediabetes. In accordance, serum CD26/DPP4 activity was increased in prediabetic individuals. Moreover, we found that CD26KO animals revealed a lower glucose excursion and increased insulin secretion in response to OGTT, reinforcing the notion that the CD26 gene has a measurable effect in glucose metabolism.

Conclusion: Together these results revealed that the CD26 gene region is controlling glucose metabolism through regulation of incretin effects under normoglycemia. Nevertheless, prediabetes states appear to override these mechanisms of metabolic control, possibly due to an increased expression of CD26/DPP4 activity in subjects with prediabetes. We hypothesize that in prediabetes, CD26 expression may be under epigenetic effects that may have an impact on therapies based in DPP4 inhibitors.

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Disclosure: **R.S. Patarrão:** None.

PS 015 Beta cell signal transduction II

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Key role of beta-arrestin 1 in preserving the function and mass of pancreatic beta cells in vivo

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Background and aims: Pancreatic beta cell function and mass alterations play a central role in the pathogenesis of type 2 diabetes (T2D). Our previous work suggests that beta-arrestin 1 (ARRB1), an ubiquitously expressed scaffold protein, plays a role in insulin secretion and beta cell survival. To characterize the precise role of ARRB1 in beta cells, we analyzed the physiological effects of conditional invalidation of Arrb1 specifically in beta cells (βArrb1KO mice).

Materials and methods: Mice invalidated for Arrb1 specifically in beta cells (βArrb1KO) were generated using the Cre-loxP system under the control of the Ins1 promoter. Male mice βArrb1KO and βArrb1WT (controls) were fed with standard or high-fat diet (HFD) for 11 weeks.

Results: Under standard diet, βArrb1KO mice displayed similar weight gain, fasting and postprandial glucose levels as well as glucose tolerance compared to βArrb1WT mice. In contrast, insulin secretion in response to glucose was altered both *in vivo* ($p<0.05$) and *in vitro* ($p<0.01$). In addition, morphometric analysis of pancreatic histological sections from βArrb1KO mice indicated a significant reduction in islet mass ($p<0.05$), with a population significantly enriched in small islets but depleted in large islets. On the other hand, under HFD, βArrb1KO mice displayed a comparable overweight to βArrb1WT mice but deficient compensatory hyperinsulinemia ($p<0.05$) and aggravated oral glucose intolerance ($p<0.05$). In addition, βArrb1KO mice did not show compensatory adaptation in islet mass unlike βArrb1WT mice. Interestingly, ARRB1 protein expression was increased in islets from prediabetic db/db mice. Conversely, ARRB1 protein expression was reduced in islets from mice under insulin resistance conditions (HFD, aged C57BL/6J, diabetic db/db mice).

Conclusion: Our results demonstrate the critical role of ARRB1 in the function, maintenance and plasticity of beta cell mass *in vivo*. A lack of ARRB1 protein expression in the states of insulin resistance could lead to defective compensatory increase in beta cell functional mass leading to T2D.

Disclosure: **J. Obeid:** None.

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How does MICU2 regulate stimulus secretion coupling in pancreatic beta cells?

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Background and aims: MICU2 and its homologue MICU1 heterodimerize and act as molecular gatekeepers of the mitochondrial calcium uniporter supercomplex (mtCUC). Although, MICU1 has been implicated in stimulus-secretion coupling, the role of MICU2 in pancreatic β-cell function is unclear. In this project, we specifically aimed to: Determine functional and bioenergetic consequences of MICU2 perturbation in rodent INS-1 832/13 and human EndoC-βH1 cell lines.

Materials and methods: Standard assays of insulin secretion, mitochondrial respiration measurement and live cell imaging tools and techniques were used in this study.

Results: Our experiments revealed that both the basal 2.8 mM and 16.7 mM glucose stimulated mitochondrial calcium ($[Ca^{2+}]_{mito}$) elevations were reduced by 33% (n=36 cells, $p<0.0001$) and 51% (n=36 cells, $p<0.0001$) respectively in INS-1 832/13 cells. Similar to our data in INS-1 832/13 cells, we obtained a 52% (n=23 cells, $p=0.0169$) and 62% (n=23 cells, $p=0.0066$) reduction in basal 2.8 mM and 20 mM glucose stimulated $[Ca^{2+}]_{mito}$ elevation in

EndoC-βH1 cells. Furthermore, Micu2 knock down hyperpolarized the inner mitochondrial membrane 50% (n=164 cells, p=0.0001) less than control INS-1 832/13 cells. Moreover, basal 2.8 mM glucose and 16.7 mM glucose stimulated ATP/ADP ratio rises were diminished by 20% (n=20 cells, p<0.0001) and 92% (n=20 cells, p<0.0001), respectively, in INS-1 832/13 cells. In contrast, in EndoC-βH1 cells, 2.8 mM glucose and 20 mM glucose stimulated ATP/ADP ratio elevation declined by 24% (n=21 cells, p=0.018) and 50% (n=21 cells, p=0.0078), respectively. In addition, 2.8 mM glucose and 16.7 mM glucose stimulated insulin secretion were diminished by 40% (n=3, p<0.0001) and 38% (n=3, p=0.0026), respectively, in INS-1 832/13 cells. Furthermore, fold change in insulin secretion from 2.8 mM to 16.7 mM glucose was diminished by 53% (n=3, p=0.0018) in EndoC-βH1 cells upon Micu2 knock down. $[Ca^{2+}]_{mito}$, and cytosolic calcium ($[Ca^{2+}]_c$) peaks were abrogated by 59% (n=109, p<0.0001) and 68.9% (n=217, p<0.0001) respectively upon 36 mM K^+ stimulation in INS-1 832/13 cells. Moreover, subplasmalemmal calcium ($[Ca^{2+}]_{mem}$) upon 36 mM K^+ stimulation was enhanced by 56.5% (n=64, p<0.0001) in Micu2 knock down INS-1 832/13 cells.

Conclusion: MICU2-associated $[Ca^{2+}]_{mito}$ mtCUC might be locally interacting and maintaining the plasma membrane Ca^{2+} channels desensitized in pancreatic β-cells. The perturbation of MICU2 might lead to dysfunctional $[Ca^{2+}]_c$ redistribution, disrupting metabolic control of insulin secretion in pancreatic β-cells.

Disclosure: **N.V. Vishnu:** None.

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Abn-CBD stimulates insulin secretion and promotes beta cell proliferation in human and mouse islets through GPR55-independent signalling

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Background and aims: GPR55 belongs to the highly specialised G-protein coupled receptor family. We and others have reported that its activation increases insulin secretion. The aims of the current study were to investigate the effects on insulin secretion and proliferation of Abn-CBD, an apparently selective GPR55 agonist, and to gain new insights into the signalling pathways operating downstream of Abn-CBD in human (H) and mouse (M) islets.

Materials and methods: Islets isolated from WT C57BL/6J and GPR55KO mice and human organ donors were perifused in the absence or presence of Abn-CBD, and insulin secretion and $[Ca^{2+}]$i were quantified by RIA and microfluorimetry respectively. The effect of Abn-CBD on beta cell proliferation (BrdU incorporation) in GPR55WT and KO M islets was evaluated over 5 days via fluorescence microscopy. PhosphoAKT and CREB, and total AKT and CREB expression were also evaluated by western blotting in H and M islets using Abn-CBD.

Results: 1μM Abn-CBD potentiated glucose-induced insulin secretion in WT and GPR55KO mice islets (WT: 9±1.5 pg/islet/min maximum increase (MI) above 20mM glucose response (20GR); KO: 9±1.2) and the effect of 10μM Abn-CBD was significantly increased in islets from GPR55KO mice (WT: 3±0.4 pg/islet/min MI above 20GR; KO: 13±0.5; n=3-4, p<0.0001). These results on insulin secretion were in agreement with the changes in $[Ca^{2+}]$i after treatment with 1 and 10μM Abn-CBD in WT and GPR55KO islets (WT, 1μM Abn-CBD: 0.02±0.01 fluorescence 340/380, MI above 20GR, 10μM Abn-CBD: 0.02±0.01; KO, 1μM Abn-CBD: 0.02±0.01, 10μM Abn-CBD 0.03 ±0.01). In addition, Abn-CBD stimulated concentration-dependent potentiation of glucose-induced insulin secretion from H islets (vehicle: 18±0.4 pg/islet/min, 0.1μM Abn-CBD: 48±0.4, 1μM Abn-CBD: 60 ±1.4, 10μM Abn-CBD: 83±1.1; n=4, p<0.0001). Similar stimulatory effects of Abn-CBD on $[Ca^{2+}]$i were observed in H islets (0.1μM Abn-CBD: 0.03±0.01 fluorescence 340/380, MI above 20GR, 1μM Abn-CBD: 0.04±0.02). Abn-CBD also significantly increased beta cell proliferation of islets isolated from WT mice (vehicle: 0.8±0.1 BrdU+ insulin cells, 1μM Abn-CBD: 1.6±0.3, p<0.0001, n=60) and it increased islet area (vehicle: 8,000μm^2, 1μM Abn-CBD: 10,500, p<0.01, n=60). Islets from GPR55KO mice showed significantly reduced basal BrdU incorporation compared to WT islets (0.2±0.03 *vs.* 0.8±0.07 BrdU+ insulin cells, p<0.001), but Abn-CBD was able to promote beta cell proliferation in GPR55KO mice islets (0.9±0.03 BrdU+ insulin cells, p<0.0001). 10μM Abn-CBD increased CREB phosphorylation by 30% in M islets and AKT phosphorylation by 20% in H islets.

Conclusion: Our results indicate that Abn-CBD increases insulin secretion and $[Ca^{2+}]$i in M and H islets. It also induced increases in CREB and AKT phosphorylation, in beta cell proliferation and in enhancing islet area. The data obtained with islets isolated from GPR55KO mice indicate that Abn-CBD exerts these stimulatory effects in a GPR55 independent manner. A broader knowledge of the novel mechanisms of action of Abn-CBD might offer new approaches to treat type 2 diabetes.

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Disclosure: **I. Ruz-Maldonado:** None.

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Extracellular nicotinamide phosphoribosyltransferase (eNAMPT) plays a dose- and time- dependent role in pancreatic beta cell function

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Background and aims: Serum levels of nicotinamide phosphoribo syltransferase (eNAMPT; visfatin/PBEF) are elevated in patients with Type 2 diabetes (T2D). However, the role of eNAMPT in pathophysiology remains unclear. Here we examined acute and chronic dose-dependent effects of eNAMPT on pancreatic islet function.

Materials and methods: Islets from mice were isolated and treated with recombinant eNAMPT for 5 minutes-1 hour and 24-72 hours, at concentrations of 0.1-10ng/ml. Glucose-stimulated insulin secretion (GSIS) was measured by radioimmunoassay. Intracellular calcium ($[Ca^{2+}]_{cyt}$) was measured in MIN6 cells using Fura-2 and wide-field microscopy. Apoptotic activity was measured using Caspase-Glo 3/7 assay and cellular NAD/NADH by colorimetric assay. Gene expression was assessed by qPCR and proliferation by BrdU staining.

Results: Insulin secretion in response to elevated glucose (2 vs 20mM) was largely unchanged after less than 24 hours of eNAMPT exposure except for when treated with 0.1ng/ml eNAMPT for 30 minutes (no treatment: 0.474± 0.045ng/ml vs 0.1ng/ml: 1.157 ± 0.195ng/ml, n=8, 4 size matched islets/well, P<0.05). However, GSIS was increased after 24-72 hours treatment with 1ng/ml eNAMPT (no treatment: 1.948 ± 0.313ng/ml vs 1ng/ml: 4.600 ± 0.816ng/ml at 48 hours, n=8, P<0.05) via NAD-dependent effects. In contrast, islets treated with higher concentrations of eNAMPT (P<5ng/ml) showed NAD-independent impaired levels of GSIS (5ng/ml: 1.347 ± 0.120ng/ml, NS) and reduced intracellular $[Ca^{2+}]_{cyt}$ (no treatment: 111.7 ± 0.921% vs 5ng/ml: 106.5 ± 0.595%, n=22, P<0.05) after 48 hours treatment. Gene expression analysis revealed that exposure to eNAMPT (P<5 ng/ml) led to reduced expression of *Pdx1* (no treatment: 1.013 ± 0.096 vs 5 ng/ml: 0.636 ± 0.067 normalised to *Gapdh*, NS) and to an enhanced inflammatory response (increased mRNA levels of *Mcp1*: no treatment: 1.075 ± 0.196 vs 5ng/ml: 5.482 ± 1.258, P<0.001; *Il1β*: no treatment: 1.138 ± 0.291 vs 5ng/ml: 5.459 ± 2.002, P<0.05; *Tnfα*: no treatment: 1.258 ± 0.553 vs 5ng/ml: 2.503 ± 0.902, NS, all normalised to *Gapdh,* n=5). Correspondingly, apoptosis is enhanced in islets treated with high concentrations of eNAMPT in the presence of cytokines (no treatment: 103492 ± 11398 vs 5ng/ml: 169693 ± 20836 luminescence (RLU), n=8, 6 size matched islets/well, P<0.05). Numbers of proliferating beta cells are unchanged after eNAMPT treatment (n=4).

Conclusion: Chronic eNAMPT exposure at levels similar to those observed in T2D serum is detrimental to normal beta cell function. However, moderate eNAMPT exposure at levels similar to those observed in non-diabetic serum promotes insulin secretion. This data begins to clarify previous conflicting findings regarding eNAMPT in T2D. Additional mechanisms of eNAMPT action are being investigated including the role of monomeric eNAMPT in beta cell function as well as treating with eNAMPT *in vivo*.

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Disclosure: **S. Sayers:** None.

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Using recombinase-mediated cassette exchange to enginner MIN6 insulin secreting cells with a tetracycline-regulated expression system

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Background and aims: It has been widely accepted that pancreatic β-cell impairment, whether in terms of function, mass or both, is of central importance for the development not only of type 1 but also type 2 diabetes mellitus. Recent advances in genome-wide association analysis and a number of studies analyzing transcriptomes or proteomes in insulin secreting cells have revealed genes to possibly be involved in impaired β-cell function and/or mass. However, research progress beyond identification of these candidate genes has been hampered and analyses of the functional impacts of these abnormalities have been limited. Although imperfect, utilization of highly differentiated insulinoma cell lines, such as MIN6, is an approach to studying gene functions in insulin secretion. However, one of the disadvantages encountered in using these cells is the low transfection efficiency of nucleotides. When investing effects of abnormalities in candidate genes, 70-80% of cells, perhaps more, need to be genetically modified, because physiological phenotypes are not anticipated to be particularly large. A viral vector approach is one method overcoming this difficulty. However, producing viral vectors is not very easy. Therefore, generating stable cell lines would be preferable, offering the advantages of accuracy and reproducibility of experiments, although generating such cell lines from highly differentiated insulin secreting cells would also be time-consuming and laborious. We herein report a very efficient system based on the recombinase-mediated cassette exchange (RMCE) for generation of a MIN6-derived master cell line for gain-of-function and loss-of-function studies of candidate genes.

Materials and methods: A recipient platform for RMCE was generated that has the zeocin-resistant gene flanked with a set of hetero-specific yeast flippase recognition target (FRT) sites. MIN6 cells, first engineered to have a tetracycline-regulatable expression system, were randomly integrated the recipient platform and searched for clones expressing GFP uniformly and at a high level. Clones with multiple platform integration were eliminated by sequential RMCE and analysis of antibiotic sensitivity. Overexpression and suppression of gene-of-interest are achieved by delivery with an exchange vector of cDNAs and shRNAs, respectively, flanked with FRT sites together with a flippase expressing plasmid.

Results: We selected one clone based on their morphological features resembling those of parental MIN6 cells and their robust and uniform GFP expression after doxycycline induction and named MIN6CE. MIN6CE cells exhibit an approximately 10-fold induction of insulin secretion in response to high glucose concentrations. Using this cell line, we showed that engineered clones with induced or suppressed expression of glucokinase were generated within 6 weeks. Moreover, we could simultaneously generate clones overexpressing either *Fdft1, Gprc5c, Prss8, Gm773, Igsf21,* or *Serping1*, whose expressions are different between MIN6 subclones with high and moderately-low glucose responsiveness in our preliminary microarray analysis. We found that *Gprc5c* and *Prss8* overexpressions increased insulin secretion.

Conclusion: We established MIN6CE as a powerful tool for studying molecular mechanisms of insulin secretion.

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Loss of intra-islet heparan sulfate represents a novel marker for the progression of type 1 diabetes in humans

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Background and aims: Human type 1 diabetes (T1D) is an autoimmune disease and multiple destructive mechanisms are likely. In addition, a residual beta cell mass can exist at diagnosis, highlighting the potential for rescue by novel therapies. We have previously demonstrated unusually high levels of the polysaccharide heparan sulfate (HS) and heparan sulfate proteoglycans (HSPGs) inside mouse beta cells, a critical role for intracellular HS in beta cell survival, the progressive loss of islet HS during T1D progression in NOD mice and expression of the HS-degrading enzyme heparanase (Hpse) by islet-infiltrating leukocytes. Treatment of NOD mice with PI-88, a Hpse inhibitor/HS mimetic, reduced the incidence of T1D by 50% and preserved intra-islet HS, suggesting that Hpse-mediated loss of beta cell HS contributes to T1D disease. In this study we examined the clinical relevance of HS for the viability of human beta cells and as a target for destruction in human T1D.

Materials and methods: HS, HSPG core proteins (collagen type XVIII (Col18), syndecan-1 (Sdc1)), insulin, glucagon and Hpse were stained by immunohistochemistry/ immunofluorescence in paraffin sections (post-antigen retrieval) of normal (n=8) and T1D (n=8) human pancreases with insulin+ve islets, obtained from the JDRF Network for Pancreas Organ Donors with Diabetes (nPOD, USA); the stained islet area was quantified using Image J software. 10E4 anti-HS mAb was used to localise highly sulfated HS and HP130 mAb identified Hpse. Isolated human islets were dispersed into single cells (using Accutase) for flow cytometry analysis of HS/HSPGs in beta cells and of beta cell viability after culture with HS mimetics (heparin or PI-88) ± acute treatment with 30% hydrogen peroxide. Beta cells were identified using Newport Green (NG) and damaged cells were stained using 7AAD or Sytox Green.

Results: Localisation of HS, Col18 and Sdc1 in normal human pancreas correlated with insulin-containing beta cells. For insulin-positive T1D islets, the insulin-stained islet area was 85% of normal islets. However, the area stained for HS, Col18 and Sdc1 was significantly reduced to 41% ($P<0.0001$), 55% ($P<0.0001$) and 42% ($P<0.0001$) of normal islets, respectively. Insulitis leukocytes showed cell surface staining for Hpse. Flow cytometry analyses showed that 84.1±3.3% of human islet cells were beta cells and contained intracellular HS and HSPG core protein. Uptake of HS mimetics during culture of human beta cells significantly improved beta cell viability by 1.6-fold ($P<0.001$) i.e., as HS replacers, significantly reduced the proportion of damaged/non-viable beta cells to 25-31% of controls ($P<0.001$) and significantly reduced hydrogen peroxide-induced death by 3-fold ($P<0.001$).

Conclusion: These findings suggest that in human T1D, the loss of beta cell HS could be mediated by leukocyte-derived Hpse and result in increased susceptibility to oxidative damage. Dual activity Hpse inhibitors/HS replacers could therefore represent a novel class of T1D therapeutic.

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Degradation of the histone acetyl transferase p300 by the ubiquitin-proteasome pathway contributes to beta cell injury in type 2 diabetes environment

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Background and aims: In type 2 diabetes (T2D), chronic hyperglycemia, lipotoxicity and pro-inflammatory cytokines are detrimental to beta-cells, causing apoptosis and impaired insulin secretion. The histone acetyl transferase p300, involved in remodelling of chromatin structure by epigenetic mechanisms, is a key activator of the transcriptional machinery. We previously showed that p300 plays a key role in pancreatic beta-cell survival and function. The aim of the study is to understand the mechanisms that control p300 regulation in beta-cells exposed to pathological conditions.

Materials and methods: Experiments were performed with the pancreatic beta-cell line (INS-1E), isolated mouse pancreatic islets and human pancreatic islets. p300 and histone H4 acetylation levels were evaluated by western blot. Gene expression was analyzed by RT-PCR. p300 mRNA levels were evaluated using available islet transcriptomics analysis of T2D subjects from two independent datasets (Gene Expression Omnibus (GEO) repository: GSE20966 and GSE38642). Apoptosis was detected by cleaved caspase-3 emergence.

Results: Chronic exposure of INS-1E cells to high glucose or pro-inflammatory cytokines resulted in decreased p300 protein expression ($p<0.001$) associated with decreased histones H4 acetylation (targets of p300) and apoptosis. In isolated human islets, p300 protein levels were also decreased under high glucose exposure (30 mM glucose for 72h), chronic exposure to palmitate (0.5 mM palmitate for 72h) and this decrease was exacerbated under glucolipotoxicity (30 mM glucose + 0.5 mM palmitate) ($p<0.05$). Treatment of INS-1E cells with the proteasome inhibitor MG-132 prevented the decrease in p300 content induced by high glucose or pro-inflammatory cytokines exposure. However, p300 mRNA levels were not altered in INS-1E cells, and islet transcriptomics analysis of T2D subjects from two independent datasets revealed no change in p300 mRNA levels compared to normal glycemic controls. Altogether these data point to a proteasomal degradation involved in p300 loss in pathological beta-cells. The emergence of apoptosis in INS-1E cells with decreased p300 protein expression or function (knock-down by siRNA or treatment with an inhibitor of the histone acetyl transferase activity of p300 (C646), respectively) ($p<0.01$) suggested that loss of p300 integrity induced by pathological conditions contributes to beta-cell apoptosis and dysfunction.

Conclusion: This study demonstrates that p300 degradation by the ubiquitin-proteasome pathway contributes to beta-cell death and dysfunction upon physiopathological conditions mimicking the diabetic environment. Our data suggest the interest to consider this epigenetic modulator p300 as a potential therapeutic target in T2D.

Disclosure: **S. Costes:** None.

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Profound alteration in the expression of Wnt-associated gene in pancreatic islets induced by high fat/high sucrose diet and potential roles of Wnt4 in beta cells

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Background and aims: Mice fed high-fat/high-sucrose (HF/HS) diet develop insulin resistance and hyperinsulinemia exhibiting the hyperplasia of pancreatic β-cells. However, the precise mechanism by which the intake of HF/HS diet induces β-cell hyperplasia and hyperinsulinemia remains to be determined. Dysregulation of Wnt signaling pathway is associated with various diseases including type 2 diabetes. Here we investigated the alterations in the Wnt signaling pathway caused by HF/HS diet in murine islets, and analyzed the potential role of Wnt4 in β-cells.

Materials and methods: Eight-weeks-old C57BL/6 mice were fed standard chow or HF/HS diet up to the age of 20 weeks, when islets and pancreatic sections were obtained. RT-qPCR was performed for measurement of the expression of Wnt pathway-related genes in islets. Immunofluorescence staining was done to identify the localization of Wnt4 protein. Transfection of the on-target siRNAs was carried out to knockdown (KD) Wnt4 in MIN6 cells. The CCK8 assay and batch incubation were performed to assess proliferation and insulin secretion in Wnt4-KD-MIN6 cells. Students' t-test was used to compare two groups.

Results: Among the Wnt ligand family, 7 genes were expressed at detectable levels in islets obtained from 20-week-old mice. Wnt4 and Wnt5b were significantly up-regulated in the islets of mice maintained on HF/HS diet, whereas Wnt2b, Wnt11 and Wnt14 were depressed. On the other hand, 9 of 10 known Fzd receptor genes were expressed in islets, showing the down-regulation of Fzd4, Fzd5, and Fzd6 in HF/HS diet-fed mice. The expression of Lrp5 and Lrp6 both of which serve as Wnt co-receptors for the canonical β-catenin pathway were decreased in HF/HS diet-fed mice. We further analyzed the role of Wnt4 of which the expression was higher in islet. Immunofluorescence staining of islets revealed that Wnt4 was highly expressed by non-β-cells located at the periphery of islets. Most of the cells strongly positive for Wnt4 were glucagon-containing cells, but not somatostatin-containing cells. Interestingly, however, the HF/HS diet feeding resulted in the up-regulation of Wnt4 expression by the β-cells. The siRNA transfection of MIN6 cells showed that the KD of Wnt4 augmented β-cell proliferation. However, glucose-induced insulin secretion was reduced by the KD of Wnt4.

Conclusion: Long-term feeding with HF/HS diet profoundly altered the expression of Wnt signaling pathway genes. In this study we further analyzed the role of Wnt4 in islets, because Wnt3a was shown to promote β-cell proliferation through the β-catenin pathway, and Wnt4 stabilizes β-catenin antagonizing the action of Wnt3a in various cells. The HF/HS diet increased the expression of Wnt4 in β-cells in which Wnt4 expression was low in mice fed standard chow. The effects of Wnt4 KD in MIN6 cells indicated that Wnt4 likely has a role in the regulation of β-cell proliferation and insulin secretion, and is involved in the impaired β-cell function in patients with type 2 diabetes.

Disclosure: **T. Ohki:** None.

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Phenotypic characterisation of islet function in both male and female MIP-CreERT^{1Lphi} mice, a beta cell specific transgenic tool

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Background and aims: The MIP-CreERT^{1Lphi} mouse has mouse insulin 1 promoter-driven Cre-recombinase expression, and offers the most tissue specific Cre model currently available for generating beta-cell specific gene knockouts. However, reports have described differences in beta-cell function in male MIP-CreERT^{1Lphi} mice, attributed to the expression of a human Growth Hormone (*hGH*) mini-gene in the Cre transgene cassette. hGH is capable of signalling via the prolactin receptor to increase levels of serotonin. Understanding this phenotype in both males and females is important for interpreting mouse models produced using this line. We here seek to confirm these previous findings and address whether the MIP-CreERT^{1Lphi} background influences studies of beta-cell function in female mice.

Materials and methods: Mice were split into four groups; wild type mice (WT control), tamoxifen-injected wild type mice (WT TMX), heterozygous MIP-CreERT^{1Lphi} mice (Cre control) and tamoxifen-injected MIP-

CreERT^{1Lphi} mice (Cre TMX). Tamoxifen was administered over 4 consecutive days at 6 - 8 weeks (20mg/ml, 100µl i.p.). Both an intraperitoneal glucose tolerance test (IPGTT) and intraperitoneal insulin tolerance test (IPITT) were carried out on all mice following 6 h fast. Islets were then isolated for *in vitro* assessment of glucose sensitivity, insulin content and mRNA expression of *hGH*, serotonin synthesis enzymes; Tryptophan hydroxylase 1 and 2 (*Tph1*, *Tph2*), and both anti-apoptotic and proliferative markers.

Results: TMX females displayed a trend towards improved glucose homeostasis in both WT TMX (AUC 1169±39) and Cre TMX (AUC 1240±57) groups compared to non-injected mice (AUC WT: 1337±69, Cre: 1377±56; *n*=6-9). This pattern was also weakly apparent in males. However, female Cre mice displayed significantly increased insulin sensitivity in IPITT experiments compared to WT controls (AUC WT control: 369±18 *vs* Cre control: 313±15, *P*<0.05; WT TMX: 399±23 *vs* Cre TMX: 335±12, *P*=0.06, two-way RM ANOVA, *n*=6-9). Expression of *hGH* (Male WT: 0.05±0.02, Cre: 1.6±0.5, *P*<0.05; Female WT: 0.05±0.02, Cre: 0.5±0.1, *P*<0.05, t-test), *Tph1* (Male WT: $0.7x10^{-8}$±$0.5x10^{-8}$, Cre: $4.2x10^{-8}$±$3.7x10^{-8}$, NS; Female WT: $0.6x10^{-3}$±$0.2x10^{-3}$, Cre: $9.8x10^{-3}$±$3.3x10^{-3}$, *P*<0.05, t-test) and *Tph2* (Male WT: $0.3x10^{-4}$±$0.1x10^{-4}$, Cre: $7.1x10^{-4}$±$2.8x10^{-4}$, *P*<0.05; Female WT: $1.0x10^{-4}$±$0.7x10^{-4}$, Cre: $2.9x10^{-4}$±$1.2x10^{-4}$, NS, t-test) are increased in all Cre mice compared to WT. However, mRNA levels of proliferative or anti-apoptotic markers (e.g. *Ccna2*, *Ccnd1*, *Ccne1*, *Birc5*) are not significantly altered.

Conclusion: Glucose tolerance in MIP-CreERT^{1Lphi} mice was unchanged from that of WT in males and females; however, insulin sensitivity was significantly increased in MIP-CreERT^{1Lphi} females. Pancreatic islets of MIP-CreERT^{1Lph} mice have increased expression of *hGH,* and serotonin-producing enzymes *Tph1* and *Tph2*, confirming hGH signalling by the Cre transgene. However, both male and female MIP-CreERT^{1Lphi} islets appeared to have similar expression profiles to WT mice for a range of anti-apoptotic, cell growth and proliferative factors. These data suggest that the phenotype previously identified in the male MIP-CreERT^{1Lphi} mice may be more pronounced in females. Though a mild effect, this phenotype should be considered when interpreting data from beta-cell specific transgenic mice generated using this model and the use of separate Cre and tamoxifen control groups is suggested.

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Islet heparan sulfate and heparan sulfate proteoglycans in type 2 diabetes

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Background and aims: Type 2 diabetes (T2D) is a metabolic disorder characterised by insulin resistance, hyperglycaemia and lipidaemia. Endoplasmic reticulum (ER) stress, induced by insulin resistance, results in defective protein maturation and beta cell failure. Heparan sulfate (HS) is a polysaccharide normally expressed at unusually high intracellular levels in beta cells and is essential for beta cell survival. HS is synthesised directly onto core proteins, forming HS proteoglycans (HSPGs). This study examined whether (i) ER stress contributes to the loss of intra-islet HS and HSPG core proteins during T2D development and (ii) HS replacement preserves T2D beta cell viability and function.

Materials and methods: HS and HSPG core proteins (collagen type XVIII, syndecan-1 and CD44) in pancreases of wildtype (wt) and T2D-prone db/db mice at 3 to 20 weeks were examined by immunohistochemistry. Islets were isolated from wt and normoglycaemic or hyperglycaemic db/db donors (5-8 weeks) and dispersed into single cells using Accutase. The expression of ER stress-associated genes (CHOP, Bip, P58 and ATF3) were analysed by real-time RT-PCR. Intracellular HSPG core proteins and HS were examined by flow cytometry on day 0 and day 2 post-culture. Beta cell viability was assessed on day 0 and day 2 ± culture with heparin (HS mimetic; 50 µg/ml) using the fluorescent dyes calcein (Cal) and propidium iodide (PI) or Sytox Green. Db/db mice (n=8-10/group) were treated from 3.5-4 weeks of age with HS mimetic PI-88 (10 mg/kg/day i.p), TUDCA (chemical chaperone; 150 mg/kg/day i.p) or saline for 28-35 days. Non-fasting blood glucose was measured 3x/week and HbA1c levels were analysed on day 28-35. In parallel, ER stress was induced in MIN6 beta cells using thapsigargin (50 nM) and tunicamycin (2 µM). Intracellular HSPG core proteins and HS levels were examined by flow cytometry.

Results: HS and HSPG core proteins in db/db islets were reduced to 44% and 22-46% (p<0.001) of controls, respectively, by 6 weeks of age. Isolated db/db islets showed a 1.5, 4.7 and 6.3 -fold increase in CHOP, BiP (p<0.001) and P58 (P<0.001) mRNA, respectively, compared to controls. HS and HSPG core proteins in db/db beta cells were reduced to 17-37% of wt controls on day 2. Wt and db/db beta cells cultured with heparin showed a 3.5-fold and 1.8-2.8-fold increase in viable $Cal^{+}PI^{-}$ beta cells, respectively, compared to controls. Heparin-treatment reduced H_2O_2 -induced death in wt and db/db beta cells to 15% and 32-60% of controls, respectively. PI-88 treatment significantly reduced HbA1c levels in 5/10 db/db mice (5.0±0.3% vs 6.1±0.2%; P=0.02) and improved blood glucose levels; similar effects were observed after TUDCA treatment. Like beta cells, MIN6 cells treated with pharmacological ER stress inducers showed induction of ER stress genes and significantly reduced levels of HS and HSPG core proteins by day 3.

Conclusion: Diminished levels of HS and HSPG core proteins in db/db islets during the progression of T2D are consistent with ER stress effects on HSPG and HS synthesis. Treatment with HS mimetics rescued db/db beta cell viability and protected against acute oxidative damage in vitro and improved glycaemic control of db/db mice in vivo. HS mimetics acting as HS replacers may represent a new class of therapeutic for preserving beta cell survival and function in T2D.

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PS 016 Stem cells and beta cell differentiation

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Gata6 controls insulin biosynthesis and secretion in adult pancreatic beta cells

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Background and aims: Previous studies, including ours, have shown that GATA4 and GATA6 are crucial for pancreas organogenesis in both mouse and human. In mice, while single inactivation of either Gata4 or Gata6 has not impact in pancreas formation, the inactivation of both in the pancreatic primordia leads to pancreatic agenesis indicating a functional redundancy for these transcription factors during pancreas formation. Recently, GATA6 mutations have also been linked to adult-onset diabetes with subclinical or no exocrine insufficiency suggesting an important role for GATA6 in human β cell physiology. We investigate the potential contribution of GATA6 to adult ß cell function.

Materials and methods: We used Gata6 $^{flox/flox}$;Pdx1-Cre conditional knock-out and Gata6 $^{flox/flox}$ mice and performed glucose and insulin tolerance tests. Dissected adult pancreata were used to histological analysis and pancreatic insulin content. Isolated islets were used for quantitative PCR, Transmission Electronic Microscope (TEM) and insulin content analysis. We also used transgenic reporter mice in which Pdx1 regulatory sequences direct LacZ expression in adult ß cells.

Results: GATA6-deficient mice develop glucose intolerance at six month of age. We show that islets deficient in GATA6 activity display decreased insulin content and impaired glucose-induced insulin secretion. Ultrastructure micrographs of Gata6-deficient ß cells reveal a markedly reduction of mature insulin granules. In agreement, islets lacking GATA6 are defcient in the expression of in insulin biosynthesis, secretion and maturation markers. Gata6 KO ß cells display swollen mitochondrial and dilated endoplasmic reticulum (ER) cisternae in Gata6 KO ß cells, suggestive of mitochondrial and ER stress. Finally, we demonstrate that Pdx1 expression in adult ß cells depends on GATA sites in transgenic reporter mice.

Conclusion: These findings might explain the diabetic phenotype in patients harboring GATA6 mutations. Mutations resulting in decreased GATA6 expression or activity postnatally might lead to ß cell dysfunction by impairing insulin biosynthesis and secretion.

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Characterising the role of the adhesion receptor GPR56 in islet development

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Background and aims: GPR56 is an adhesion receptor that is known to regulate proliferation, apoptosis and organ development. Studies in neuronal development have indicated that it is critical for the proper formation of the cerebral cortex. Outside the CNS, GPR56 plays structural functions in seminiferous tubule development where its absence during embryogenesis leads to male infertility. It has been shown that GPR56 is highly enriched in endocrine progenitors in the developing pancreas. We have previously reported that GPR56 is abundantly expressed in islets where it regulates β-cell proliferation, but its function in islet development is not yet known. The aim of this study was to investigate the expression and function of GPR56 at different stages of islet development using GPR56 KO mice.

Materials and methods: Pregnant dams and post-natal day 9 (P9) mice were injected with BrdU (50mg/kg) intraperitoneally. Using fluorescence microscopy, GPR56 expression and its colocalisation with endocrine progenitor markers (PDX1, NGN3 and SOX9) were investigated in WT fixed-frozen pancreas sections at embryonic days E11, E13, E15, E18 and at P9. WT and GPR56 KO pancreas sections were immunoprobed for Ki67, BrdU, insulin, glucagon, and the neuronal and vascular markers TUJ1 and CD31. Immunostained images were quantified by Image J.

Results: Immunostaining revealed that GPR56 was expressed by PDX1$^+$, NGN3$^+$ and SOX9$^+$ endocrine progenitor cells. It was strongly expressed at the early days of pancreas development and became downregulated as the cells differentiated (% area GPR56$^+$ cells; E11:0.87±0.04, E13:0.85±0.06, E15: 0.36±0.08, E16: 0.15±0.05). It was then upregulated at the stage of β-cell replication (% area GPR56$^+$ cells; E18: 0.15±0.05, P9: 0.47±0.07, n=10 sections). At E16, there was an increased number of NGN3$^+$ progenitors in GPR56 KO pancreas (NGN3$^+$ cells/mm^2; WT: 3,012±211, KO: 3,880±266, n=6-12 replicates, p<0.05), suggesting reduced differentiation. However, there was no change in proliferation of progenitor cells at E16 (BrdU$^+$ cells/mm^2; WT: 475.8±44.6, KO: 448.2±18.2, n=6-12 replicates, p>0.2). The number of cells proliferating and still in the cell cycle was significantly lower in KO islets at P9 (BrdU$^+$Ki67$^+$ cells/mm^2; WT: 188.4±29.6, KO: 82.7±10.3, n=3 mice/genotype, p<0.05), leading to less β-cells (% β-cells/islet; WT: 68.5 ±0.8, KO: 54.8±3.0, n=3 mice/genotype, p<0.05), but higher numbers of α-cells in KO islets (% α-cells/islet; WT: 17.7±0.9, KO: 33.7±2.8, n=3 mice/genotype, p<0.01). There were no differences in islet capillary density (number/μm^2; WT: 0.03±0.007, KO: 0.02±0.009, n=3 mice/genotype, p>0.2) or percentage islet nerve area (WT: 0.75±0.10, KO: 0.72±0.06, n=3 mice/genotype, p>0.2).

Conclusion: Our data suggest that GPR56 is differentially expressed during mouse islet development, where it is required at key stages of proliferation and differentiation of endocrine cells. However, GPR56 is not required for islet innervation and vascularisation of the developing endocrine pancreas.

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Disclosure: **O.E. Oladapo:** None.

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Purification of replicative pancreatic beta cells for gene expression studies

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Background and aims: β-cell proliferation is a rare event in adult pancreatic islets and varies within a range of 0.4% to 6% of β-cells per day depending on age. This low proportion of replicative beta cells precludes molecular analysis of β-cell replication-related pathways in entire islets, since it may be masked by the highly abundant post-replicative islet cells. Nucleoside analogues (ie. BrdU, CldU and IdU) have been extensively used for the identification of replicative cells. All of them require the use of DNA denaturation facilitating sterical access of antibodies to the nucleosides that may preclude downstream gene expression analysis. Alternatively, 5-ethynyl-20-deoxyuridine (EdU) is structurally similar to the natural nucleosides but its detection is based on a copper-catalysed covalent reaction between a dye-conjugated azide and the alkyne group of the EdU, known as Click chemistry. The small sized dye-azide complex allows for efficient EdU detection avoiding harsh conditions that degrade the structure of the cells. We sought to develop a method for replicative β-cells sorting based on EdU incorporation, suitable for gene expression analysis that could be used in a variety of experimental designs and species.

Materials and methods: Replicating cells were identified by EdU incorporation and Newport green in cultured rat islets. β-cell viability and replication of

islets exposed to EdU was determined by TUNEL and BrdU incorporation, respectively. For β-cell separation islet cells were sorted by size, granularity and Newport Green fluorescence emission that was combined with emitted fluorescence for EdU-labelled replicative cells sorting. The purity of the resulting sorted populations was evaluated by insulin staining and by EdU for β-cell and replicative cell identification respectively. Total RNA was isolated from purified cell-sorted populations and mRNA was linearly amplified using T7-RNA-polymerase based in vitro transcription. RNA quality was assessed in the Bioanalyzer 2100. cDNA synthesis was performed from 200ng of amplified RNA and qPCR was run in a 7900HT Fast Real-Time PCR system.

Results: β-cell viability and replication were not affected by islet exposure to EdU for 1 week in culture. However longer exposure was detrimental for β-cell viability (β-cell apoptosis in control: 0.65%±0.17%; 10μM EdU: 1.1%±0.17%; p=0.02). Accordingly, the percentage of EdU labelled β-cells at the end of the 7 day-culture was approximately 7-fold higher than the 1 day labelling with BrdU (24h-BrdU pulse: 0.51±0.08% labelling; 7 days-EdU pulse: 4.3±0.48% labelling) but a longer pulse of EdU did not result in higher percentage of labelled β-cells (14days-EdU pulse: 2.45±0.94% labelling). Cell sorting of dispersed islet cells resulted in 96.2% purity for insulin positivity in the collected β-cell fraction and 100% efficiency of the EdU-based cell separation. RNA integrity was similar between FACS-sorted replicative and quiescent β-cells. Differential gene expression analysis of replicative vs quiescent β-cells showed the expected pattern of cell cycle-related mRNAs; increased ki67, pcna, the epigenetic modulators ezh2 and bmi1, and decreased foxo1 and ins2 in replicative β-cells.

Conclusion: We have developed a method that achieves efficient purification of replicative β-cells that maintains the integrity of the RNA for downstream gene expression analysis.

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Disclosure: **N. Tellez:** None.

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A high-throughput phenotypic screen identifies selective modulators of human pancreatic beta cell proliferation for type 2 diabetes

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Background and aims: Identification of novel compounds to selectively induce pancreatic beta cell proliferation has the potential to restore functional beta cell mass and insulin secretory demand in type 2 diabetes. Success has been limited due to lack of relevant cell lines for high-throughput in vitro screening and limited quantities of primary beta cells for hit validation and translational efficacy. The genetically engineered human pancreatic β-cell line, EndoC-βH1 (EndoCell) was employed to generate a high-throughput phenotypic screen to enable the identification of novel modulators of beta cell proliferation. A multiparametric high throughput high content dispersed primary islet assay was developed to facilitate detailed characterisation of hits in multicell type islet cell cultures.

Materials and methods: The EndoC-βH1 cells were shown to exhibit glucose-inducible insulin secretion and express β-cell transcription factors including PDX1 and NKX6-1 at levels similar to those in human islet cells. The newly developed HTS assay facilitated exposure to a large range of diverse chemical compounds previously unexplored in this context. An image based high throughput screen of 120K chemically diverse small molecules employed EdU incorporation and cell count end points to positively identify compounds which increase EndoC-βH1 proliferation. The hits were further profiled in pancreatic ductal cells for understanding of non-beta cell proliferative capacity. A 384-well multi-parametric dispersed primary islet assay employed EdU, insulin and Hoechst endpoints for identification of proliferating beta cell and non-beta cell sub populations and was sufficiently robust to enable concentration response data.

Results: Several chemical series were identified as robust proliferators of the EndoC-βH1 cells. Further analysis of proliferative capacity in pancreatic ductal cells facilitated prioritisation of compounds which specifically drive beta cell proliferation. The development and application of high-throughput translational primary islet assays enabled validation of hit compounds that selectively enhance beta cell proliferation and counts without effects on the non-beta cell population. Analysis of the EC50 of compounds across the EndoC-βH1 cell assay and the primary islet assay showed strong correlations of compound efficacy and potency.

Conclusion: A phenotypic screen identified novel compounds to increase pancreatic beta cell proliferative capacity. The multi-parametric concentration-response data in primary islets allowed detailed characterisation of compound efficacy and potency in ex vivo primary tissue, enabling better prediction of drug action and safety in future in vivo studies and in the clinic. The generation of concentration response curves has allowed direct comparisons of proliferative effect for compounds in primary tissue and diabetes relevant cell-lines, providing important insights into immortalised cell-line and primary cell translatability of novel targets.

Disclosure: **N. Mullooly:** None.

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Modulation of lamin isoforms expression protects from type 2 diabetes and favor beta cells biogenesis after STZ-induced type 1 diabetes

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Background and aims: LaminA, laminC and progerin are alternative RNA processing of the *LMNA* gene. We investigated the function of laminC in aging and metabolism using mice expressing only LaminC (LCS). Intriguingly, these mice live longer, have decreased energy metabolism, increased weight gain, reduced respiration and decreased mitochondrial biogenesis. Consistently, transcriptome analysis of LCS adipose tissue reveal high variations in the expression of key regulators of energy expenditure. Interestingly, we observed that aged LCS mice are obese but remain glucose tolerant. The aim of the project is to investigate how LCS mice, that become obese under chow diet, remain protected from T2DM.

Materials and methods: All animal procedures were conducted in strict adherence with the European Community Council Directive of November 24, 1986 (86-609/EEC). Intra Peritoneal Glucose Tolerance Test, Insulin Tolerence Test, isolated islets, histological studies were all conducted on 25-weeks (young) and 75-weeks (old) old mice (n=5). T1DM was induced by one i.p. injection of Streptozotocin (STZ, 200mg/kg). Histological studies were performed on paraffin embedded pancreas and stained with either HE or by IHC using insulin, Glucagon, Phospho histone H3, Lectin antibodies or TUNEL staining. MIN6 LCS cell line was generated using CrispR/Cas9 technology. MIN6 LCS proliferation rate was monitored by Iprasense Technology. MIN6 LCS apoptosis rate was measured by FACS after AnnexinV staining.

Results: The metabolic status of young vs old LCS mice shows that old LCS mice become obese but stay more glucose tolerant because they exhibit a higher insulin secretion level than WT mice. Histological pancreas analysis of LCS mice shows an increased β cell mass since the number and the size of the islets increases in old LCS mice. Otherwise, isolated islets from old LCS mice exhibit a higher secretion capacity compared to WT isolated islets. Using the CrispR/Cas9 gene editing technology, we generated a specific expression of LaminC in the MIN6 β cell line. We confirmed that LCS MIN6 cells exhibit a higher insulin secretion in response to glucose stimulation. On the other hand, we cannot show any difference in cell proliferation or resistance to apoptosis induced by STZ, cytokine or H2O2. All these results suggest that LCS mice exhibit a better tissue-regenerative capacity. To investigate this point, we perform a STZ-induced T1DM. We show that exclusive laminC expression rescues STZ-induced diabetes since 5 weeks after STZ injection, LCS mice are normoglycemic whereas WT mice are still diabetic. In STZ-treated LCS mice we observed, using apoptosis and proliferation staining, a mild protection from apoptosis and a high number of proliferating cells including β cells duplication.

Conclusion: We demonstrate that mice expressing exclusively laminC become obese but are protected from diabetes because they develop islets

hyperplasia and have a higher insulin secretion capacity. We are now investigating, by genome wide analysis, the molecular mechanisms underlying this phenotype. Taken together, our results demonstrate that LMNA encodes functionally distinct isoforms that plays an important role on β cell plasticity.

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Disclosure: **C. Chavey:** None.

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Forced maturity in pancreatic beta cells impairs islet function

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Background and aims: It is widely assumed that beta cell maturity is permissive for normal islet function. Indeed, type 2 diabetes (T2D) is regarded as a state of beta cell de-differentiation. However, recent RNASeq and in situ imaging studies have shown the existence of discrete beta cell subpopulations, including putative pacemakers. Using a viral strategy to "force" maturity throughout the beta complement, we sought to understand how heterogeneity may influence normal islet function and insulin release.

Materials and methods: Mouse islets were transduced with either control, adenovirus harboring a polycistronic construct for Ngn3, MafA, Pdx1 and mCherry (Ad3-NPM), or adenovirus harboring PATagRFP (Ad-PATagRFP), which encodes photoactivated TagRFP. Immunohistochemistry against Pdx1 and insulin was performed using specific antibodies and images captured using a Zeiss LSM780 confocal microscope. Gene expression was determined using QRT-PCR with SYBR Green chemistry. Islet-wide Ca^{2+} dynamics were analyzed using a Crest X-Light spinning disk head coupled to a Lumencor SPECTRA X light engine. Insulin secretion was measured by HTRF assay. All animal studies were regulated by the Home Office according to the Animals (Scientific Procedures) Act 1986 of the United Kingdom.

Results: Treatment with Ad3-NPM induced 2- and 8-fold increases in MafA and Pdx1 expression, respectively, in adult mouse islets. Ngn3 levels remained unchanged probably due to cross-repression mechanisms. Immunohistochemical analyses of Pdx1 showed that overexpression occurred preferentially in immature beta cells, forcing homogenous maturity throughout the population. Fast multicellular imaging approaches revealed sharply blunted Ca^{2+} responses to glucose and KCl (ΔF= 0.81 vs 0.44 AU, respectively; $P<0.01$), as well as a reduction in beta cell-beta cell coordination (12.0 vs 8.0 %; $P<0.05$), in Ad3-NPM-treated islets. The ensuing loss of functional connectivity was associated with a reduction in the number of hubs (12.6 vs 5.6 % hubs, Con vs Ad3-NPM, respectively; $P<0.05$), immature beta cells previously shown to act as pacemakers. Forced beta cell maturity increased basal insulin secretion, leading to impairments in glucose-stimulated insulin secretion (7.5 vs 5.0-fold, Con vs Ad3-NPM WT, respectively; $P<0.05$). Suggesting a specific role for transcription factor overexpression, Ad-PATagRFP was unable to affect Ca^{2+} fluxes, coordination indices, hub proportion or insulin secretion.

Conclusion: Loss of beta cell heterogeneity through homogenous de-differentiation (e.g. during T2D) or maturity (i.e. herein) is associated with islet failure. Thus, re-creation of subtle differences in the islet transcriptional and functional landscape- or so-called "islet identity"- may be required for the proper restoration of beta cell function during type 2 diabetes.

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Pax8, a prosurvival gene in islets potentially implicated in gestational diabetes

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Background and aims: We previously demonstrated that expression of the transcription factor PAX8 is barely to non-detectable in human islets and pancreatic neuroendocrine tumors. Notwithstanding, single nucleotide polymorphisms (SNPs) in the vicinity of the pax8 gene have been associated with Type 2 Diabetes Mellitus while expression of this factor is robustly induced in mouse islets during gestation. Thus the functional role of PAX8 in islet physiology and Diabetes including gestational Diabetes Mellitus (GDM) remains controversial. Herein we address this question by: 1) lentiviral-mediated forced expression of PAX8 in islets, 2) heterozygous deletion of the pax8 gene in mice 3) Screening for novel SNPs within the pax8 gene that may correlate with GDM in a human cohort.

Materials and methods: Islet metabolic activity (MTT assay), apoptosis, glucose-stimulated insulin secretion (GSIS) and gene expression profiling was performed on isolated islets 96 hours post PAX8 lentiviral-mediated transduction. Oral glucose tolerance test (OGTT) were performed on Pax8 heterozygous mice before and during gestation and treated or not with polyI:C (mimicking viral infection). The complete coding region of the pax8 gene was sequenced on candidates that fulfilled strict selective criteria.

Results: Islet metabolic activity was preserved subsequent to PAX8 overexpression (100% in control versus 117±35% in PAX8 over-expressing islets; $p=0.64$), consistent with unaltered GSIS (fold induction 2.65±0.4 in control vs. 2.65±0.53 in PAX8 over-expressing islets; $p=0.99$). In contrast, islet viability subsequent to isolation was significantly increased by PAX8 (100% in control versus 64±11% in PAX8 over-expressing islets; $p=0.02$), correlating with a reduced percentage of cleaved caspase 3-positive cells (17±1.6% in control versus 8.4±2.2% in PAX8 over-expressing islets; $p=0.04$). Accordingly, inflammatory signaling pathways such as interferon-gamma were significantly modulated in PAX8 overexpressing islet ($p<0.05$). Heterozygous PAX8 females treated or not with polyI:C exhibited similar OGTT at 14.5 gestation as compared to wild type mice. Notwithstanding, genetic screening in 7 families complying with the selection criteria revealed a yet uncharacterized SNP within the pax8 gene of one pedigree that correlated with GDM. This SNP generates an amino acid substitution (PAX8-T356M) within the C-terminal trans-activation domain (TAD) of PAX8. Accordingly, in silico analysis using web-based tools (PolyPhen, SIFT and PredictSNP) predicted a deleterious impact of this SNP.

Conclusion: Our data clearly demonstrate that increased expression of PAX8 impacts islet viability, an essential attribute to maintain glucose homeostasis in response to altered metabolic demand such as during pregnancy. Normal glucose tolerance during gestation in heterozygous pax8 mice might be explained by gene haplosufficiency. However, deleterious mutations within the TAD potentially resulting in a dominant negative phenotype may contribute to the pathogenesis of GDM. This premise is currently being investigated for PAX8-T356M.

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Sfrp5 increases glucose-stimulated but not basal insulin secretion in rat INS-1E cells

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Background and aims: Secreted frizzled-related protein (Sfrp)5 belongs to the Sfrp family, the largest family of Wnt inhibitors. Protein and mRNA levels of Sfrp5 were downregulated in pancreatic islets from obese rodents and humans. In rodent models, Sfrp5 inactivated the canonical Wnt signaling pathway, reduced beta-cell proliferation and increased the ratio of proinsulin to C-peptide. However, the impact of Sfrp5 on insulin secretion in pancreatic beta-cells has not been analysed yet. Therefore, we aimed to investigate (i) whether Sfrp5 has an impact on cell proliferation and apoptosis, (ii) how Sfrp5 influences basal and glucose-stimulated insulin secretion and (iii) whether Sfrp5 acts via inhibiting the Wnt signaling pathway in rat beta cells.

Materials and methods: We treated rat INS-1E cells without or with (i) 1000 ng/ml or (ii) 5000 ng/ml recombinant Sfrp5 for 24h. We determined protein or mRNA levels of the proliferating cell nuclear antigen (PCNA), cyclin B1 and Ki-67 as markers for cell proliferation and cleaved caspase-3, cleaved caspase-9, B-cell lymphoma 2 (BCL2) and p53 upregulated modulator of apoptosis (PUMA) as markers for apoptosis using Western blotting and real-time PCR. To determine the effect of Sfrp5 on basal and glucose-stimulated insulin secretion we stimulated the cells with 2.5 mmol/l and 20 mmol/l glucose, respectively. The concentrations of insulin in the supernatant and cell lysate were measured by ELISA. To analyse whether Sfrp5 regulates the canonical Wnt signalling pathway we measured the protein levels of the active form of β-catenin and mRNA levels of the transcription factor 7-like 2 (TCF7L2). In addition, we determined the impact of Sfrp5 on the phosphorylation levels of cAMP response element-binding protein (CREB)-Ser133 representing an important transcriptional factor in the non-canonical Wnt signaling pathway regulating insulin production and processing.

Results: Glucose-stimulated insulin secretion was increased by 25% for 1000 ng/ml Sfrp5 (p=0.05) and by 34% for 5000 ng/ml Sfrp5 (p=0.02) compared to control, whereas basal insulin secretion was not affected at both concentrations. At 5000 ng/ml Sfrp5, protein levels of PCNA were decreased by 28% (p=0.04) and there was a trend towards a reduction of the mRNA levels of cyclin B1 by 17% and Ki-67 by 14%. Sfrp5 did not change mRNA or protein levels of cleaved caspase-3, cleaved caspase-9, BCL-2 and PUMA. Moreover, Sfrp5 neither altered the protein levels of the active form of β-catenin and mRNA levels of TCF7L2 nor the phosphorylation level of CREB-Ser133.

Conclusion: Sfrp5 increased glucose-stimulated but not basal insulin secretion without activating the canonical or non-canonical Wnt signalling pathway. While future experiments are necessary to confirm this finding in human beta cells, we report an incretin-like effect of Sfrp5 on insulin secretion in rat beta cells.

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Islet heterogeneity in the healthy human pancreas

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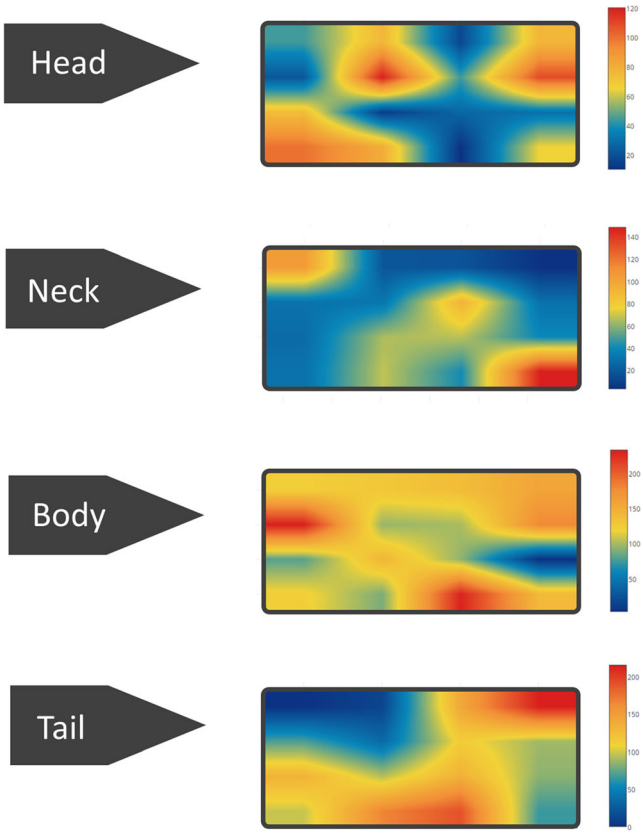
Background and aims: Previous studies related to the anatomy of the islets have been focused on the mice pancreas or the human diabetic pancreas (either from Type 1 diabetes or Type 2 diabetes patients). The spatial distribution of the islets in the human pancreas was until recently relatively unknown. Some of the obstacles have been often represented by an apparent lack of islet organization based on previous studies. The aim of our study was to find the anatomical organization of these micro-organs in a healthy pancreas.

Materials and methods: A healthy human pancreas from a 35 years male donor has been fixated and sectioned into four anatomical regions, namely the head, neck, body and the tail. These regions were further divided into a square matrix of 16 slides. Two parameters have been measured on each histological slide, namely the area and the perimeter of the islets. By using classical formulas and new algorithms for analysis, we have determined a series of parameters, such as the average value of the islet diameter, the mean islet volume, the total number of islets inside the pancreas, the total islet volume inside the pancreas, and the islet percentage from the total volume of the pancreas. 2D heat maps of the four slices have been built and analyzed.

Results: We have analyzed a total of ~5400 islets along the pancreas. The islet percentage from the total pancreas volume (45 cm^3) showed an approximate value of ~ 4.5% (~3.2 million islets). The mean islet diameter (108.92 μm (± 6.27)) and the mean islet volume (686,994.29 μm^3 ±107,297.82) have been used in conjunction with new algorithms for image analysis for the construction of four 2D heat maps. Our analysis have shown a clear cords-like structures in the organization of the islets along the pancreas. (Figure). The figure shows islet densities on four slices (red - high density of islets, blue - low density of islets). Figure reference system: the top of each slice represents the dorsal pancreas.

Conclusion: Our conclusion on the organization of the islets indicated a non-random spatial distribution. Furthermore, the spatial distribution of the islets, derived from 2D heat maps suggests cords-like structures with clear anatomical paths along the pancreas.



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Morphometric analysis of stem/progenitor cell phenotypes in human diabetic pancreas

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Background and aims: Diabetes mellitus may represent a unique condition to estabilish whether organ pathology is primarily due to changes in the functional properties of stem or parenchymal cells or both. The variability in tissue response to chronic hyperglycaemia makes it difficult to sustain a unified hypothesis on diabetes multiorgan damage. The impairment of tissue homeostasis is still incompletely understood. The basic pathogenic process of type 2 diabetes is widely described as islet beta cell loss and dysfunction. However, a detailed tissue characterization of the human pancreas in diabetes is still lacking. In addition, we attempted to provide evidence of the hypothesis that in diabetes tissue homeostasis is compromised by alterations in progenitor cells.

Materials and methods: Our study describes structural changes in pancreas of 20 type 2 diabetic patients compared to 20 controls. Exocrine and endocrine parenchyma, and abundance and distribution of stem cell associated

phenotypes were documented in different portions of diabetic and normoglycemic pancreas.

Results: The morphometric assessment of the number and dimension of insulin or glucagon expressing islets documented a significant reduction in diabetic tissue ($p<0.05$). However, a significant increase in individual Ins ^{pos} and glucagon ^{pos} cells was observed in diabetes compared to control . Moreover, a lower dimension of endocrine islets was measured in diabetic pancreas compared to hyperglycemic tissues. Interestingly, the number and distribution of cells carrying the stem cell associated antigen c-kit ^{pos} were decreased ($p<0.05$) in diabetic pancreas compared to controls. c-kit ^{pos} cell compartment was found to be reduced in both interstitial and insular areas of the diabetic pancreas. Similarly, diabetes affected the number of individual CD34pos cells distributed throughout the pancreatic tissue. Conversely, the quantity of CD133 ^{pos} cells was unchanged in diabetic pancreas.

Conclusion: Our results suggest that changes in number of stem/progenitor cells occurs in huma pancreas subjected to diabetes. This finding may provide an innovative pathogenetic view with clinical implications.

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PS 017 Insulin secretion and exocytosis I

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Human islets exhibit variability in macronutrient-stimulated insulin release that cannot be explained by gross donor characteristics

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Background and aims: Type 2 diabetes (T2D) and obesity frequently occur together. The molecular mechanisms associated with the pathogenesis of these two conditions remain unclear. However, dysfunctional insulin-secreting beta cells are critical to the development and progression of both conditions. In pre-diabetes and early stage T2D, this dysfunction is associated with hyperinsulinemia. Central to this, obesity is prevented in mice that are genetically incapable of developing hyperinsulinemia. In human studies, long-term insulin therapy induces weight-gain, and drugs that reduce insulin secretion can aid in weight-loss. As such, a reduction in insulin levels may be beneficial to the metabolic health of individuals with hyperinsulinemia. This can be achieved with ketogenic diets which limit ingested carbohydrates, keeping insulin levels low. However, it is not known to what extent non-carbohydrate substrates such as lipids and amino induce insulin release, and whether individual-to-individual variations are seen in *ex vivo* human islet studies. Such variations could explain why 'low-carb' diets are only effective in a subset of individuals. Here we report dynamic insulin secretion measurements from multiple human islet donors in response to various macronutrient stimuli, and correlate these responses to islet culture time and gross donor characteristics.

Materials and methods: Human islets from 13 non-diabetic donors were from the IsletCore program (Alberta Diabetes Institute) or the Ike Barber human islet transplant laboratory (Vancouver General Hospital). Insulin secretion in response to high glucose (15 mmol/l), the amino acid leucine (5 mmol/l) or the free fatty acids oleate/palmitate (1.5 mmol/l; 1:1 mix) was measured via perfusion assay and assayed for insulin via RIA. Islet samples were flash-frozen to be used for parallel genetic, transcriptomic and metabolomic profiling.

Results: Unique human islet samples released insulin in a highly variable manner. In response to glucose (15 mmol/l), peak insulin release was 2 to 24-fold (over basal), with an average release of 10-fold. Leucine (5 mmol/l) induced peak insulin release by 2 to 21-fold (over basal), with an average release of 7-fold. Islets from 3/13 donors released insulin in response to oleate/palmitate (1.5 mmol/l; 1:1 mix) by 4 to 16-fold. We correlated the insulin response to each macronutrient stimulus with gross donor characteristics (age, sex, BMI), and the *ex vivo* culture time of the islets. No significant correlations were seen, but there was a moderate positive correlation between glucose-stimulated insulin release and BMI ($r^2=0.46$, $p=0.096$). We also examined intra-nutrient correlations and found a mild positive correlation between leucine-stimulated and glucose-stimulated insulin release ($r^2=0.29$, $p=0.056$). Ongoing studies are correlating insulin responses to genetic, genomic and metabolomic factors.

Conclusion: Our data now shows donor-donor variation in insulin release from isolated islets in response to the three main macronutrient categories. This variation could explain how macronutrient intake can have distinct effects on weight loss and diabetes risk in individuals.

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CD36 is increased in human islets from obese type 2 diabetes donors, and affects beta cell function by the modulation of exocytotic proteins and insulin content

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Background and aims: CD36 is known as a scavenger receptor involved in lipid influx and immunity. However, the role of CD36 in beta-cell function and its pathophysiological significance in type 2 diabetes (T2D) have not been fully elucidated. Our previous studies on mouse lines with different susceptibilities to diet-induced diabetes (SDG-P/R mice) demonstrated that islets from the pre-diabetic SDG-P mice have hereditary defects in insulin secretion with an increased gene expression level of CD36. Here, we aim to evaluate the expression level of CD36 in diabetic islets and to investigate the influence of CD36 on insulin secretion using clonal beta-cells.

Materials and methods: CD36 mRNA expression level in human islets was derived from RNA-seq data. Gene expression and protein levels of CD36 in the GK and Wistar rat islets were evaluated by qPCR and western blot. Insulin secretion at 16.7 mmol/L glucose for 1hr or at 50 mmol/L K^+ for 15 min, gene expression (qPCR) and protein levels (western blot) were evaluated in CD36 over-expressing INS-1 cells. CD36 over-expression was controlled by the Tet-on system using doxycycline (500 ng/mL for 72 hr).

Results: Human islets from obese donors with impaired glucose tolerance (IGT) or T2D (n=19) had an increased CD36 mRNA expression level (15%, $p<0.05$) as compared to those from obese donors with normal glucose tolerance (n=12). The GK rat islets (n=7) had increased gene expression (70%; $p<0.01$) and protein levels (25%; $p=0.085$) of CD36 as compared to the Wistar rat islets (n=8). CD36 over-expression in INS-1 cells reduced insulin secretion at 16.7 mmol/L glucose by 45% (n=5, $p<0.01$ v.s. control cells). Also, depolarization-induced insulin secretion (50 mmol/L K^+) was reduced by 25% (n=5, $p<0.01$). In agreement with the reduced depolarization-induced insulin secretion, gene expression of Snap25, Vamp2 and Syt7 (Synaptotagmin 7) was decreased by 35%, 15% and 15%, respectively (n=6, $p<0.05$ for each mRNA). Protein level of SNAP25 was decreased by 25% (n=5, $p<0.05$). Furthermore, CD36 over-expression reduced insulin content by 20% (n=5, $p<0.01$) and insulin gene (Ins1) expression by 30% (n=6, $p<0.05$).

Conclusion: CD36 was increased in pre-diabetic/diabetic islets, and over-expression in INS-1 cells reduced glucose-/depolarization-induced insulin secretion and insulin content. We suggest that CD36 causes defective insulin secretion by the reduction of exocytotic proteins and the inhibition of insulin gene expression in beta-cells. Given that the islets from T2D donors have decreased levels of exocytotic proteins and low insulin content, CD36 could be a potential therapeutic target to rescue the beta-cells in diabetic conditions.

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Expression and function of Cl^- loaders, extruders and channels in pancreatic islet beta cells: implications for intracellular Cl^- regulation and insulin secretion

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Background and aims: The population with the highest risk for developing age-dependent diabetes is patients with Cystic Fibrosis (CF). Almost 50% of the cases develop diabetes by the 4th decade of life. Hyperglycemia in these patients worsens lung function and nutritional status and increases the number of hospitalizations resulting in higher mortality. Recent data suggests that patients with pancreatic insufficiency are at a higher risk to develop CF-related diabetes (CFRD) due to β-cell dysfunction. Glucose metabolism and plasma membrane depolarization are responsible for the initial secretory response in pancreatic β-cells and regulated primarily, though not exclusively, by the ATP-sensitive K^+ channel (K_{ATP}). In these cells, the intracellular chloride concentration ($[Cl^-]_i$) is kept above thermodynamic equilibrium providing an additional driving force for the depolarization and prolongation of action potentials necessary for sustained insulin secretion. In other cell types, at least seven cation-chloride co-transporters of the solute carrier protein family 12 group A (*Slc12a*) and some members of the *Slc4a* and *Slc26a* families are considered regulators of $[Cl^-]_i$. Whilst some of these transporters actively accumulate Cl^- ions, others extrude them, creating a Cl^- gradient across the plasma membrane which in turn is dissipated by functioning anion channels. The net functional balance between Cl^- loaders, extruders and channels determines whether Cl^- has a depolarizing, hyperpolarizing or no action in a given cell. The aim of this study was to systematically confirm, determine or extend the expression pattern of proteins involved in the regulation of $[Cl^-]_i$ in mammalian β-cells and to determine their role in the secretory response.

Materials and methods: We have used conventional and quantitative RT-PCR, Western blotting and immunolocalization studies in human and rodent pancreatic islets and clonal β-cell lines and available pharmacological tools to identify Cl^- transporters and channels involved in the secretory response.

Results: Insulin-secreting β-cells express *Slc12a1* and *Slc12a2* the prototypical Cl^- loaders, the Cl^- extruders *Slc12a4*, several splice variants of *Slc12a5, Slc12a6* and *Slc12a7* and channels such as *Cftr*, *Ano1, Ano2* and all the subunits of the volume-regulated anion channel *Vrac i.e.*, *Lrrc8a*, *Lrrc8b*, *Lrrc8c*, *Lrrc8d* and *Lrrc8e*. Treatment of β-cells with the diuretic bumetanide, inhibitor of *Slc12a1* and *Slc12a2* or with ML077, a recently developed selective inhibitor of *Slc12a5*, blocks or stimulates, respectively, insulin secretion in response to glucose. The stimulation of the secretory response observed upon inhibition of *Slc12a5* with ML077 did not require K_{ATP}-channels, as the effect still occurred in the presence of glibeclamide, but it was reduced by the addition of $CFTR_{inh172}$ and Ani9, inhibitors of *Cftr* and *Ano1/Vrac*, respectively.

Conclusion: Our experiments suggest that insulin-secreting β-cells tightly maintain an outwardly directed Cl^- gradient and that impairing its dissipation through inhibition of *Cftr, Ano1* and *Vrac* blunts insulin secretion in response to glucose.

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Disclosure: **M. Di Fulvio:** Grants; ADA 1-17-IBS-258, WSU 226132.

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Chronic fructose potentiates insulin secretion from beta cells through intra and extracellular ATP signalling mediated respectively by AMPK and P2Y receptors

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Background and aims: Fructose massively appeared as a sweetener additive in the Western diet in the late 70's, preceding the high incidence of obesity and type 2 diabetes. In the hypothalamus, a major intracellular effect of chronic exposure to fructose is energy-sensing alterations mediated by AMPK activation. In pancreatic beta-cells, AMPK is activated in conditions of nutrient deprivation and renders the cell more sensitive to glucose. Although fructose *per se* does not acutely stimulate insulin exocytosis, we investigated the effects of chronic exposure to fructose in standard culture medium on beta-cell function.

Materials and methods: INS-1E beta-cells or freshly isolated human islets and islets from WT or pannexin1 knockout mice were exposed for 4 days to 5.5 mM fructose in their respective standard medium. At the end of this pre-

treatment period, we measured AMPK phosphorylation, mitochondrial respiration, intracellular ATP, calcium levels and insulin secretion. We performed immunodetections of KIR6.2, pannexin1 channels and the calcium-mobilizer purinergic P2Y1 receptor.

Results: Chronic exposure of INS-1E beta-cells, WT mice and human islets for 4 days to fructose induced AMPK phosphorylation (1.6-fold, $p<0.05$) and exaggerated insulin secretion in response to intermediate 8.3-11 mM glucose (1.6-fold, $p<0.05$). Correlating with AMPK activation, fructose pre-treatment in INS-1E beta-cells reduced intracellular ATP levels (-30%) without affecting mitochondrial respiration and caused translocation of KIR6.2 to the cell membrane. Stronger plasma membrane depolarization and faster cytosolic calcium oscillations were observed in the fructose-treated cells. Importantly, fructose exposure increased by 30% INS-1E extracellular ATP under stimulatory glucose conditions. This cellular ATP release, potentially mediated by pannexin1 channel, may activate purinergic P2Y receptors. Immunoblot and immunofluorescence analyses showed the presence of pannexin1 and P2Y1 in INS-1E cells and islets. Addition of the ecto-ATPase inhibitor ARL67156 or the P2Y agonist 2MeSADP to naive INS-1E cells and mouse or human islets potentiated insulin secretion stimulated by intermediate glucose, mimicking fructose pre-treatment. Conversely, the pannexin1 inhibitor mefloquin and the P2Y1 antagonist MRS2179 reversed the potentiated secretory response induced by fructose in INS-1E cells and human islets. These results were confirmed using Panx1-KO islets. Moreover, clearance of extracellular ATP and ADP by apyrase also inhibited fructose-potentiating effects.

Conclusion: Fructose treatment induced intracellular starving-like phenotype in INS-1E beta-cells and islets, resulting in AMPK activation and potentiation of intermediate GSIS. This effect was mediated by activation of purinergic P2Y receptors through increased release of cellular ATP by pannexin1 channel.

Disclosure: **T. Brun:** None.

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At low glucose arginine stimulates and sulfonylureas or K^+ depolarisation inhibit glucagon secretion by perifused mouse islets

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Background and aims: Like pancreatic beta-cells pancreatic alpha-cells release their peptide hormone by depolarization-triggered exocytosis and both alpha- and beta-cells express KATP channels. However, the sulfonylurea gliclazide has been described to directly inhibit glucagon release, which might explain its more favorable clinical results as compared to other sulfonylureas. Here, we have investigated the role of depolarizing stimuli and glucose on the interaction between alpha- and beta-cells within isolated islets.

Materials and methods: Batches of 50 freshly isolated NMRI mouse islets were perifused with a HEPES-buffered Krebs-Ringer medium (2 mg/ml BSA) saturated with 95% O_2 and 5% CO_2, which contained the respective secretagogues. The contents of insulin and of glucagon were determined by ELISA (Mercodia) from the same samples of the fractionated efflux.

Results: Decreasing the glucose concentration after 60 min of perifusion with 10 mM glucose to 1 mM led to a transient increase of glucagon secretion before the insulin secretion was diminished. Raising subsequently the glucose concentration from 1 to 30 mM increased the insulin secretion before the glucagon secretion was diminished. In the presence of 1 mM glucose 20 mM arginine had a moderate stimulatory effect on glucagon secretion but not on insulin secretion. Adding either 30 μM gliclazide or 500 μM tolbutamide or 15 mM KCl to the perifusion medium increased insulin secretion before it diminished glucagon secretion. The combined action of 20 mM arginine and 30 μM gliclazide in the presence of 1 mM glucose led to a simultaneous increase of glucagon and insulin release. This increase was modest and transient for glucagon, whereas the insulin secretion remained moderately elevated after an initial peak. Adding 1 μM of the α_2-adrenoceptor agonist clonidine in the continued presence of arginine and gliclazide suppressed insulin secretion and at the same time markedly increased glucagon secretion.

Conclusion: Stimulated secretion of glucagon appears to be an autonomous function of the alpha-cells, which can be abrogated by the paracrine effect of concomitantly stimulated insulin secretion. α_2-Adrenoceptor agonism inhibits insulin but not glucagon secretion. There is no evidence for a direct inhibitory effect of gliclazide on glucagon secretion.

Disclosure: **E. Frueh:** None.

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Inhibition of fatty acid synthase impairs insulin secretion in islets from mice fed both a normal and high-fat diet

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Background and aims: NADPH is an important player in the antioxidant system. It has been proposed to be a mediator in glucose-stimulated insulin secretion in amplifying pathway from pancreatic β-cells. Here we investigate if secretory capacity is correlated with the redox state of the β-cells within intact islets. To address this we measured the acute effect of a FAS (fatty acid synthase) inhibitor - C75 - on insulin secretion, both in chow- and high fat diet (HFD)-fed mice and electrical activity.

Materials and methods: Insulin secretion was measured in isolated islets from chow and HFD (60% fat for 8 weeks) mice with or without C75 (150 μM), at 1 and 11 mM glucose. The membrane potential was recorded in the presence of 11 mM glucose from microdissected mouse islets, using high-resistance microelectrodes, at 11 mM glucose.

Results: C75 reduced insulin secretion at 11 mM glucose by >80 % ($p<0.001$) in both chow and HFD islets; basal insulin secretion was not affected by C75. These results show that FAS inhibition has similar effects under normal and diabetogenic (HFD) conditions in pancreatic β-cells. C75 hyperpolarized (20%; i.e. made more negative) the membrane potential during the inactive phase of the oscillatory electrical activity evoked by 11 mM glucose ($n=4$, $p<0.001$), an effect that peaked at 10-12min. Burst duration (active phase), increased burst frequency (+28%) and reduced the duration of the silent intervals between two successive bursts (-26%). In addition, C75 decreased spike amplitude as well as the threshold by 25% ($n=4$, $p<0.001$) and 17% ($n=4$, $p<0.05$), respectively. The latter parameter reflects a reduction in voltage threshold for action potential firing. Rather different results were obtained upon application of NADPH (150 μM), which increased the interburst potential by 13% ($n=4$, $p<0.001$) and increased burst duration by 45% without affecting the burst frequency or action potential amplitude.

Conclusion: These data are in agreement with the observation that C75 and NADPH have opposing effects on insulin secretion. These results suggest that the metabolic balance in β-cells 'jumps' between FAS and β-oxidation. By inhibiting FAS, pancreatic β-cells become less electrically active which lead to a decrease in insulin secretion. Our observations suggest that FAS inhibition has a deleterious effect on β-cell electrical activity and insulin secretion.

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Disclosure: **J.I. Real:** None.

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Dual amylin and calcitonin receptor agonists exert direct peripheral effects on beta cells, muscle and adipose tissue contributing to their beneficial effects on metabolism

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Background and aims: Dual amylin and calcitonin receptor agonists (DACRA) are novel candidates for treatment of obesity and type 2 diabetes due to their impressive effects on body weight, glucose control and insulin sensitivity. However, the mechanisms behind the DACRA-mediated metabolic improvements are unclear. Here we investigate in which tissue the DACRAs, KBP-042 and KBP-089, have direct effects

using a combination of in vivo activation and ex vivo cultures of healthy and diabetic adipose tissue, as well as pancreatic islets.
Materials and methods: To investigate DACRA mediated tissue activation in vivo, rats were treated with a single subcutaneous dose of KBP-042 (5 μg/kg) followed by collection of metabolic tissue and analysis of signaling pathways by western blot. To investigate whether DACRAs directly mediated secretory function of the pancreatic islets, we used isolated islets from healthy adult rats. The effect of various KBP-042 concentrations (0.1, 1, 10 and 100 nM) were tested on glucose stimulated insulin secretion (GSIS) and arginine induced insulin secretion (ASGS) in freshly isolated islets and islets, which were cultured under glucolipotoxic conditions (GLTX) (22 mM glucose and 500 μM palmitate) with islets cultured in 5.5 mM glucose media as controls. To explore whether DACRAs had direct effect on metabolic activity in adipose tissue, epididymal and perirenal fat pads were surgically removed from lean rats treated with cultured in the presence of KBP-089 and oxidation was measured using Alamar Blue.
Results: Administration of KBP-042 (5 μg/kg) acutely activated Akt signaling in both muscle and epididymal adipose tissue in vivo. We did not observe any differences in Erk and STAT3 signaling activation in muscle and adipose tissue from rats treated with KBP-042. KBP-042 dose-dependently reduced GSIS and ASGS in freshly isolated islets. In the islets cultured under glucolipotoxic conditions, GSIS was significantly attenuated compared to the islets cultured in normal control media. Impressively, KBP-042 was able to preserve the secretory function of the GLTX islets when co-cultured for 24 hours. The GSIS was dose-dependently higher in the islets, which had KBP-042 present during culturing. Finally, KBP-089 significantly increased the metabolic activity in both perirenal and epididymal adipose tissue, hence indicating an increased energy expenditure.
Conclusion: In conclusion, we show that KBP-042 preserves the secretory function in GLTX cultured islets. Furthermore, KBP-042 acutely activates Akt signaling in muscle and epididymal adipose tissue, indicating a beneficial metabolic effect of KBP directly in metabolic tissues, and finally, that KBP-089 might have a direct effect on mitochondrial oxidation. Thus, KBP-042 directly mediates activation in metabolic tissues and directly modulates pancreatic islets, which is highly promising and warrants for further studies.
Disclosure: **K. Henriksen:** Stock/Shareholding; Nordic Bioscience.

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Effect of ARHGAP21 reduction upon glucose-insulin homeostasis and body composition of diet-induced obesity in C57BL/6 mice

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Background and aims: GTPase activating proteins (GAP) seems to impact glucose-insulin homeostasis. Inhibition of GAP ARHGAP2 in pancreatic islet from neonate mice, improves pancreatic beta cell function. However, the possible role of ARHGAP21 in glucose homeostasis remains unknown. Here we used an ARHGAP21 haplodeficient mice, aiming to explore the involvement of ARHGAP21 in glucose-insulin homeostasis.
Materials and methods: 30 days old C57BL/6 mice, controls and ARHGAP21 haplodeficient mice were fed on chow diet (Ctl and Het) or a high-fat diet (Ctl-HFD and Het-HFD) during 10 weeks. We analyzed body composition considering body weight gain during all experimental period and also measuring perigonadal fat content after euthanasia. We also evaluated the glucose-insulin homeostasis through glucose and insulin tolerance test, as well as assessing fasted glycemic and insulinemic levels. Further, we analyzed the pancreatic islet function and morphology focuses on pancreatic beta cell insulin secretion and size. To analyze the data we used the Student´s t-test. Data are mean ± SEM, and the difference between the groups were considered statistically significant if $P \leq 0.05$.
Results: We did not observe any difference between the Ctl and Het animals fed on chow diet. As expected, Ctl-HFD mice developed the deleterious effects inherent in the consumption of the high fat diet. Interestingly, Het-HFD animals did not become obese (34,96 g ± 0,83 Ctl-HFD x 28,93 g ± 1,07 Het-HFD) and had less accumulation of fat deposits (4,58 % body weight ± 0,25 Ctl-HFD x 2,66 % body weight ± 0,54 Het-HFD), which probably contributed to lower insulinemia of fasting (0,75 ng/μL ± 0,11 Ctl-HFD x 0,37 ng/μL ± 0,09 Het-HFD), insulin sensitivity (1,46 kITT ± 0,36 Ctl-HFD x 3,83 kITT ± 0,78 Het-HFD) and glucose tolerance (30194 AUC ± 1727 Ctl-HFD x 24111 AUC ± 1381 Het-HFD). As no compensation was required for the pancreas, these animals had lower insulin secretion (0,93 ng/μL ± 0,08 Ctl-HFD x 0,65 ng/μL ± 0,03 Het-HFD) and lower beta cell area (2470 square μm ± 64 Ctl-HFD x 1268 square μm ± 114 Het-HFD).
Conclusion: Taken together, our study indicates a possible role of ARHGAP21 in whole body metabolism, impacting on body weight gain, as well as glucose-insulin homeostasis in high fat diet-fed mice. The data suggest that GAP protein member as a potential candidate to prevent and treat obesity and related diseases.
Clinical Trial Registration Number: 3783-1
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Disclosure: **G.M. Soares:** Grants; CNPq, Capes, FAPESP.

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The effect of islet culture on the biphasic pattern of fuel-induced insulin secretion

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Background and aims: The insulin secretion pattern by mouse islets is reported to differ from that of human and rat islets in that a strong first phase is followed by an elevated plateau instead of a slowly ascending second phase. This has led to the argument that mouse islets or beta cells may not be a good experimental model. Here, we have tested the role of islet culture on shaping the kinetics of insulin secretion.
Materials and methods: Mouse islets were isolated by collagenase digestion and selected by hand-picking. The culture duration was 22 h in RPMI 1640 with 10% FCS. The glucose concentration was 5 mM throughout. The kinetics of secretion was measured by islet perifusion and ELISA of the fractionated efflux. The kinetics of the cytosolic Ca^{2+} concentration and of the NAD(P)H- and FAD-autofluorescence were measured by epifluorescence microscopy of perifused islets.
Results: Raising the glucose concentration from 0 to 30 mM produced a modest initial increase in insulin secretion (from 11 to 24 pg x $islet^{-1}$ x min^{-1}) of freshly isolated islets, which was followed by a continuous increase up to 95 pg x $islet^{-1}$ x min^{-1}. Using the same protocol with cultured islets 30 mM glucose generated a marked first phase (70 pg x $islet^{-1}$ x min^{-1}) which after 15 min receded back to a moderately elevated plateau (27 pg x $islet^{-1}$ x min^{-1}). When 10 mM α-ketoisocaproic was the stimulus, fresh islets responded with a prompt, strong and sustained secretion, whereas cultured islets generated a pattern which closely resembled that of 30 mM glucose. The kinetics of the NAD(P)H/FAD- autofluorescence differed in that the NAD(P)H-increase but not the FAD-decrease by 30 mM glucose occurred about 5 min earlier and was significantly larger in cultured islets, but the overall pattern was unchanged by the cell culture period. Similarly, the modest increase of the NAD(P)H/FAD- autofluorescence by KIC was markedly enhanced in cultured islets, but retained its overall pattern, in particular the sluggish decrease upon wash-out. Finally, $[Ca^{2+}]_i$ measurements showed that the resting values were lower after culture, but the square wave increase upon glucose

exposure and wash-out was visible with both, freshly isolated and cultured islets.
Conclusion: Since freshly isolated mouse islets show an ascending second phase with glucose and a fast and strong initial response with KIC, the uniform response pattern with a plateau after a marked first phase is shaped by the cell culture period. Cell culture may not simply relieve the stress of collagenase isolation but also leaves an imprint on the metabolic memory of the beta cell. The effect on the secretion kinetics is not reflected by the parameters of oxidative phosphorylation and $[Ca^{2+}]_i$ and may therefore be mediated by the signals of metabolic amplification.
Disclosure: **M. Morsi:** None.

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Formation of peudoislets induces incretin responsiveness in incretin-unresponsive clonal beta cells: possible role of an amino acid transporter

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Background and aims: Cell-to-cell interactions between pancreatic beta cells are essential for normal regulation of insulin secretion. We previously found that incretin responsiveness was induced by formation of pseudoislets (PIs) in otherwise incretin-unresponsive monolayer-cultured cells (MCCs). In this study, we tried to elucidate the mechanism of the induction of incretin responsiveness in PIs.
Materials and methods: PIs were formed from incretin-unresponsive mouse clonal beta cells (MIN6-K20) by seven-day culture on ultra-low attachment dishes. We examined morphology, contents of ATP, cAMP and insulin, and insulin secretion, and also performed RNA sequencing and metabolome analysis. We evaluated the effect of oxamic acid (OA), an inhibitor of the malate-aspartate shuttle, on glutamate production and insulin secretion. We also examined a possible role of amino acid transporters in insulin secretion from MCCs and islets of ob/ob mice, an animal model of obesity and diabetes.
Results: PIs showed well-developed mitochondria and increased contents of cellular metabolites, ATP, cAMP, and insulin. Both GLP-1 and GIP did potentiate insulin secretion from PIs in a concentration-dependent manner. OA treatment inhibited glutamate production by glucose and abolished incretin/cAMP-induced insulin secretion. Expression of a neutral amino acid transporter was markedly up-regulated in MCCs while it was down-regulated in PIs. Inhibition of the transporter increased cellular glutamate content and induced incretin responsiveness in MCCs. The inhibition also increased incretin-induced insulin secretion from islets of ob/ob mice.
Conclusion: Our study indicates that increased cellular metabolism by formation of PIs confers incretin responsiveness, in which down-regulation of an amino acid transporter contributes to the accumulation of cellular glutamate.
Supported by: MEXT
Disclosure: **N. Yokoi:** None.

PS 018 Insulin secretion and exocytosis II

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The selective serotonin reuptake inhibitor fluoxetine improves glucose homeostasis by promoting insulin secretion and maintaining functional beta cell mass

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Background and aims: Management of depression using the serotonin reuptake inhibitor fluoxetine has been reported to cause reductions in plasma glucose and glycated haemoglobin that are independent of changes in food intake and body weight. Fluoxetine reaches steady state concentrations in plasma of approximately 2μM. Its use at concentrations up to 75μM is reported to be cytotoxic in β-cell lines *in vitro*, but there is no information on whether physiologically relevant concentrations of fluoxetine play a role in regulating β-cell function and glucose homeostasis. This was investigated in the current study.
Materials and methods: MIN6 β-cells or isolated mouse islets were exposed to increasing concentrations of fluoxetine (0.1μM-10μM) for 24-72h. Cell viability was assessed by ATP quantification, β-cell proliferation was determined by quantifying BrdU incorporation and islet cell apoptosis was examined by measuring caspase 3/7 activities. Dynamic insulin secretion was quantified by radioimmunoassay after perifusion of isolated mouse and human islets in a physiological salt solution in the absence or presence of 1μM fluoxetine. In *in vivo* experiments, ob/ob mice were administered 4 doses of fluoxetine (10mg/kg body weight) or DMSO (vehicle) intraperitoneally over the course of 2 weeks prior to being subjected to intraperitoneal glucose tolerance tests following a single administration of glucose (2g/kg body weight) in the presence of fluoxetine or DMSO.
Results: While 24h exposure to 10μM fluoxetine significantly reduced ATP synthesis (control: 4,538,119±193,788 luminescence units; +10μM fluoxetine: 154,230±18,935 n=5; P<0.01) β-cell viability was not compromised by 0.1 and 1μM fluoxetine, even after incubation for 72h (0.1μM fluoxetine: 4,521,279±134,752; 1μM fluoxetine: 4,410,727 ±115,886; n=5; P>0.5). In perifusion experiments 1μM fluoxetine induced rapid and sustained potentiation of glucose-induced insulin secretion from mouse (AUC: 20mM glucose: 654±78; +1μM fluoxetine: 1202 ±108; n=4; P<0.05) and human (AUC: 20mM glucose: 1320±17; +1μM fluoxetine: 2567±73; n=4; P<0.01) islets. Furthermore, 1μM fluoxetine induced a 1.10±0.03 fold increase in BrdU incorporation (n=10; P<0.05) in MIN6 β-cells and a 38±9% reduction in caspase 3/7 activities in mouse islets (n=8; P<0.01). *In vivo* experiments indicated that intraperitoneal administration of fluoxetine improved glucose handling in ob/ob mice (blood glucose concentrations, control vs fluoxetine; T=0: 6.7±0.8mM vs 6.5±0.5; P>0.5; T=210min: 36.3±8.5mM vs 16.5±2.4; P<0.05; n=5).
Conclusion: These data demonstrate that physiologically relevant concentrations of fluoxetine are well tolerated by β-cells *in vitro* for at least 72 hours. Acute exposure to fluoxetine stimulated insulin secretion, increased β-cell proliferation and reduced apoptosis, while delivery to mice *in vivo* improved glucose tolerance. These data are consistent with a role for fluoxetine in regulating glucose homeostasis through direct effects at β-cells. Repurposing of fluoxetine thus represents a novel therapeutic strategy for the management of type 2 diabetes.
Supported by: Diabetes UK
Disclosure: **B. Liu:** None.

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Mechanotransduction is involved in glucose-stimulated insulin secretion

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Background and aims: The consensus model for glucose-stimulated insulin secretion is incomplete, ion channels other than the K_{ATP} channel are needed for generating the glucose-dependent depolarization. Several reports indicate that glucose stimulation causes a slight increase in beta cell volume. Cell swelling increases membrane tension and such a mechanical signal can be transduced into electrical signals by mechanosensitive ion channels. Piezo1 and 2 were discovered as a new class of mechanosensitive channels and non-selectively allow passage of Ca^{2+}, K^+ and Na^+, which generates an overall depolarizing effect. These channels could provide the ion conductance missing in the beta-cell consensus model. Here we have explored the role of mechanotransduction in insulin secretion.
Materials and methods: RNA sequencing; qPCR; Western blot; ELISA; Ca^{2+} imaging
Results: PIEZO1 exhibited 20% increasingly altered expression in islets from individuals with known type 2-diabetes (T2D) (among the 7.6% most differentially expressed genes, p=0.012; non-diabetes: n=114, diabetes: n=17). PIEZO1 positively associated with INSULIN (R^2=0.33, p=6.1e-13, n=131). The mRNA expression level of Piezo1 in db/db mice showed similar tendency by increasing 82% (WT: 0.034 vs db/db: 0.062, p<0.05, n=5, 3, respectively). In contrast, prolonged (48h) glucose treatment in INS-1 832/13 cells significantly decreased the expression of Piezo1 by 65% comparing with 2.8 and 20mMG treatment (2.8mMG: 100%, 5mMG: 80.19%, 10mMG: 43%, 20mMG: 34%, n=3), as well as the protein level (2.8mMG: 1.2, 5mMG: 0.9, 10mMG: 0.7, 20mMG: 0.5, n=5). Insulin secretion was markedly inhibited in Piezo1-silenced cells (NC: 359 vs KDp1: 199, p <0.05, n=4), but not when Piezo2 was silenced (NC: 359 vs KDp2: 350, n=4). Piezo1-agonist Yoda1 (25 μM) stimulated insulin secretion at basal (2.8mMG; DMSO: 32.5 vs Yoda1: 64.7 ng insulin /mg protein /h, p<0.001) and stimulated conditions (16.7 mMG; DMSO: 318.4 vs Yoda1: 527.2 ng /mg /h, p<0.05, n=7). Percentage of secreted insulin was significantly suppressed by Piezo1 inhibitors at basal (2.8mMG; untreated: 0.57% vs GsMTx4: 0.4%, p<0.01, Ruthenium Red: 0.3%, p<0.0001) and stimulated conditions (16.7mMG; untreated: 5.9% vs GsMTx4: 3%, p<0.05, RR: 3.2%, p<0.05). Silencing of Piezo1 did not affect depolarization-evoked Ca^{2+} signaling (peaks; NC: 4.4 vs KDp1: 4.5, n= 29), when stimulated using a high-K^+ solution of the same osmolarity. By contrast, glucose-stimulated Ca^{2+}-signals were impaired by Piezo1 knockdown (glucose AUC; NC 829 vs KDp1: 642, p<0.0001, n= 46). Piezo1- agonist Yoda1 stimulated Ca^{2+} signals significantly (peaks; DMSO: 1.44 vs Yoda1: 2.58, p<0.0001, n=61).
Conclusion: We explore the involvement of mechanotransduction in the stimulus-secretion coupling of the pancreatic beta cell. Our findings suggest that: 1) Piezo1 current contributes to the basal depolarizing tone, in particular in INS-1 832/13 cells that have modest K_{ATP} currents and exhibit spontaneous action potentials also at basal glucose; 2) Piezo1 is part of the nutrient-sensing machinery of the beta-cell. These results rectify many of the shortcomings of the established consensus beta-cell model and offer strong support for mechanotransduction playing an important role in glucose stimulated insulin secretion, as well as being involved in determining the risk for T2D. Further experiments are underway to find the reason of upregulation of PIEZO1 expression in T2D and explore the regulation network of PIEZO1.
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Disclosure: **Y. Ye:** None.

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Knockdown of beta1-integrin affects exocytotic proteins and integrins expression in pancreatic beta cells

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Background and aims: The β1-integrin receptor provides a physical basis for cell-cell and cell-ECM contact and influences cell differentiation, migration, proliferation, function, and survival. *In vitro* functional blockade of β1-integrin results in inhibition of islet cell adhesion, islet architecture disruption, and a significant increase in islet cell death in both human and rodent systems. Mouse models of conditional postnatal β1-integrin deficiency in collagen I producing cells (β1KO) or beta-cells (MIPβ1KO) demonstrated a significant reduction in beta-cell mass with impaired glucose tolerance and insulin secretion. However, the role of beta-cell β1-integrin in the recruitment of exocytotic molecules that are required for insulin release and trafficking of other integrins expression remains to be determined. The aim of the current study is to examine whether loss of β1-integrin in beta-cells influences exocytotic protein recruitment and integrin expression in β1-integrin KO islets and cultured INS-1 cells.
Materials and methods: Adult pancreata and islets (10-12 weeks of age) were collected from both inducible β1KO or MIPβ1KO mice and their counterpart wild-type (WT) littermates. INS-1 cells were cultured on matrix-coated plates with or without an immunoneutralizing β1-integrin antibody treatment. Expression of exocytotic molecules and integrins in the islets and INS-1 cells were analyzed by quantitative RT-PCR and immunofluorescence.
Results: Both β1KO and MIPβ1KO mice displayed impaired glucose-stimulated insulin secretion (GSIS) and a concomitant reduction in Munc18-1, Snap25, and Vamp2 mRNA and protein levels, suggesting an important role for β1-integrin in the regulation of insulin exocytotic machinery. β1KO and MIPβ1KO islets also showed lower levels of α6 and αV integrins on beta-cell membranes compared to WT islets, and, since both α subunits are associated with β1 and other β subunits, indicates that integrin expression and trafficking to the plasma membrane may depend on exocytotic machinery. INS-1 cells cultured on matrix proteins had high adhesion to collagen I and IV matrices and increased exocytotic proteins expression, which correlated with enhanced insulin secretion at basal and GSIS. Blocking β1-integrin decreased INS-1 cell adhesion to the collagen I matrix and reduced the expression of Munc18-1 and SNARE proteins along with other integrins, resulting in significantly lower insulin secretion following GSIS.
Conclusion: We have found that beta-cell β1-integrin deficiency causes dysfunctional insulin secretion that is associated with the impaired recruitment of exocytotic proteins and other integrins. This observation suggests that β1-integrin on beta-cells is a crucial regulator of the exocytotic machinery, integrin expression and trafficking.
Supported by: Natural Sciences and Engineering Research Council of Canada
Disclosure: **J. Li:** Grants; Natural Sciences and Engineering Research Council of Canada.

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Role of StarD10 in beta cell physiology

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Background and aims: Risk alleles in the *STARD10* locus, associated with type 2 Diabetes (T2D), impair glucose-induced insulin secretion and are associated with decreased proinsulin:insulin ratios. We have recently shown that the T2D risk associated with variation at this locus was most probably mediated through reduction in *STARD10* expression in the β-cell. Here we investigate the mechanisms by which STARD10 could affect insulin secretion.
Materials and methods: Islets were isolated from StarD10$^{fl/fl}$-ins1-cre male mice (β*StarD10*KO). Electrophysiology was performed in perforated patch-clamp configuration, on dissociated islets. Correlation analysis of Ca^{2+} signalling was performed using a Pearson R coefficient. Transmission Electron Microscopy (EM) images were obtained from isolated islets after chemical fixation and sectioning using an ultramicrotome.

Results: We have previously shown a decrease in calcium signals induced by glucose in isolated islets of β*StarD10*KO. However, current clamp recordings of isolated β-cell from these animals showed no change in the membrane potentials in response to 3 and 17 mM glucose addition, compared to WT littermates (n=2 mice/genotype). We observed similar levels of cell-cell coordination as measured by correlation analysis of Ca^{2+} signalling in presence of 3 or 17 mM glucose or 10mM KCl. Interestingly, Transmission electron microscopy images of β-cell isolated from β*StarD10*KO mice exhibited altered dense core granule appearance. These animals showed a significant increase in granules showing a rod like structure (WT: 3.5 vs. KO: 13.5 % total granules; $p<0.5$) coupled with a reduction in granules showing the usual dense core, when compared to the WT littermates (WT: 84.2% vs. KO: 72.2%; $p<0.5$).
Conclusion: These data indicate a role of StarD10 in normal insulin crystallisation and granule dense core formation, potentially responsible, at least in part, for the defect in insulin secretion previously observed in islets of β*StarD10*KO mice. The rod like structure in the granules is reminiscent of the phenotype observed in *ZnT8*KO mice, suggesting a potential regulation of Zn transport by STARD10.
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Disclosure: **G. Carrat:** Grants; MRC, Welcome Trust, Royal Society, SFD.

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The HDAC inhibitor MC1568 rescues Hdac7-induced beta cell impairment by improving mitochondrial function
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Background and aims: It is becoming increasingly clear that epigenetics contribute to beta-cell dysfunction in type 2 diabetes (T2D). For example, we recently showed that HDAC7 is upregulated in human pancreatic islets from donors with T2D, and overexpression of Hdac7 in rat islets and clonal beta-cells impaired insulin secretion and cell survival. We could further show that treating Hdac7-overexpressing cells with the HDAC inhibitor MC1568, or co-transfecting the cells with a siRNA targeting Hdac7, rescued the secretory impairment. These findings support the development of HDAC7 specific inhibitors for the possible use in treatment of T2D. The aim of the current study was to further investigate the mechanisms by which MC1568 improves beta-cell function.
Materials and methods: Hdac7 was overexpressed in 832/13 INS-1 beta-cells via plasmid transfection and the cells were treated with 1μM MC1568. Glucose-stimulated insulin secretion (GSIS) was determined by standard methods and mitochondrial function was determined by measurement of cellular ATP and oxygen consumption. beta-cell number and apoptosis were determined by crystal violet staining and measurement of Caspase-3/-7 activity, respectively.
Results: We could verify that Hdac7 overexpression results in impaired GSIS (~50% reduction, $p<0.05$) and this was completely reversed by treatment with MC1568. The inhibitor had no effect on GSIS from control transfected cells however. Hdac7 overexpression also resulted in reduced ATP levels at high glucose levels (~30% reduction, $p<0.05$) and impaired mitochondrial respiration (~25% reduction, $p<0.05$). Treatment with MC1568 normalized ATP levels and improved mitochondrial respiration in Hdac7 overexpressing cells, again without effect on control transfected cells. Lastly, treatment with MC1568 normalized the number of beta-cells in vitro via a complete rescue of Hdac7-induced apoptosis.
Conclusion: In summary, our data suggest that treating Hdac7 overexpressing beta-cells with MC1568 rescues insulin secretion via improving mitochondrial function. MC1568 also improves beta-cell survival. These data further support the development of HDAC7 specific inhibitors as novel therapeutics for T2D.
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Disclosure: **K. Bacos:** None.

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Expression of S561F CDKAL1 variant modifies the constitutive trafficking and affects insulin release in INS1E cells
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Background and aims: Congenital hyperinsulinism (CHI) is a rare disorder (OMIM#256450), characterized by hypoglycaemia due to inappropriate insulin secretion. A Whole Exome Sequencing (WES) analysis performed on CHI patients lacking mutations in ABCC8/KCNJ11 identified a polimorfism in the CDKAL transcript (S561F-CDKAL1 variant). CDKAL is a methylthiotransferase that modifies tRNA(Lys) to enhance translational fidelity of transcripts, including the one encoding proinsulin. Interestingly, CDKAL is a susceptibility gene for type 2 diabetes and CDKAL knock-out (cdkal1 -/-) mice showed impaired glucose homeostasis, thus indicating the protein involvement in beta-cell function. Aim of this work was to understand the impact of the CDKAL1 variant S561F on the insulin content, trafficking and release in pancreatic beta cells.
Materials and methods: Clonal INS1-E cells expressing Wild Type (WT) or S561F CDKAL1 were generated and used as a model to characterize S561F-CDKAL1 impact on beta cell function. The localization of the variant protein was monitored by immunofluorescence and insulin content and release were measured with ELISA. An acridine orange assay was performed to evaluate the constitutive and regulated trafficking and possible alterations in vesicle protein expression were evaluated by western blotting.
Results: Wild type CDKAL1 overexpressed in INS1-E cells localized in the reticular compartment. The S561F variant was similarly confined to the reticular compartment, although its localization was enriched in spot-like structures distributed in the perinuclear region. Insulin content was increased by overexpression of WT CDKAL1 (2 fold over INS1E, $p<0.05$) while it was decreased by S561F-CDKAL1 variant expression. Conversely, insulin release measured in overnight culture medium or in 30 minutes static incubation in normal glucose concentrations was increased in the S561F-CDKAL1 as compared to WT clones (2 to 4 folds increase over WT; $p<0.05$), thus suggesting a different insulin processing/secretion in the mutant CDKAL1. An acridina orange assay performed to measure the constitutive and regulated trafficking in INS1E cells revealed more basal exocytosis in S561F-CDKAL1 than WT clones. Interestingly, the basal release was not further increased by potassium chloride or high glucose. Western blotting experiments revealed up-regulation of proteins involved in the secretion machinery in mutant clones compared to WT.
Conclusion: The S561F-CDKAL1 variant expression leads to an increased basal insulin release in INS1E cell line. Such an increase is associated to a defect in the vesicle trafficking and correlates with altered expression of proteins involved in the secretory machinery , further studies are needed to clarify molecular mechanisms linking CDKAL to insulin processing and membrane trafficking. Our findings confirm the importance of CDKAL1 in insulin release and suggest a possible mechanism by which this variant can participate to the development of congenital hyperinsulinism.
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Disclosure: **E.S. Di Cairano:** None.

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A robust in vitro islet model: long-lived, standardised and glucose-responsive human islet microtissues
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Background and aims: Inherent heterogeneity in pancreatic islet size, cellular composition and function as well as the short *ex vivo* lifespan of both intact and dispersed islets, pose a significant challenge for their use as an *in vitro* model system in diabetes research.
Materials and methods: To address this issue, we developed a standardized 3D islet model by controlled reaggregation of dispersed primary islet cells. Reaggregated islets - homogenous in size, cellular composition and tissue architecture - were cultured in 96-well-plates in a single islet per well format, enabling high-throughput data acquisition with low intra-assay variability.
Results: Our model displayed uniform, highly reproducible and robust glucose-regulated insulin and glucagon secretion across donors for more than 28 days in culture. In perifusion experiments, the step from 2.8 to 16.7 mM glucose induced biphasic insulin secretion with a prominent first phase (35-fold increase) and a sustained, pulsatile second phase (8-fold increase) potentiated by glucagon-like peptide 1, closely mimicking dynamic in vivo insulin secretion. Relative proportion of endocrine cells, quantified by morphometric analysis of tissue sections, closely reflected fractions found within the human pancreas (54.6% β, 35.5% α and 7.2% δ cells). Basal β-cell proliferation observed in reaggregated islets from multiple donors was comparable to previously described rates and was successfully increased with various stimulators. Long-term exposure to β-cell stress inducers, such as increased glucose, free fatty acid or cytokine concentrations, impaired β-cell function and/or islet viability. This impairment was partially restored upon removal of the stress inducer or addition of β-cell supportive compounds.
Conclusion: These results demonstrate that our model is suitable for high-throughput and long-term study of islet function and regeneration in health and disease.
Disclosure: **B. Yesildag:** Employment/Consultancy; InSphero AG.

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In vitro amino acid restriction: a new tool to study protein malnutrition-induced beta cell programming predisposing the development of type 2 diabetes

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Background and aims: Malnutrition predisposes to develop obesity and diabetes. Protein malnutrition reduces pancreatic beta cell mass and proliferation as well as insulin secretion, calcium handling, and the content of proteins related to glucose metabolism and secretion machinery. To deepen the study of mechanisms involved in beta cell programming by protein malnutrition, we aimed to validate an *in vitro* model of amino acid restriction on INS-1E cell line and isolated mouse pancreatic islets.
Materials and methods: INS-1E cells and islets isolated from C57bl/6 mice (by collagenase) were treated for 48 hours in RPMI 1640 medium supplied with 5% FCS and 11 mmol/l glucose (INS-1E) or 10% FCS and 5.6 mmol/l glucose (islets), with 100% (Control - Ctl) or 25% (Malnutrition - Mnt) of amino acids solution (RPMI 1640 Amino Acid Solution 50x R7131 - Sigma) at 37°C in a humidified atmosphere of 95% O_2 and 5% CO_2. We evaluated insulin secretion (RIA), calcium handling (fura-2/AM), protein content (western blot) as well as gene expression (RT-PCR real- time) involved with apoptosis, beta cell metabolism and insulin secretion. Results were expressed as mean ± SEM. Statistical significance was determined using Two-tailed unpaired t-test. $p < 0.05$ was considered significant.
Results: The restriction of amino acid did not alter cleaved caspase 3 protein content (Ctl: 99.98±0.03 %; Mnt: 93.80±8.8 % P value: 0,618). Insulin secretion was reduced in both, INS-1E and islets treated with Mnt solution in presence of stimulatory glucose concentration (INS-1E: Ctl: 0.063±0.006 ng/μg*ml^{-1}; Mnt: 0.038±0.006 ng/μg*ml^{-1}; P value: 0,006. Islets: Ctl: 0.066±0.010 ng/islet*h; Mnt: 0.042±0.008 ng/islet*h; P value: 0,0477). The frequency of calcium oscillation in response to glucose (22.2 and 11 mmol/l) was increased both in Mnt-treated INS-1E and islets (INS-1E: Ctl: 4.486±0.4 oscillation/min; Mnt: 7.462±0.3 oscillation/min; P value: <0,001. Islets: Ctl: 2.778±0.7 oscillation/min; Mnt: 6.300±1.0 oscillation/min; P value: 0,0118). Amino acid restriction, in INS1-E cells, altered the protein content of Sirt 3 (Ctl: 99.9±0.01 %; Mnt: 78.75±8.8 %; P value: 0,0326), PKC (Ctl: 99.9±0.02 %; Mnt: 80.13±5.4 %; P value: 0,0034), phosho-Eif2alpha (Ctl: 99.9±0.03 %; Mnt: 173.8±22.7 %; P value: 0,0311) and Snap 25 (Ctl: 99.9±0.02 %; Mnt: 73.06±5.9 %; P value: 0,0005), proteins known to be modulated by protein malnutrition. Moreover, the expression of insulin gene was reduced in pancreatic islets exposed to amino acid restriction (Ctl: 0.998±0.002; Mnt: 0.787±0.033 relative quantification; P value: <0,001).
Conclusion: We validate a viable *in vitro* model of amino acid restriction in INS-1E cells and isolated mouse pancreatic islets. This model offers a new tool to investigate the mechanisms by which protein malnutrition impairs beta cell mass and function, favoring the development of type 2 diabetes.
Supported by: Capes/ FAPESP/ CEPID-OCRC
Disclosure: **L.M. Gonçalves:** None.

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Metabolism-secretion coupling and kinase array profiling reveal selective changes upon chronic hyperglycaemia and exposure to C16/C18: n fatty acids in INS-1E beta cells

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Background and aims: Chronic exposure to elevated glucose levels and/or free fatty acids impairs beta-cell function, leading to insulin secretion defects and beta-cell death, potentially prompting diabetes. These effects, referred to as glucolipotoxicity, and mechanisms induced by saturated versus unsaturated fatty acids remain unclear. Using a semi-high throughput approach, we investigated the effects of chronic exposure of INS-1E beta-cells to saturated and unsaturated fatty acids, associated with high glucose, on mitochondrial function, glucose-stimulated calcium rise, kinetics of insulin secretion, and untargeted kinase activity.
Materials and methods: INS-1E beta-cells were cultured for 3 days at low 5.5 mM, standard 11.1 mM (control) and high 25 mM glucose in the presence or absence of BSA-complexed 0.4 mM saturated (palmitate), monounsaturated (oleate) or polyunsaturated (linoleate, linolenate) fatty acids. Metabolic fitness was determined by redox activity (MTT assay) and mitochondrial function by oxygen consumption rate (OCR), ATP generation, and proton leak (OCRs) using 96-well Seahorse monitoring. INS-1E beta-cell function was assessed by 96-well online measurements of both intracellular calcium (Fura-2) and kinetics of insulin secretion (luciferase-based C-peptide substitution) upon glucose stimulation. Kinase array (PamStation-12, 144 peptides per array) was used to profile phosphotyrosine (PT) and serine/threonine (ST) kinase activity.
Results: Metabolic fitness of the cells increased along with chronic high glucose. Chronic palmitate (C16:0) and polyunsaturated fatty acids, linoleate and linolenate (C18:2, C18:3), decreased the metabolic fitness of INS-1E cells by 30% at normal and high glucose, while oleate (C18:1) affected this parameter twice as less. Glucose-stimulated OCR was not changed by chronic high glucose, by fatty acids, or the combination of both. However, both palmitate and oleate decreased ATP production by increasing proton leak at high glucose. Palmitate and oleate were also the only fatty acids reducing markedly the calcium signal by nearly 90% against 60-70% for polyunsaturated fatty acids. These effects were not additive to glucotoxicity regarding the secretory function as palmitate and oleate did not alter further insulin secretion under glucotoxic conditions. However, oleate was deleterious at low and normal glucose culture conditions, while palmitate potentiated the secretory response. The different metabolic stresses affected mainly the activity of kinases involved in

signal transduction, cell cycle and stress response. When combined with high glucose, oleate specifically increased both PTK and STK activity in INS-1E beta-cells.

Conclusion: Under chronic high glucose, both the saturated fatty acid palmitate and the monounsaturated fatty acid oleate altered the first steps of metabolism-secretion coupling, while polyunsaturated did not. Such alterations were differently reproduced on the complete development of the secretory response, showing that the different fatty acids selectively affected the amplifying pathway, an observation made at any concentrations of glucose culture. Kinase activity profiling also provides new insight on the differential effects of metabolic stresses revealing a specific signature of glucose and fatty acids on kinase activity in INS-1E beta-cells.

Supported by: CTI

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Increased secretagogin secretion as a response to human beta cell stress

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Background and aims: Therapies promoting the preservation and restoration of functional pancreatic β-cells are desirable for the treatment of type 2 diabetes mellitus (T2D). A challenge for the development of such therapies is the lack of biomarkers to assess changes in β-cell stress with therapeutic intervention. Secretagogin (SCGN) is a Ca^{2+} binding protein, expressed in endocrine cells, particularly in human islets. The exact function of SCGN is still unknown. A previous study has shown significantly elevated SCGN plasma levels in T2D compared to non-T2D individuals. We hypothesized that increased SCGN secretion in T2D individuals may reflect β-cell stress. The aim of this study was to evaluate SCGN as a potential biomarker of β-cell stress using human EndoC-βH1 cells, islets and pancreatic tissue, as well as human islet xenografts transplanted (Tx) mice, subjected to stress conditions.

Materials and methods: Human tissue and primary islets were purchased from Prodo Laboratories Inc. providing islets isolated from donor pancreases with consent from Organ Procurement Organizations. Human pancreatic tissue was double stained using mouse anti SCGN ab and guinea pig anti Insulin ab. Human islets were cultured for 24 hours and subjected to normal (5.8 mM) and high (22 mM) glucose conditions. EndoC-βH1 were transfected with SCGN and Scrambled siRNA oligos and stress was induced by either thapsigargin, tunicamycin or cytokine cocktail (IFN-γ, IL1-β, TNF-α) (n=4). To study human islet in vivo under stress, human islets (1000 IEQ) was transplanted under the kidney capsule to diabetic and non-diabetic nu/nu mice (n=7). At termination, plasma samples were collected. ELISA kits were used to quantify human SCGN, Insulin and C-peptide in plasma samples and culture media. Apoptosis activity was measured using Caspase-Glo 3/7 assay.

Results: Immunohistochemistry confirmed that SCGN co-localized with insulin in the β-cells of the human endocrine pancreas but also with the other islet cell type, while, exocrine pancreatic tissue had no SCGN expression. SCGN was not secreted merely in an insulin dependent manner as determined by correlation to the insulin or C-peptide release in EndoC-βH1 cells, human primary islets and plasma from Tx mice. However, secretion of SCGN from EndoC cells was significantly increased in response to all stressors, including cytokine cocktail (2662 ± 138 pg/mL, $p<0.0001$), tunicamycin (1932 ± 75 pg/mL, $p<0.0001$), thapsigargin (1689 ± 136 pg/mL, $p<0.0001$), compared with DMSO ctrl. (973 ± 17 pg/mL). Silencing of SCGN in EndoC β-cells, resulted in significantly increased apoptosis when induced by ER, but not by inflammatory stress, compared with cells treated with scrambled siRNA stress (tunicamycin 978 ± 12 RLU vs. 667 ± 24 RLU, $p<0.0001$; thapsigargin, 1277 ± 35 RLU vs. 1101 ± 29 RLU, $p = 0.003$; cytokine, 690 ± 84 RLU vs. 691 ± 78 RLU, $p = 0.99$; DMSO ctrl; 430 ± 33 RLU vs. 372 ± 53 RLU, $p = 0.2$). In plasma from Tx mice, hSCGN inversely correlated with hC-peptide levels, showing that a poor glycemic control was related to high levels of hSCGN ($r = -0.79$, $p = 0.036$). SCGN may be released to protect the islets from increased stress but ultimately the engraftment of islets failed.

Conclusion: This study showed for the first time that SCGN showed interesting features as a novel biomarker reflecting islet and/or β-cell stress. However, further mechanistic and T2D cohort studies will be required to confirm these findings.

Disclosure: **S.F. Hansson:** None.

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Complement components C3 and C5 improve islet beta cell function

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Background and aims: The complement system represents one of the major effector mechanisms of the innate immune system. Complement components 3 and 5 (C3 and C5) play essential roles in the complement system, generating C3a and C5a peptides that act as chemotactic and inflammatory factors. These active peptides are mainly associated with immune responses, but there is some evidence that they also regulate glucose homeostasis. The aims of the current study were to determine the mRNA and protein expression of C3/C3a and C5/C5a in islets, and identify the effects of C3a and $C5a_1$ receptor activation on human and mouse islet secretory function and viability.
Materials and methods: The mRNA expression of genes encoding C3 and C5 proteins, as well as CFD and C2 (key elements in the complement system cascade for activation of C3 and C5), and C3a, $C5a_1$ and $C5a_2$ receptors was quantified by qPCR in human and mouse islets. Distribution of C3 and C5 proteins in human and mouse islets was determined by IHC, with insulin, glucagon and somatostatin co-staining. Effects of C3 and C5 proteins and receptor agonists sc-214644 (C3a) and 65121-ANA ($C5a_1$) and antagonists SB 290157 (C3a) and PMX 205 ($C5a_1$) on static and dynamic glucose-stimulated insulin secretion (GSIS) from human and mouse islets were measured by radioimmunoassay. Intracellular calcium and apoptosis were assessed by standard techniques. Activation of C3a and $C5a_1$ receptor was assessed using DiscoverX β-arrestin assays.
Results: C3 and C5 complement proteins and their receptors are expressed by human and mouse islets, and C3 and C5 are mainly co-localised in β- and α-cells. Complement proteins and receptor agonists potentiated GSIS from human islets (20mM glucose: 1.13±0.05 ng/islet/hour; +100nM C3: 1.50±0.10; +100nM C5: 1.49 ±0.08; +1μM C3a: 1.81±0.08; +1μM C5a: 1.87±0.12; p< 0.001) but GSIS was inhibited by 1μM SB 290157 (0.80 ±0.05) and 1μM PMX 205 (0.76±0.05). Similar effects were observed in mouse islets. Single cell microfluorimetry experiments indicated that 1μM C3a and $C5a_1$ receptor agonists increased intracellular calcium at 20mM glucose in mouse islets. C3a and $C5a_1$ receptor agonists also protected human islets against apoptosis induced by a pro-apoptotic cytokine cocktail (No cytokines control: 100±9.5%; cytokines control: 204±23.3%; 1μM C3a + cytokines: 115.0±7.4%; 1μM C5a + cytokines: 109.3±9.3%; p< 0.001) and palmitate (No palmitate control: 100±8.0%; palmitate control: 144± 4.3%; 1μM C3a + palmitate: 99.9± 8.0%; 1μM C5a + palmitate: 110.8± 7.5%; p< 0.001). Similar effects were observed in mouse islets. Conditioned media from islets exposed for 1 h to 5.5 and 20mM glucose stimulated C3a and $C5a_1$ receptor-mediated β-arrestin recruitment.
Conclusion: Our data reveal that C3 and C5 complement proteins and their receptors are expressed by human and mouse islets, and that islets secrete activating receptor ligands in a glucose-dependent manner. Activation of the C3a and $C5a_1$ receptor in islets results in potentiation of glucose-induced insulin secretion, protection against cytokine- or palmitate- induced apoptosis and increased intracellular calcium levels. These observations demonstrate a functional link between activation of components of the innate immune and improved human and mouse β-cell function, suggesting that low level chronic inflammation may improve glucose homeostasis through direct effects on β-cells.
Disclosure: **P. Atanes:** None.

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The p66Shc protein mediates saturated fatty acid-induced insulin resistance in pancreatic beta cells

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Background and aims: Insulin-secreting pancreatic beta-cells are a target for insulin that acts on cell growth, insulin biosynthesis and release. Prolonged exposure of beta-cells to high levels of saturated fatty acids (SFAs) impairs insulin signaling and the insulin-induced increase in proinsulin mRNA. The p66Shc protein is a redox sensor and radical oxygen species (ROS) producer and mediates obesity-induced insulin resistance in adipose tissue and endothelial cells. In this study, we explored the role of p66Shc in SFAs-induced insulin resistance of beta-cells.
Materials and methods: The effects of insulin on insulin biosynthesis and secretion were investigated in rat insulin-secreting INS-1E cells and in human pancreatic islets exposed to 0.5 mM palmitate for 24 h or left untreated. Insulin-induced insulin biosynthesis and secretion were evaluated by detection of insulin cellular content and C-peptide released in the medium, respectively, using specific ELISA kits. The role of p66Shc in palmitate-induced beta-cell insulin resistance was investigated in INS-1E cells both by siRNA-mediated p66Shc gene silencing and adenovirus-mediated p66Shc overexpression, respectively.
Results: When INS-1E cells and human islets were pretreated with 0.5 mM palmitate for 24 h the effects of insulin to augment insulin protein levels and to stimulate C-peptide secretion were abrogated. Under these conditions, we found that palmitate increased p66Shc mRNA and protein levels. The role of p66Shc in palmitate-induced beta-cell insulin resistance was thus investigated. Knockdown of p66Shc resulted in increased C-peptide levels both under basal conditions ($p<0.05$ vs vehicle) and following insulin stimulation ($p<0.05$ vs control), and restored the insulin-induced C-peptide secretion that was reduced by palmitate ($p<0.05$ vs vehicle). By contrast, overexpression of p66Shc inhibited insulin-induced C-peptide secretion under basal conditions and in the presence of palmitate ($p<0.05$ vs mock). Similarly, knockdown of the p66Shc protein restored the insulin-mediated increase in insulin content that was reduced by palmitate ($p<0.05$ vs vehicle), while p66Shc overexpression reduced it both under basal conditions and in the presence of palmitate ($p<0.05$ vs mock). Moreover, palmitate enhanced p70S6K phosphorylation on Thr389 and IRS-1 phosphorylation on Ser307, reducing IRS-1 protein levels due to its degradation, and impaired basal and insulin-stimulated AKT phosphorylation ($p<0.05$ vs control). However, following p66Shc protein knockdown, the effects of palmitate on p70S6K Thr389 and IRS-1 Ser307 phosphorylation were attenuated ($p<0.05$ vs vehicle). By contrast, p66Shc overexpression augmented IRS-1 Ser307 phosphorylation and reduced the ability of insulin to stimulate AKT phosphorylation both under basal conditions and in the presence of palmitate ($p<0.05$ vs mock).
Conclusion: The p66Shc protein plays an inhibitory role in insulin signaling, insulin-induced insulin biosynthesis and C-peptide secretion in beta-cells and mediates SFAs-induced insulin resistance.
Disclosure: **G. Biondi:** None.

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Identification of Negr1 as an age-regulated gene in mouse islets with a role in glucose homeostasis

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Background and aims: The restoration of a functional beta cell mass is a therapeutic target in diabetes. Thus, a deeper knowledge of the mechanisms governing beta cell proliferation is needed. The postnatal stage is a valuable context for the study of factors involved in the regulation of beta cell replication, since a significant expansion of the beta cell mass occurs during this period. Then, beta cell proliferation drops progressively and it is almost nonexistent during adulthood. In this study, we used high-

throughput RNA expression profiling in mouse islets of various ages in order to identify intrinsic factors involved in the decline of beta cell proliferation rates.
Materials and methods: We performed gene expression analysis in pancreatic islets isolated from 2, 4 and 20 week-old (WO) C57B/6J mice using Affymetrix GeneChip HT MG-430 PM Array Plate. We obtained *Negr1* Knockout (KO) mice and studied glucose tolerance and insulin resistance by i.p. glucose tolerance test (GTT) and insulin tolerance test (ITT). Hepatic glucose production was assessed by i.p. pyruvate tolerance test (PTT). Beta cell mass was quantified histologically by morphometry. RNA expression in islets from *Negr1* KO mice was assessed by qPCR.
Results: Microarray results revealed *Negr1* as one of the top upregulated genes with age in mouse islets. *Negr1* followed an opposed pattern of expression to that of the proliferation markers *Ki67* and *Pcna,* which were highly expressed in 2 WO islets and decreased progressively in 4 and 20 WO islets. We measured body weight, glycaemia and performed morphometric analysis of the pancreas in *Negr1* KO mice during the postnatal stage to establish whether these mice have a beta cell phenotype. As reported in previous literature, 4 WO *Negr1* KO mice presented lower body weight. Interestingly, at this age these mice also had lower glycaemia than controls (Ctrl vs KO: 145±4 mg/dl vs 120±5 mg/dl, $p<0.001$). Next, we analyzed glucose homeostasis in 8 WO *Negr1* KO mice. GTT showed that these mice had impaired glucose tolerance (AUC Ctrl vs KO: 9236±959 vs 13445±1079, $p<0.05$). On the other hand, ITT and PTT tests revealed no alterations in insulin resistance or hepatic glucose production in *Negr1* KO mice. Beta cell mass as determined by morphometric analysis was similar in 4 WO *Negr1* KO and littermate controls. Yet, when we assessed the expression of beta cell function and identity genes in isolated islets from this age, we found an increase in the expression of *Mafa* (Ctrl vs KO: 1±0.12 vs 1.9±0.25, $p<0.01$), a significant reduction in the levels of *G6pc2* (Ctrl vs KO: 1±0.09 vs 0.73±0.05, $p<0.001$) and *Slc2a2* (Ctrl vs KO: 1±0.06 vs 0.79±0.06, $p<0.05$).
Conclusion: Impaired glucose tolerance in the absence of insulin resistance and altered hepatic glucose production in *Negr1* KO mice suggests that *Negr1* has a role in the regulation of functional beta cell mass. At the molecular level, increased *Mafa* and decreased *G6pc2* islet gene expression might be involved in the *Negr1* KO phenotype. Intriguingly, *G6pc2* KO mice present lower fasting glycaemia, reduced body weight and slightly impaired glucose tolerance, a similar phenotype to that of *Negr1* KO mice. Further experiments including metabolic tests in high-fat diet fed mice and the study of the regenerative capacity of the beta cells in *Negr1* KO after STZ treatment are needed to describe in detail the role of *Negr1* in beta cells.
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Disclosure: **J. Mir-Coll:** None.

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PKC-mediated control of glutamate signalling in islet of Langerhans in response to hyperglycaemia

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Background and aims: Islets of Langerhans use a sophisticate system of paracrine and autocrine signals to synchronize their activities, among these is glutamate which controls hormone release and β-cell viability, by activating specific glutamate receptors. The extracellular glutamate concentration is shaped by the glutamate transporter GLT1 but the precise mechanisms by which it modulates islet functions is poorly understood. Aim of the proposed research was to verify the impact of acute changes of glucose concentrations on GLT1 function and glutamate signalling in the islet and to verify the molecular mechanisms of this modulation.
Materials and methods: Mouse βTC3 cells and human islets were incubated under different glucose concentrations (from 3 to 16.7 mmol/l glucose for up to 30 minutes), and the localization and activity of plasma membrane glutamate transporters was studied by in vivo imaging, Total internal reflection microscopy (TIRFM) and [3H]-Glutamate uptake. Hormone release was detected by means of ELISA assays.
Results: We found that the acute exposure of βTC3 cell lines human β-cells to high glucose concentrations caused the transporter relocalization in intracellular compartments (assessed by TIRFM). Accordingly, GLT1 transport activity, measured by [^{3}H]D-glutamate uptake, was inhibited by 25±5%. The GLT1 relocalization was prevented by inhibition of PKC activation, a kinase implicated in the control of vesicular trafficking and upregulated by high glucose, indicating that PKC controls the localization and/or activity of this transporter. In line with this possibility, PKC activation by TPA treatment caused a statistically significant reduction in the glutamate uptake. Accordingly, we found that βTC3 pre-treatment with the PKC inhibitor Bisindoleymide abolished the glutamate uptake down-regulation induced by 20 mM glucose incubation. Furthermore, no additive effects were observed when βTC3 cells, maintained in 20 mM glucose, were incubated with the PKC activator TPA
Conclusion: In the context of the islet physiology, glutamate is an important paracrine signals that positively modulate the somatostatin secretion. Being GLT1 the main regulator of extracellular glutamate concentration, its relocalization in intracellular compartments would potentiate the activation of glutamate receptors on δ-cells, thus stimulating the somatostatin release. Understanding the molecular mechanisms controlling glutamate release and clearance in islet of Langerhans may be important to control glucose homeostasis in health and disease.
Disclosure: **C. Perego:** None.

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Pkcδ-induced glucagon secretion in alpha cells

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Background and aims: Type 2 diabetes is known as "bi-hormonal disorder" due to dysregulated insulin and glucagon secretion. Although, protein kinase cδ (Pkcδ) associates with glucagon secretion in pancreatic α cells, the precise molecular mechanism of Pkcδ in α cells remains unclear. Agouti related peptide YY (PYY) hormone expresses in neuron, gut and pancreatic islet δ and PP cells which reduces glucagon secretion by bariatric surgery. To our knowledge, however, the association between Pkcδ and diabetes/PYY is unknown. Thus, the aim of this study is to elucidate Pkcδ-dependent glucagon secretion in pancreatic α cells.
Materials and methods: Glucagon secreting αTC1 cells were incubated with 11 and 25 mM glucose conditions, and subjected to diacylglycerol (DAG) EIISA analysis and western blotting for Pkcδ. Pkcδ was knocked down with shRNA for Pkcδ by using lentiviral vector in αTC1 cells. Pkcδ function was studied by pharmacological and genetic inhibitions by rottlerin and shPkcδ, respectively. Activation of Pkcδ was assessed by cleavage (catalytically active fragment, Pkcδ-CF) and phosphorylation for Pkcδ by western blot. Lentiviral vector harboring wild type (WT) and caspase-3-insesitive Pkcδ (Pkcδ-D327A) were infected to αTC1 cells. Neuropeptide Y 1 receptor (Npy1r) agonist PYY and antagonist BIBP-3226 were employed to evaluate Pkcδ-dependent glucagon secretion in αTC1 cells. Isolated mouse pancreatic islets were incubated with insulin antibody to neutralize insulin action. Glucose and Npy1r antagonist induced glucagon secretion were studied in the presence of insulin antibody with/without rottlerin in isolated islet.
Results: High glucose induced glucagon secretion accompanied with increased DAG production and Pkcδ activation in αTC1 cells. Pkcδ knockdown prevented glucose-induced glucagon secretion in αTC1 cells. Interestingly, high glucose condition resulted in cleavage of Pkcδ in αTC1 cells. Overexpression of caspase-3-insesitive Pkcδ (Pkcδ-

D327A) showed same glucagon secretion pattern compared to that of WT, indicating cleavage of Pkcδ is epiphenomenon regarding to glucagon secretion in αTC1 cells. Npy1r agonist PYY suppressed glucagon secretion in αTC1 cells. On the other hands, Npy1r antagonist increased glucagon secretion and DAG production in αTC1 cells. Analogous to high glucose condition, Npy1r antagonist activated Pkcδ. Isolated mouse pancreatic islets were used to further elucidate the molecular mechanism of Pkcδ. In ex vivo islet study, high glucose reduced glucagon secretion due to increased insulin secretion. Thus insulin antibody was supplemented to neutralize insulin action in isolated islet. Compared to control IgG antibody, presence of insulin antibody was resulted in augmented glucagon secretion in both euglycemia and high glucose conditions, confirming insulin as a suppressor of glucagon secretion. Analogous to αTC1 cells, high glucose and Npy1r agonist induced glucagon secretion in the presence of insulin antibody in islet cells. Furthermore, Pkcδ inhibitor rottlerin suppressed this phenomenon, indicating Pkcδ regulates glucagon secretion in α cells especially in insulinopenic high glucose condition and PYY-Npy1r pathway.
Conclusion: High glucose and suppression of PYY-Npy1r pathway induced glucagon secretion in α cells. Targeting dysregulated glucagon secretion via Pkcδ in diabetes (especially in insulinopenic type 1 diabetes) might be promising approach.
Disclosure: **F. Kei:** None.

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CD36 dependent Vav2, a guanine nucleotide exchange factor activation promotes inflammatory activation in pancreatic beta cells

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Background and aims: Altered metabolism has been implicated in the pathogenesis of beta-cell failure in type 2 diabetes. Of them, many clinical studies have confirmed a positive correlation between plasma and tissue levels of several ceramide species play a major role in inflammatory and stress responses that induce type 2 diabetes. CD36, a class B scavenger receptor initiates the inflammatory signaling in adipocytes and macrophages in diet-induced obesity and in the pathogenesis of type 2 diabetes. Here we investigated the role of CD36 signaling and its impact for the beta-cell inflammation induced by ceramide.
Materials and methods: To address this question, we used INS-1, rat islets and human 1.1b4 pancreatic beta-cells with C2-ceramide (N-acetyl-sphingosine). NF-kB-p65 DNA-binding activity was measured by ELISA-based transcription factor assay. Gene expression was assessed by real-time RT-PCR. Apoptosis was determined by TUNEL In-Situ cell death detection kit. Rac1 activation was measured by non-radioactive Rac1 activation kit (Millipore). NADPH oxidase activity was measured by Lucigenin based chemiluminescence assay. Protein expression levels of Src, Vav2, TXNIP and insulin signaling were measured by western blot.
Results: Exposure of INS-1 and human 1.1b4 beta cells to C2-ceramide (50uM) induced a time dependent increase in Src mediated Vav2 tyrosine phosphorylation, which activates its GEF activity accompanied by expression of active Rac1-GTP ($p<0.05$). Rac1-GTP activation enhanced NADPH oxidase activity (5 fold $p< 0.001$) with a 4.5 fold ($p<0.005$) increase in ROS production. Further, NADPH oxidase activity potentiated C2-ceramide induced nuclear factor NF-kB transcriptional activity (3.5 fold $p<0.001$). This effect was associated with upregulation of TXNIP mRNA levels ($p<0.005$) and downregulation of Insulin ($p<0.05$) and PDX1 mRNA ($p<0.001$) levels. Interestingly, pharmacological inhibition of CD36 by Sulfo-N-succinimidyl oleate (SSO) blocked C2-ceramide induced Src activation and reduced Vav2 GEF activity by decreasing Vav2 tyrosine phosphorylation. Further, reduced expression of active Rac1-GTP resulted in decreased NADPH oxidase activity by ~ 50% ($p<0.005$). Under these same conditions, nuclear factor NF-kB transcriptional activity was strongly inhibited by 2 fold ($p<0.001$). Moreover, inhibition of CD36 downregulate TXNIP mRNA ($p<0.05$) and upregulate the levels of Insulin, PDX1 mRNA ($p<0.05$). Finally, inhibition of CD36 by SSO or Src activation by SU6656 (10uM) reduced INS-1 and human pancreatic 1.1b4 beta cell apoptosis.
Conclusion: Ceramide triggers the CD36 mediated upregulation of Vav2 guanine nucleotide exchange factor (GEF) activity by Src activation. This is associated with enhanced Rac1-GTP- NADPH oxidase activity and a rise in nuclear factor NF-kB transcriptional activity with cell apoptosis. Collectively, our results unveil a novel role of CD36 in early molecular events leading to the ceramide-induced pathogenesis of pancreatic beta-cell dysfunction and failure.
Disclosure: **S. Elumalai:** None.

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RNA-seq analysis of human islets exposed to gluco-lipotoxic conditions identifies transcriptomic changes associated with transient or persistent beta cell dysfunction

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Background and aims: Several gluco-lipotoxic conditions (GLtox) reduce glucose-stimulated insulin secretion (GSIS) and promote beta cell death, which, in association with genetic predisposition, can lead to type 2 diabetes (T2D). We have previously observed that reversal of functional damage of human islets (HI) induced by GLtox occurred after removal of some, but not all, the tested deleterious conditions. Here we describe the transcriptome profile of HI with transient or persistent beta cell dysfunction induced by GLtox.
Materials and methods: For the purpose of the present study we used HI isolated from 8 non-diabetic organ donors (age, 70±11 yrs; gender, 4M/4F; BMI, 23.2±3.4 kg/m^2) cultured with/without 0.5 mM palmitate (P) or P + 22.2 mM glucose (P+G). Islets were studied basally (Bas), after 2 days (2d) of GLtox incubation and following 4 additional days in normal medium (wash-out, WO). Islet RNA (RIN ≥ 7.9) was sequenced by Illumina HiSeq2500; reads (≥198M/sample) were mapped using the OmicSoft Aligner.
Results: Compared to Bas values, GSIS decreased significantly at 2d with both P and P+G exposure. Normalization of GSIS was observed at WO vs 2d with P, but not with P+G. With P-exposed islets, RNA-seq analysis showed that the transcripts differentially (FDR<0.05) expressed were 161 (84 up and 77 downregulated) with 2d treated vs 2d control HI and 3,452 (1,733 up and 1,719 downregulated) with WO vs 2d treated HI. The comparison of 2d P-treated vs 2d untreated islets retrieved 35 pathways (DAVID version 6.8): 8 were upregulated (including Fatty acid metabolism and PPAR signalling) and 27 downregulated (including Cell adhesion molecules and Complement and coagulation cascades). In the comparison of WO vs 2d P-treated HI, 92 pathways were identified: 24 were upregulated (including: Synaptic vesicle cycle, MODY, Insulin secretion, ABC transporters, Insulin signalling, Calcium signalling) and 68 downregulated (including PI3K-Akt signalling, Hippo signalling, MAPK signalling, Notch signalling, Wnt signalling and Apoptosis). With P+G-exposed islets the transcripts regulated were 1,048 (572 up and 476 down) with 2d P+G-treated vs 2d control HI and 20 (4 up and 16 down) with WO vs 2d treated HI. The comparison of 2d P+G-treated vs 2d untreated islets indicated 57 pathways, of which 27 were upregulated (including TNF signaling pathway, Protein processing in ER, NF-kB signalling, Fatty acid biosynthesis and Adipocytokine signalling) and 30 downregulated (including Glutathione metabolism, Metabolic pathways, MODY and Glycolysis/Gluconeogenesis). Only minor changes were seen in the analysis of WO vs 2d P+G-treated HI.
Conclusion: At our experimental conditions, lipotoxicity (0.5 mM P) induced transient deterioration of GSIS from human islets; however,

lipo-glucotoxicity (0.5 P + 22.2 mM G) resulted in persistent functional damage. Different transcriptomic changes were found to associate with the two conditions, indicating pathways to be possibly targeted for prevention or reversal of GLtox-induced beta cell damage.
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Disclosure: **L. Marselli:** Other; Research Support.

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Somatostatin protects against palmitate-induced apoptosis and ER stress in pancreatic beta cells

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Background and aims: Somatostatin (SST) released from pancreatic delta cells inhibits insulin secretion from pancreatic beta cells but little is known about the impact of the peptide on beta cell survival. Anti-proliferative and anti-apoptotic properties have been reported but protective effects of SST have also been observed in retinopathy, acute liver damage and in models of neurodegeneration. Here we investigate a potential role of SST in beta cell survival.
Materials and methods: mRNA expression was assessed by qPCR, beta cell proliferation by immunocytochemical measurements of BrdU incorporation and apoptosis by assessment of 3/7 caspase activity in MIN6 beta cells or by TUNEL in cells from dispersed mouse islets.
Results: MIN6 beta cells and primary mouse islets expressed mRNA for somatostatin receptor 2, which has previously been linked to apoptotic pathways. Pre-treatment of MIN6 cells for 48h with SST14 (10nM-1μM) dose-dependently reduced palmitate-induced 3/7 caspase activity in MIN6 cells (0.5mM palmitate, 20h; 10nM SST: 102±3% palmitate only; 100nM: 92±3, 1μM: 80±3; n=8, P<0.001) and in dispersed mouse islet cells (100 nM SST: 50±9% palmitate only, n=8, P<0.001). In contrast, SST14 (10nM-1μM) did not affect the proliferative rate of MIN6 cells (n=8, P>0.2). Consistent with previously-reported induction of ER-stress in beta cells by palmitate, the mRNA expression of heat shock protein 70 (*Hspa1a*) and the pro-apoptotic, ER stress-induced transcription factor, CHOP (*Ddit3)*, were up-regulated in MIN6 beta cells following 20h incubation with 0.5mM palmitate (301±80 and 313±76% control, respectively). 48h pre-treatment with 1μM SST14 decreased this effect by 27±5 and 15±3%, respectively (n=4, P<0.05). Prolonged (48h) incubation with 2% fetal bovine serum (FBS) similarly led to increased mRNA expression of *Ddit3* (194±20% control, P<0.05), and this was also reduced by SST14 (48h, 1μM: 53±3% 2% FBS only, n=4, P<0.05). Reduction of palmitate-induced 3/7 caspase activity by SST14 was not reversed by incubation in the presence of CYN154860, a SSTR2 antagonist (1μM SST: 71±2% palmitate only; 1μM SST + 100nM CYN: 72±2%; 1μM SST + 1μM CYN: 65±2; 1μM SST + 10μM CYN: 69±3, n=8, P>0.2). In addition, mRNA expression of SSTR2 was not affected by incubation with palmitate (20h) nor with 1 μM SST14 (48h, n=4, P>0.2).
Conclusion: These results suggest that SST14 partially protects against palmitate-induced apoptosis and ER stress and that this effect is not mediated via the SSTR2 receptor.
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Disclosure: **A.C. Hauge-Evans:** None.

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The mitochondrial Ca^{2+} uniporter (MCUa) is required for glucose-stimulated mitochondrial Ca^{2+} uptake and insulin secretion in mouse pancreatic beta cells

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Background and aims: Oxidative metabolism is central to the stimulation of insulin secretion by glucose. Whether mitochondrial Ca^{2+} uptake in pancreatic β-cells potentiates this process, however, remains unclear. MCUa is believed to form the main route of entry for Ca^{2+} into the mitochondria. To test this hypothesis, we have generated β-cell specific MCUa knockout mice and assessed the impact of blocking mitochondrial Ca^{2+} uptake on β-cell function and whole body glucose homeostasis.
Materials and methods: C57BL6 mice bearing MCUa alleles with *floxP* sites flanking exons 11 and 12 were bred with mice bearing Cre recombinase inserted at the Ins1 locus (Ins1Cre). This strategy allowed highly β-cell selective deletion of both MCUa splice variants (KO). Mice bearing floxed MCUa alleles but lacking Cre recombinase were used as littermate controls (WT). Intraperitoneal glucose (1 g/kg) tolerance was measured every four weeks from eight weeks of age, and insulin sensitivity (0.75U/kg) was determined at 8-10 weeks of age. Glucose-stimulated insulin secretion (3 to 17 mM) was examined *in vitro* during perifusion at 37 °C in Krebs-bicarbonate medium. Adenovirus-mediated delivery of a targeted recombinant Ca^{2+} probe, R-GECO, and Fura-Red, were used respectively to record mitochondrial and cytosolic free Ca^{2+} changes in dissociated β-cells during fluorescence microscopy (Olympus IX81 microscope, 40x objective).
Results: Glucose (3 vs 17 mM)-stimulated mitochondrial Ca^{2+} uptake was markedly reduced in KO vs WT mice (AUC: WT, 24.06 ± 0.67 vs KO, 22.12 ± 0.42, $p<0.05$, $n=5$, 3 mice per genotype) while the cytosolic Ca^{2+} responses were unaffected. Glucose (3 vs 17 mM)-stimulated insulin secretion was also impaired from isolated islets of KO mice in comparison to littermate controls (AUC: WT, 0.035 ± 0.001 vs KO, 0.017 ± 0.005, $p<0.05$, $n=$ 3-4, 3 mice per genotype). Paradoxically, male KO mice displayed significantly improved glucose tolerance compared to WT mice at eight weeks of age (AUC: WT, 1329 ± 47.78 vs KO, 1113 ± 46.2 mmol/L*min, $p<0.01$, n=11-15). However, this difference normalised from twelve weeks of age. Body weight, fed and fasting glycaemia and insulin sensitivity did not differ significantly between genotypes.
Conclusion: MCUa is crucial in pancreatic β-cells to allow glucose-stimulated Ca^{2+} uptake by mitochondria and normal glucose-stimulated insulin secretion. These findings thus support the view that increases in mitochondrial matrix Ca^{2+} are required for a full activation of oxidative metabolism, the generation of increases in ATP/ADP and potentially of other mitochondrially-derived coupling factors in response to stimulation with glucose. The apparent compensatory mechanisms which allow maintained or improved insulin production in MCUa KO mice *in vivo* remain to be established.
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Disclosure: **E.A. Haythorne:** None.

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Light-induced pancreatic beta cell proliferation through endogenous opsin signalling

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Background and aims: Diabetes is a disease characterized by the loss of function and number of pancreatic beta-cells with no available cure. A key therapeutic concept is based on regenerating beta-cell mass by islets transplantation or inducing proliferation of beta-cells by growth factors. These therapies, however, are either highly invasive or lead to non-specific induction of cell proliferation and thus linked to carcinogenic potential. Here we propose an alternative approach based on light induced proliferation of beta-cells that offers spatial and temporal precision.
Materials and methods: Using RT-PCR, we studied the expression of opsin proteins in rodent and human pancreatic islets. By using a nucleotide incorporation assay we analyzed the proliferative responses of cell lines, primary murine and primary human pancreatic islets to illumination with blue-green light. The activation of the major proliferative MAPK/Erk and anti-apoptotic PI3K-/Akt pathways after illumination

was assessed by Western blot. Furthermore, we optimized the illumination protocol regarding wavelength- and intensity-dependence for potent proliferative pathway activation.
Results: We found that several opsins, especially panopsin, and to lower levels melanopsin and rhodopsin, are present in rodent and human pancreatic islets. To test whether these opsins have a functional role, we analyzed the response of primary murine and human pancreatic islets to illumination with blue-green light. We determined the percentage of proliferating cells in islets and found increased proliferation after illumination relative to control groups. Illumination furthermore resulted in elevated activation of the major proliferative MAPK/Erk and anti-apoptotic PI3K/Akt pathways, as determined by Erk1/2 and Akt phosphorylation levels.
Conclusion: Taken together, our results show that pancreatic β-cell proliferation can be induced with high spatial and temporal precision using visible light, without addition of exogenous factors or gene transfer, indicating a potential mechanism for a novel therapeutic strategy.
Disclosure: **E. Reichhart:** None.

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Protective role of complement C3 against cytokine-mediated beta cell apoptosis

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Background and aims: Type 1 diabetes is a chronic autoimmune disease characterized by pancreatic islet inflammation and β-cell destruction by pro-inflammatory cytokines and other mediators. The complement system, a major component of the immune system, has been recently shown to also act in metabolic organs, such as liver, adipose tissue, and pancreas. In the present study we identified complement C3 as an important hub of a cytokine-modified complement network in human islets and characterized the role of C3 in β-cell survival.
Materials and methods: RNA sequencing and protein-protein interaction analyses of ten human islet preparations left untreated or treated with pro-inflammatory cytokines IL-1β + IFN-γ were used to identify gene networks modified by cytokines. INS-1E cells, primary rat β-cells, and dispersed human islets were transfected with small interfering RNAs targeting C3 (inhibition of >50%) and subsequently exposed to the same cytokines. Viability assays were performed by nuclear dyes (Hoechst/Propidium Iodide). C3, insulin and CXCL10 secretion were evaluated by ELISA kits. mRNA expression was evaluated by RT-PCR, and modulation of signaling pathways was assessed by Western blot analysis.
Results: C3 was identified as a key hub for a cytokine-regulated network containing 216 proteins. Proinflammatory cytokines induced C3 expression (>6 fold increase, n=4-9; $p<0.05$) and secretion (1.6-7 fold increase, n=6; $p<0.05$) in rodent β-cells and dispersed human islets. C3 inhibition did not modify insulin accumulation in the medium or insulin and PDX1 mRNA expression. On the other hand, C3 silencing exacerbated cytokine-induced inflammation, as evaluated by CCL2, CCL5 and CXCL10 expression and CXCL10 secretion (1.3-8 fold increase, n=4-6; $p<0.05$), and apoptosis both under basal conditions and upon cytokine exposure (1.5-2.0 fold increase, n=4; $p<0.05$). These effects were due to JNK activation and AKT inhibition, as shown by mechanistic experiments based on parallel inhibition (JNK) or up-regulation (AKT) of the downstream signaling molecules. Exogenous C3 addition protected against cytokine-induced β-cell death (30-40% protection, n=4-5; $p<0.05$) and partially rescued the effects of C3 inhibition.
Conclusion: Locally produced C3 is an important prosurvival protein in pancreatic β-cells and its deficiency renders β-cells vulnerable to inflammatory stress.
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Disclosure: **R.S. Dos Santos:** None.

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Localisation of a novel Mst1 inhibitor for the therapy of diabetes by MALDI imaging

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Background and aims: MALDI imaging mass spectrometry (MALDI-IMS), has been established for an unbiased protein distribution analysis in various organs including the mouse pancreas. In this study, we aimed to use MALDI-IMS for a specific localization identification of neratinib, a tyrosine kinase inhibitor, which was identified in a biochemical kinase inhibition screen as potent MST1 inhibitor. Neratinib is currently in Phase III clinical trials for breast cancer targeting Her2/EGFR dual kinases.
Materials and methods: Beta cell survival and protein expression upon neratinib exposure of human islets and INS-1E cells was analysed by western blotting. Glucose homeostasis was analysed by in-vivo GSIS in daily neratinib or vehicle injected multiple low-dose streptozotocin-induced and obese $Lepr^{db/db}$ mouse models of type 1 and type 2 diabetes. MALDI spectra from 10 μm cryo sections were recorded using a Bruker autofleX speed mass spectrometer.
Results: Neratinib inhibited activated MST1 and restored beta cell survival under multiple diabetogenic conditions in vitro (H_2O_2, high glucose and free fatty acid concentrations and inflammatory cytokines IL-1β and IFNγ) in human islets and INS-1E cells. In vivo, neratinib restored normoglycemia, beta cell function, survival and beta cell mass in type 1 (multiple low-dose streptozotocin-induced) and type 2 (obese diabetic $Lepr^{db/db}$) diabetic mice. In both models, daily intraperitoneal injections of neratinib over 30 days significantly reduced fasting glucose levels and improved glucose tolerance, the insulin/glucose ratio, and glucose-stimulated insulin secretion during a glucose tolerance test. The characteristic isotope distribution of neratinib could be detected by MALDI-IMS in single spectra of high neratinib intense regions in the pancreas 4h after neratinib injection in the mice, while no signal came from vehicle injected control mice. The pancreas was the organ with the highest neratinib specific peak intensity.
Conclusion: We show that neratinib, a novel inhibitor of MST1, can be localized in the pancreas after i.p. injection. It represents a robust proof-of-concept for MST1 inhibition therapy in vivo in rodent models of both type 1 and type 2 diabetes.
Supported by: JDRF
Disclosure: **K. Maedler:** None.

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A novel human $CD3^+CD56^+$ regulatory subset: involvement in the pathogenesis of type 1 diabetes

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Background and aims: The key role of regulatory populations in the prevention of autoimmunity and immune mediated diseases has been largely shown. A number of observations indicates that normal peripheral blood of healthy individuals contains a small percentage of lymphocyte population co-expressing the CD56 and CD3 surface markers that characterize human natural killer (NK) and T cells, respectively. Although the biological hallmarks and functional properties of $CD3^+CD56^+$ cells are poor defined, experimental evidence associated this lymphocyte subset with different pathophysiological conditions. Recently we described that the absolute number of circulating $CD3^+CD56^+$ T at disease onset, associated with a higher β-cell activity in T1D one year later. Moreover, we found that number of $CD3^+CD56^+$ cells was reduced in T1D subjects. Overall, these findings suggest a possible regulatory role for this cell subset in T1D pathogenesis. We aim at investigating the functional properties, the precise role and the mechanism of action of $CD3^+CD56^+$ cells in both physiological and autoimmune diabetes.
Materials and methods: $CD3^+CD56^+$ cells were isolated from peripheral blood mononuclear cells (PBMCs) of human healthy subjects (HS) and T1D, by high-performance cell sorting. Phenotype and functional properties of human $CD3^+CD56^+$ cells was evaluated *in vitro*.
Results: In HS, we found that flow-sorted $CD3^+CD56^+$ cells inhibited proliferation, cytotoxicity and interferon (IFN)-gamma production of $CD8^+$ T cells activated via T cell receptor (TCR), both in autologous and allogeneic conditions. Furthermore, it has been observed that down-modulation of $CD8^+$ T cell effector functions required cell to cell contact and not associated with apoptosis induction. Finally, frequency and suppressive ability of $CD3^+CD56^+$ cells were reduced in T1D compared with HS.
Conclusion: Our data unveil a novel lymphocyte subset with regulatory properties that specifically modulate $CD8^+$ T cell response, which numbers and function were impaired in T1D. Our results are conceivable with the hypothesis that altered $CD3^+CD56^+$ number and activity may account for the deranged effector function of $CD8^+$ T lymphocytes, typical of T1D. Thus, monitoring and therapeutic manipulation of $CD3^+CD56^+$ cells may represent an innovative approach to restore immune-tolerance in autoimmunity.
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Double insulin-glucagon positive pancreatic islet cells in type 1 diabetes and the role of cytokines

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Background and aims: Pancreatic islet cells double-positive for insulin and glucagon (DPIGc) have been described in subjects with type 2 diabetes or insulin resistance. Information on DPIGc in human type 1 diabetes (T1D) is limited. We explored the presence of DPIGc in islets of T1D organ donors and studied the effects of a pro-inflammatory milieu.
Materials and methods: Pancreas samples and/or isolated islets from 3 T1D (age: 45±25 yrs; BMI: 25.6±0.5 kg/m^2; duration of diabetes: 12±11 yrs) and 12 non-diabetic (ND; age: 54±19 yrs; BMI: 23.6±4.2 kg/m^2) multi-organ donors were used. Pancreas specimens collected from normoglycemic non-obese diabetic (NOD) mice (16 weeks of age), the human EndoC-βH1 cell line and the rodent INS-1E and MIN6 beta cell lines were also used in selected experiments. Immunofluorescence, confocal and electron microscopy (EM) assessments were performed with tissue samples. Isolated islets from ND were cultured up to 120h with or without the pro-inflammatory cytokines (cyt) IL-1β (50 U/ml) and IFN-γ (1,000 U/ml) and analysed by EM. Beta cell lines were exposed to cyt for 24h and glucagon gene expression was then determined by RNA-Seq and/or quantitative RT-PCR.
Results: Light microscopy studies revealed the presence of DPIGc in T1D pancreatic samples and also in NOD islets with insulitis (approximately 5% of endocrine cells), but these cells were seldom observed in ND individuals. By EM we found that DPIGc were 10±4% (out of 453 cells counted) in T1D and 1±2% (out of 538 cells) in ND islets ($p<0.01$). Cyt-exposed human islets showed a progressive and marked increase of DPIGc (3±2% at 24h, 31±11% at 120h), with decreased proportion of cells containing only insulin granules and an unchanged percentage of cells with only glucagon granules. No apoptotic DPIGc were observed. DPIGc persisted after 48h of cyt removal. On the other hand, cyt exposure did not increase glucagon gene expression in EndoC-βH1, INS-1E or MIN6 cells.
Conclusion: DPIGc are more common in T1D pancreatic islets, possibly deriving from beta cells as a consequence of the pro-inflammatory milieu.

Lack of direct effect of cyt on glucagon expression in beta cell lines suggests that factors derived from the islet microenvironment could play a role in DPIGc formation.
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Disclosure: **M. Bugliani:** None.

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Low-grade inflammatory marker profile is mainly driven by adiposity-related characteristics in patients with LADA, type 1 and type 2 diabetes

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Background and aims: Patients with LADA (latent autoimmune diabetes in adults) share some characteristics with both classical type 1 (T1D) and type 2 diabetic (T2D) subjects. Previous work has shown that the clinical and metabolic characteristics of these subjects are perched between T1D and T2D patients. Recent work reported that, apart from the characteristics of the acquired immunology traits, patients with LADA are indistinguishable from T1D diabetic subjects in terms of innate immunity. The hypothesis of this study was that patients with LADA would also show a differential profile in terms of low-grade inflammatory mediators, according to the specific adiposity- and metabolic-related traits.
Materials and methods: Serum levels of adiponectin, sTNFRII (soluble TNF-receptor II), and IL-6 were measured using ELISA, and standard laboratory procedures were used to measure hsCRP (high-sensitivity C reactive protein) and leukocytes in patients with LADA (n=82), T1D (n=73) and T2D (n=442). Multiple logistic regression and lineal models were performed to analyze associations between T1D, LADA and T2D, inflammatory markers, and clinical-metabolic variables (age, sex, GAD antibodies, BMI, WHR), systolic-BP (SBP), diastolic-BP (DBP), glucose, HbA1c, triglycerides, HDL-cholesterol, LDL-cholesterol and total-cholesterol).
Results: The clinical and metabolic variables in patients with LADA were intermediate between T1D and T2D, except for fasting glucose (higher than in T2D). Further, only sex distribution (p=0.218) and total-cholesterol (p=0.580) showed no differences among groups. There were no significant differences in IL6 (p=0.607) among groups. Levels of adiponectin, leukocytes and hsCRP were not different between T1D and LADA; when comparing T2D and LADA, no differences were found for sTNFRII. In logistic regression models: age (p=0.008), DBP (p=0.047) and HDL-cholesterol (p=0.017) were independently associated with LADA and T1D model. In LADA and T2D model, the variables independently associated were age (p=0.044), BMI (p<0.0001), glucose (p=0.025) and HDL-cholesterol (p<0.001). No association of the type of diabetes with any of the cytokines studied was found. In linear regression model, BMI (p=0.009), DBP (p=0.001), HDL-cholesterol (p=0.003), triglycerides (p=0.038), adiponectin (p=0.031), IL6 (p=0.028) and leukocytes (p=0.007) were associated with GAD levels.
Conclusion: Although patients with LADA showed a differential profile of innate inflammatory markers when compared with T1D and T2D, these differences were explained mainly by adiposity-related inflammatory markers regardless of the type of diabetes.
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Disclosure: **E. Castelblanco:** None.

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Depletion of STAT6 attenuates the cytoprotective action of interleukin-13 in pancreatic beta cells

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Background and aims: Administration of interleukin (IL)-13 prevents the development of diabetes in non-obese diabetic (NOD) mice and it also protects human and rodent pancreatic beta cells against the cytotoxic effects of pro-inflammatory cytokines. Thus, loss of IL-13 signalling may contribute to the demise of beta cells in type 1 diabetes. IL-13 signals through a cell surface receptor to promote the activation of Jak kinase signalling leading to the phosphorylation and activation of a transcription factor, STAT6. Importantly, we have found that STAT6 expression is diminished in the insulin containing beta cells of patients with Type 1 diabetes and we hypothesise that this may contribute to their loss. Here, we have examined the importance of Jak kinases and STAT6 in mediating the cytoprotective actions of IL-13 in beta cells.
Materials and methods: INS-1E cells were employed to investigate the actions of IL-13 in the presence and absence of various cytotoxic stimuli. Western blotting was used to investigate changes in total and phosphorylated-proteins, with cell viability studied by vital dye staining. Signalling via the Jak/STAT pathway was interrupted either by pharmacological inhibition or using STAT6 specific siRNA. Finally, gene expression studies were performed using a Jak/STAT specific RT-qPCR array, with results verified by primer specific qPCR.
Results: Treatment of INS-1E cells with IL-13 led to a rapid (within 15 minutes) increase in STAT6 phosphorylation at Tyr641 and protected the cells against a range of cytotoxic stimuli including; serum starvation (no serum: 46.4± 3.5% cell death, IL-13:28.0 ± 3.3%; $p < 0.001$), 0.25mM Palmitic acid (PA: 91.4 ± 1.5%, PA+IL-13: 75.1 ± 3.3%$p < 0.001$), and a pro-inflammatory cytokine cocktail (PICs (IL-1β, TNFα, IFNγ and IL-6): 64.7 ± 4.3%, PICs+IL-13: 47.4 ± 2.2% $p < 0.001$). Pharmacological intervention with a selective Jak kinase inhibitor, P6, reversed the cytoprotection by IL-13 (no serum: 30.0 ± 1.3%, IL-13: 16.9±0.8%, IL-13+P6: 30.3±1.3%; $p < 0.001$). To confirm the involvement of STAT6 in this response, siRNA mediated knockdown was used. STAT6 knockdown was achieved efficiently (75%; $p<0.05$) and sustained for 5 days. Depletion of STAT6 did not, itself, alter INS-1E cell viability directly (scrambled control: 11.3 ± 1.7%, siRNA alone: 14.3 ± 2.7%, $p>0.05$) but it markedly diminished the cytoprotective actions of IL-13: (no serum + scrambled siRNA: 27.96 ± 3.3%; no serum + STAT6 siRNA: 42.01 ± 2.6%; $p<0.005$). Analysis of gene expression by RT-qPCR array revealed that IL-13 caused the up regulation of multiple genes and, among these, the largest increase was in a gene encoding SIRPα (also called SHPS-1). This response was attenuated by STAT6 knockdown ($p<0.001$).
Conclusion: These findings demonstrate that phosphorylation and activation of STAT6 is required to mediate the cytoprotective effects of IL-13 in beta cells and reveal that this is accompanied by increased transcription of a previously unrecognised STAT6 target gene in beta cells, SIRPα. The data lend weight to the hypothesis that a reduction in STAT6 activation may contribute to beta cell loss during the progression of type 1 diabetes. Therapeutic targeting of this pathway may therefore be a novel means to reduce β-cell loss.
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Disclosure: **K.A. Leslie:** None.

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In-situ immunohistochemical detection of $ZnT8_{186-194}$ reactive $CD8^+$ T cells in the pancreas of nPOD type 1 diabetic donors

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Background and aims: Autoreactive T cells are a hallmark of type 1 diabetes (T1D) pathogenesis and represent key mediators of islet autoimmunity. Insulitic lesions from both T1D donors and NOD mice are enriched with CD8+ Tcells which lead to beta-cell destruction. Previous studies demonstrated that the human leukocyte antigen (HLA)-A2-restricted zinc transporter 8 $(ZnT8)_{186\text{-}194}$ beta-cell epitope is preferentially targeted by interferon-γ-producing CD8+ T cells in T1D patients. Although such autoimmune reactivity in T1D has been previously reported, information is lacking about the pancreatic localization of such $ZnT8_{186\text{-}194}$-reactive CD8+ Tcell in T1D. Therefore, the aim of this study was to identify $ZnT8_{186\text{-}194}$-reactive cells in the pancreas of T1D donors using HLA-A2 multimer (MMr) immunostaining.
Materials and methods: The in-situ MMr immunohistochemical detection method used herein has been previously reported and validated. OCT frozen pancreatic sections from n=4 T1D, n=4 islet-specific autoantibodies positive (aAb+) and n=4 islet-specific autoantibodies negative (CTR) non-diabetic donors were obtained from the nPOD network. PE (Phycoerythrin)-coupled $ZnT8_{186\text{-}194}$MMrs recognizing autoreactive CD8+ T cells were loaded at 1μg/section and incubated at 4°C overnight. Rabbit anti-PE and Goat anti-Rabbit-HRP were used in order to detect MMr binding.
Results: We investigated whether $ZnT8_{186\text{-}194}$-reactive cells were detected in pancreata from nPOD donors. Sections from all 4 T1D cases analyzed and from 2 of 4 aAb+ cases displayed $ZnT8_{186\text{-}194}$ MMr+ cells, while all sections from CTR cases were negative. $ZnT8_{186\text{-}194}$ MMr+ cells were found scattered either within islets or the exocrine tissue. MMr+ cell count per each section analyzed revealed an increased number of positive cells in T1D cases and aAb+ cases compared with CTR; moreover, an increased number of MMr+ cells were found in islets of T1D cases compared with aAb+ donors. Parallel staining of sections with control MMrs loaded with an irrelevant MelanA peptide did not detect any positive cells, confirming ZnT8 specificity.
Conclusion: We detected for the first time the presence of $ZnT8_{186\text{-}194}$-reactive cells in the pancreas of T1D donors, thus suggesting an unprecedented role for these cells in destructive insulitis during autoimmune diabetes.
Disclosure: **G. Sebastiani:** None.

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Increased expression of CO- and NO-producing enzymes in beta cells of obese mice with incipient diabetes

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Background and aims: Our previous observations suggested that carbon monoxide (CO) and nitric oxide (NO) serve as positive and negative modulators, respectively, of pancreatic β-cell function. Here we studied the expression levels of the CO-producing enzymes heme oxygenase 1 (HO-1) and 2 (HO-2) in relation to the nitric oxide (NO) producing enzymes NO synthase 1 (NOS-1) and 2 (NOS-2) in two different animal models with a distinct diabetic phenotype *i.e. ob/ob* and *db/db* at the age of 6 weeks (prediabetic normoglycemic stage) and at 12 weeks (initial hyperglycemic stage) to elucidate a possible role for these enzymes in the early development of spontaneous obese diabetes.
Materials and methods: Fluorescence microscopic analysis of β-cell protein expression levels of the different enzymes in relation to the prevailing plasma levels of glucose and insulin at the different stages of diabetes development. Lean control mice were included. Each group consisted of 6-12 mice.
Results: At 6 weeks the basal plasma levels of glucose were essentially within the normal range in all three groups, while the insulin levels were greatly enhanced in both *db/db* and *ob/ob*. Plasma glucose, controls 7.6 ±0.5 mmol/l vs *db/db* 9.4±0.5 mmol/l and *ob/ob* 9.1±0.6 mmol/l. Plasma insulin, controls 11.1±1.6 pmol/l vs *db/db* 18.1±2.9 pmol/l and *ob/ob* 17.8±3.1 pmol/l ($p<0.05$). β-Cell protein expression of the two NOS-enzymes, especially NOS-2, was extremely high in *db/db* and very low in *ob/ob*. NOS-1 protein expression in controls 93.3±7.2 vs *db/db* 215 ±16.7 and *ob/ob* 97.5±18 (intensity/μm^2) ($p<0.001$ vs *db/db*). NOS-2 intensity/μm^2 was 0.1±0.04 in controls, 167±22.9 in *db/db* and 11.1±3.5 in *ob/ob*, ($p<0.001$ *db/db* vs *ob/ob*). HO-1 was 0.6±0.03 in controls but highly increased in *db/db* (210±22.8) vs *ob/ob* (24.1±5.6) ($p<0.001$). HO-2 was stable in each group although slightly depressed in *ob/ob*. All fluorescence parameters were essentially the same at 12 weeks. The plasma levels of glucose and insulin were now very high in both diabetic groups. Plasma glucose, controls 7.8±0.6 mmol/l vs *db/db* 19.1±0.7 mmol/l and *ob/ob* 18.8±0.9 mmol/l ($p<0.001$). Plasma insulin, controls 17.1±0.7 vs *db/db* 33±1.7 and *ob/ob* 205.8±25.4 ($p<0.001$) for both.
Conclusion: The results suggest that the early development of β-cell demise in *db/db* mice is imposed by a marked increase in the expression of the NOS-enzymes, especially NOS-2. The increase in HO-1 expression seems not to be satisfactory as an antioxidant/antistress factor in *db/db* mice. The leptin-deficient *ob/ob* mice do not react with increased NOS expression and activity, a fact that might be of great importance for their undamaged β-cells and insulin oversecretion throughout their life time.
Disclosure: **I.M. Al-Amily:** None.

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Exendin-4 prevents palmitate-induced cell death via modulation of autophagy in INS-1 cells

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Background and aims: Western diets composed of saturated free fatty acids have been considered as environmental factor contributing to the pathogenesis of type 2 diabetes mellitus (T2DM). Exendin-4 (Ex-4), a glucagon-like peptide-1 receptor agonists (GLP-1 RAs), is an effective therapeutic agent for T2DM and has been proven to protect pancreatic β-cells. Autophagy is essential for the maintenance of β-cell function and mass. Recently, increasing attention is being focused on the effect of autophagy in T2DM. However, the effects of GLP-1 RAs on β-cell functions associated with autophagy also remain elusive. The aim of this *in vitro* study was to evaluate whether GLP-1 RAs can modulate autophagy in β-cells.
Materials and methods: INS-1 cells were treated with 0.5 mM palmitate (PA) and the resultant cell death was measured using MTT assay. Changes in autophagic signaling were measured using autophagy markers like microtubule-associated protein light chain 3 (LC3)-II and p62. To establish the function of Ex-4 on inhibition of autophagy, INS-1 cells were transfected with siRNA against mTOR and raptor, followed by treatment with PA and Ex-4.
Results: Enhanced autophagosome formation was observed upon exposure of INS-1 cells to 0.5 mM PA for 6 h. Treatment with Ex-4 improved the survival of INS-1 cells treated with PA, and reduced the levels of LC II. These findings indicate that autophagy could be activated in PA-treated INS-1 cells, and suggest that Ex-4 might play a protective role in PA-induced cell death through the reduction of excessive autophagy. Knockdown of mTOR and raptor reduced the protective action of Ex-4 against PA-induced cell death, suggesting that mTOR and raptor activation are involved in Ex-4 mediated protection from excessive autophagy in β-cells.
Conclusion: The results of the present study improve the current understanding of mechanisms involved in the protective effects of Ex-4 on pancreatic β-cells and provide evidence for the prevention of β-cell autophagy in clinical practice.
Disclosure: **K. Ahn:** None.

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Beta cells exposed to sera from obese diabetic patients undergoing improved glycaemic control exhibit enhanced survival and diminished formation of reactive oxygen species

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Background and aims: Pancreatic β-cells are highly vulnerable and react to plasma alterations and to the oxidative stress, common conditions in type 2 diabetes mellitus (T2DM) and obesity. Oxidative stress promotes the expression and activation of different matrix metalloproteinases (MMPs) involved in many cellular functions, including proliferation and apoptosis. We aimed at understanding whether factors present in the sera of (1) obese patients with T2DM and of (2) comparable patients that underwent severe weight loss and improved glycemic control affect differentially beta cells characteristics.
Materials and methods: Forty obese T2DM patients were assigned to two groups according to their treatments: (1) intensive medication for diabetes, and (2) laparoscopic sleeve gastrectomy. Serum samples collected from these patients at baseline (0 month) and 6 months after the beginning of the study were subjected to biochemical assays (the content of 8-hydroxy-2'-deoxyguanosine as an oxidative stress biomarker, and hormones, like leptin and adiponectin) and in vitro studies. For the latter, confluent human insulin-secreting 1.1B4 β-cells were chronically exposed to 10% serum samples from the two groups of patients, collected at 0 and 6 months. After 72 h, the following parameters were investigated: (i) gene and protein expression of MMP2 and MMP9 (by quantitative Real Time-PCR and Western blot, respectively), (ii) production of reactive oxygen species (ROS, estimated with DCFH-DA assay), (iii) cellular viability, proliferation and apoptosis (using MTT assay, xCELLigence system, and flow cytometry after annexin V-FITC/propidium iodide staining), and (iv) the presence of insulin (by immunohistochemistry).
Results: Patients: glucose homeostasis was significantly improved in all patients subjected to gastrectomy and only in a few diabetes-treated patients. In addition, all these patients presented significantly decreased serum levels of 8-hydroxy-2'-deoxyguanosine and leptin, whereas the adiponectin level was increased. Cells: compared to β-cells incubated with sera obtained at the beginning of the study, the β-cells exposed to sera collected after 6 months from patients who succeeded to improve their glycemic control exhibited: (i) increased cell proliferation (32%, $p<0.05$); (ii) decreased ROS production (40%, $p<0.01$) and enhanced protein expression of SOD2, indicating an increased antioxidant capacity; (iii) diminished gene expression of MMP2 and MMP9 (30%, and 7%, respectively, $p<0.05$) and (iv) increased intracellular stored insulin.
Conclusion: Together, these findings indicate that factors present in the obese T2DM patients' sera are injurious for pancreatic β-cells whereas the sera of patients who attained an improved glycemic control, diminished leptin and increased adiponectin levels are not detrimental to cells and modulate molecular mechanisms that enhance their antioxidant capacity (decreased MMP2/MMP9 and increased SOD2 expression) and promote cell survival. These results may facilitate the design of future strategies targeted to protect the β- cells and optimize the treatment of obesity T2DM.
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Disclosure: **M. Nemecz:** None.

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The macrophage stimulatory activity of the autoantigenic proinsulin B-chain peptide B11-23 is mediated by the 70 kDa heat shock protein DnaK

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Background and aims: Autologous insulin represents a dominant antigen in the development of immune reactivity against pancreatic beta cells and in the pathogenesis of type 1 diabetes. Under stress conditions as they occur during inflammatory processes, proteins increasingly associate with heat shock proteins (Hsp). Proteins/peptides bound to Hsp exhibit increased immunogenicity. By chaperoning autologous peptides, Hsp may contribute to the development of autoimmunity. As innate immune cells play an important role in the initiation of beta cell-directed immunity, we hypothesized that Hsp70, a prominent member of the HSP family, and (prepro-)insulin-derived peptides synergize in the activation of macrophages.
Materials and methods: Interactions of the 70 kDa Hsp analogue DnaK with preproinsulin-derived peptides (13 amino acids long) were assessed by competition binding assays. Cultivated cells of the monocyte/macrophage lines J774A.1 and MM6 were exposed to various concentrations of DnaK, to proinsulin-derived 13mer peptides or to combinations of DnaK and the 13mer peptides. The release of inflammatory mediators was quantified by ELISA and multiplex technology.
Results: In a screening assay, a set of overlapping 13mer peptides spanning the entire amino acid sequence of preproinsulin was tested for DnaK binding. A DnaK-binding region was identified in each of the functional preproinsulin domains, i.e. in the signal peptide, the C-peptide and the A- and B-chain. Highest DnaK affinity (K'_d 2.2 ± 0.4 μM) was observed for the B-chain core region B11-23, which had previously been identified as dominant target of (auto-)immune reactivity in type 1 diabetes. Exposure of cultivated macrophages to combinations of 10 μg/ml B11-23 and 1 μg/ml DnaK induced the release of higher levels of tumor necrosis factor α (TNFα) and interleukin 6 (IL-6) from J774A.1 (446±143 pg/ml TNFα; 35±12 pg/ml IL-6; all $p<0.05$)) and MM6 cells (757±110 pg/ml TNFα; 572±42 pg/ml IL-6; all $p<0.01$)) than the individual reagents alone (<122 pg/ml TNFα; <10 pg/ml IL-6). Combinations of DnaK and the peptide B18-30 from the C-terminal region of the B-chain had no significant effects on cytokine release. Binding assays showed a 17.5 fold higher DnaK affinity of B11-23 than B18-30 ($p<0.001$). In a screening approach, macrophage activity, as assessed by the release of inflammatory mediators, remained unaffected by DnaK in the absence or presence of a wide panel of other 13mer peptides of the proinsulin A- and B-chains and the C-peptide.
Conclusion: We conclude that the particular macrophage-stimulating potential of combinations of Hsp70 and B11-23 may contribute to the immunodominance of this insulin peptide in the development of beta cell-directed autoimmunity.
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Disclosure: **V. Burkart:** None.

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Are any differences in the clinical manifestation and the prevalence of ZnT8-ab and other antibodies depending on age at onset diabetes type 1 in adults?

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Background and aims: Autoimmune diabetes is a common form of diabetes in childhood and adolescence but it could appear at any age. The aim of the study was to assess the prevalence of diabetes-associated autoantibodies, including zinc transporter 8 antibodies (ZnT8-ab), anti-thyroid antibodies and clinical manifestation of type 1 diabetes (T1DM) (age <35years) and latent autoimmune diabetes LADA (age >35years) at onset of disease.
Materials and methods: The study comprised 119 patients, 66 T1DM (women/men: 18/48; mean age: 26±5 years), LADA (women/men: 19/34; age: 43±8 years). Diabetes was diagnosed based on WHO criteria. To confirm autoimmune diabetes origin autoantibodies were tested: GAD-ab, IA2-ab, ICA, ZnT8-ab, screening for thyroid peroxidase antibodies (ATPO) was performed.
Results: On disease onset there were no differences in BMI, glycaemia, HbA1c value, ketones in the urine, blood pH and TSH between T1DM and LADA patients (p>0,05). In LADA higher C-peptide level (1,2±0,7 vs 1,0±0,5 pmol/ml, p=0,03) was observed. The prevalence of GAD-ab, IA2-ab, ZnT8-ab was similar in both study groups (p>0,05). ICA had been reported more often in T1DM ((81,8 vs 43,3 %, p=0.0007) but ATPO in LADA (35,8 vs 21.2 %, p=0.04). The titres of diabetes-associated antibodies GAD-ab, IA2-ab and ICA were higher in T1DM than in LADA [(691,3±984,4vs280,3±294IU/ml, p=0.004), (731,4 ±1150,2vs 162,4±432,3U/ml, p=0.0001), (254,4±396,0vs49,1±79,1JDF, p=0.0003)]. The prevalence of multiple autoantibody positivity differed significantly between T1DM and LADA. The prevalence of single autoantibody positivity was rare in T1DM (4.5%; ICA only). Commonly, T1DM were positive for two (27.3%), three (40.9%) or four (19,6%) autoantibodies. Conversely, LADA often presented one (30.2%) antibodies (81.2% GAD-ab, 12,5 % IA2-ab and 6,3 % ICA). The number of positive autoantibodies was related to younger age (Rs -0,23), lower C-peptide (Rs -0,28) and higher titres of all antibodies (p<0,05). 45% T1DM and 34 % LADA subjects were positive for ZnT8-ab. ZnT8-ab positive subjects had lower C-peptide level (0,9±0,5vs1,1±0,7pmol/ml, p=0,03) and higher titre of IA2-ab (705,4±1046,2 vs 326,5±846,2U/ml, p=0.03), in T1DM group higher titre of ATPO (97,5±244,4 vs 11,0±34,1U/ml, p=0.04).
Conclusion: Adults classified as T1DM and LADA differ the β-cell function and severity of autoimmune process at the diabetes diagnosis. LADA patients are more prevalent to ATPO. ZnT8-ab positivity is common but not increases the sensitivity for diagnosis of autoimmune diabetes in adults. ZnT8-ab positivity was related to lower β -cell function and higher titre of autoantibodies.
Disclosure: **A. Rogowicz-Frontczak:** None.

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Prevalence and diagnostic value of zinc transporter 8 antibodies in Bulgarian population with type 1 diabetes

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Background and aims: Zinc transporter 8 (ZnT8) is the most recently recognized autoantigen involved in the pathogenesis of type 1 diabetes. The assessment of ZnT8 antibodies (ZnT8-Ab) has become available in Bulgaria in the past two years. To that moment there is no data for the prevalence and diagnostic value of this new immunologic marker for Bulgarian population of type 1 diabetes patients. The aim of the study is to investigate the prevalence and diagnostic value of ZnT8-Ab in a Bulgarian population of type 1 diabetes patients.
Materials and methods: 75 patients (45 males and 30 females), of mean age 37.2 ±11.4 years and mean BMI 23.4±4.3 kg/m^2, of up to 5 years duration of the disease were investigated. In 48% of the patients diabetes was newly-diagnosed or of less than a year duration. Antibodies to glutamic acid decarboxylase (GAD 65-Ab), antibodies to thyrosin phosphatase (IA 2-Ab) and ZnT8-Ab were assessed by a quantitative immunoenzyme assay ELISA. The diagnosis of idiopathic type 1 diabetes was established in antibody negative patients with the additional performance of insulin secretion stimulatory test - venous glucose tolerance test with assessment of immunorective insulin, confirming insulinopenia. Statistical analysis of data was performed with SPSS vs. 21 applying descriptive analysis, chi-square test and logistic regression - stepwise forward method.
Results: One or more of the investigated antibodies were detected in 85.7% of the participants, ZnT8-Ab being the second most prevalent - in 45.7% of the group, following GAD 65-Ab - 77.1% and exceeding the prevalence of IA-2-Ab - 34.3%. Triple negative were 14.3% of the participants. Positive to ZnT8 only were 2.9% which accounts for 16.6% of the cases of idiopathic type 1 diabetes. ZnT8-Ab were significantly more prevalent in the subgroup with newly-diagnosed diabetes and diabetes of less than a year duration - 63.6%, (p=0.04). The assessment of the classical combination GAD 65-Ab + IA-2-Ab identified 83% of type 1 diabetes cases (F=[2,3] 43.4, p<0.001, r=0.462). The use of ZnT8-Ab instead of IA 2-Ab as a second immunologic marker identified more cases of type 1 diabetes - 87% (F=[2,3] 46.6, p<0.001, r=0.486), and the combination of all the three antibodies led to the diagnosis of 92% of the type 1 diabetes cases (F=[3,3] 50.7, p=0.043, r=0.515).
Conclusion: In Bulgarian population of adult patients with autoimmune diabetes ZnT8-Ab is the second most prevalent antibody and the second in diagnostic importance after GAD 65-Ab. The assessment of ZnT8-Ab in addition to GAD 65-Ab and IA 2-Ab reduces the cases of idiopathic type 1 diabetes and the use of ZnT8-Ab as a second immunologic marker in addition to GAD 65-Ab outweighs the established combination of GAD 65-Ab+IA 2-Ab.
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Disclosure: **N. Chakarova:** None.

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The role of autoimmune markers in the development of microvascular complication in patients with diabetes

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Background and aims: The available literature contains little data on the prevalence of autoimmune markers other than antibodies against islet antigens in patients with diabetes. The aim of this study was to evaluate the prevalence of antinuclear antibodies (ANA), anti-neutrophil cytoplasmic antibodies (ANCA), antiparietal cell antibodies (APCA), anti-smooth muscle antibodies (ASMA) in patients with type 1 and type 2 diabetes. Another objective of the study was to assess the correlation between their presence and the degree of metabolic control in both studied groups.
Materials and methods: The study included 100 patients aged 25-75 years, with a body mass index (BMI) in the range of 20-30 kg / m2, hospitalized in the Department of Internal Diseases, Diabetology and Endocrinology, with previously diagnosed diabetes who were assigned to one of two 50 subjects' groups (Table 1). In all study participants performed a panel of biochemical tests - fasting blood glucose, HbA1c, lipid profile, liver enzymes, uric acid, serum creatinine, eGFR by MDRD, microalbuminuria. For the determination of antibodies by indirect immunofluorescence techniques were used (Wistar Cmd). In addition, all patients were examined for neurological diabetic neuropathy and fundus examination.
Results: The presence of ANA antibodies were found in 12 patients (24%) with type 1 and 11 patients (22%) with type 2 diabetes. In the patients with type 1 diabetes, there was a correlation between the presence of anti-ANA and the prevalence of diabetic polyneuropathy. No such relationship was observed in patients with type 2 diabetes. In both type 1 and 2 diabetes, there were no relationships between the presence of these antibodies and the presence of diabetic retinopathy and microalbuminuria. The presence of anti-p-ANCA has been found in 16

patients (32%) with type 1 diabetes and 14 patients (28%) of type 2 diabetes, c- ANCA in 5 (10%) and 2 (4%) subjects. In the group of patients with type 1 diabetes, albuminuria was significantly more frequent in patients with ANCA autoantibodies (41% vs 6%). In the case of neuropathy in patients with type 1 diabetes, the incidence reached 41% versus 15%. APCA occurred with equal frequency in both groups with type 1 and with type 2 diabetes -10%. The presence of APCA was associated with the occurrence of peripheral neuropathy, both in subjects with type 1 diabetes and type 2 diabetes.

Conclusion: Identification of ANA and APCA in patients with type 1 diabetes may be a marker used to isolate a group of patients with the risk of developing diabetic neuropathy. The presence of ANCA antibodies in patients with type 1 diabetes may indicate an increased risk of microangiopathic complications. ASMA antibody does not appear to be significant in the diagnosis of diabetes and its chronic complications.

PARAMETER	TYPE 1 DIABETES average (±SD)	TYPE 2 DIABETES average (±SD)
AGE (years)	41,28 (± 13,37)	62,08 (± 8,99)
BODY MASS (kg)	70,22 (± 1,86)	81,74 (±12,80)
GROWTH (m)	1,72 (±0,09)	1,69 (±0,10)
BMI (kg/m^2)	23,64 (±3,01)	28,31 (±2,13)
WAIST CIRCUMFERENCE (cm)	84,27 (±10,95)	103,22 (±9,71)
HIP CIRCUMFERENCE (cm)	95,19 (±8,17)	107,05 (±6,253)
WHR	0,88 (±0,07)	0,96 (±0,07)
DURATION OF DIABETES (years)	13,48 (±12,96)	11,33 (±9,77)

Disclosure: **J. Litwińczuk-Hajduk:** None.

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Response to IL-2 signalling is specifically impaired in type 1 diabetes

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Background and aims: The response to interleukin-2 (IL-2) is essential for the stability and function of regulatory T cells (Tregs) in vivo. Impaired IL-2 receptor signaling might play a crucial role in the decreased function of Tregs in autoimmunity but might also contribute to the highly increased suppressive capacity of Tregs in cancer. In this study we aimed to examine differences in the phosphorylation of STAT5 as a downstream measure of IL-2 signaling in type 1 diabetes (T1D), rheumatoid arthritis (RA), colo-rectal cancer (CRC) and healthy controls.

Materials and methods: Phosphorylation of STAT5 was examined in patients with T1D, RA, and CRC (10 participants in each group), and in 10 healthy controls. Freshly drawn whole blood samples were stimulated with increasing amounts of IL-2 [0, 0.5, 1, 5, 10 U/ml] for 30 minutes. The expression of phosphorylated STAT5 (pSTAT5) in Tregs was analysed by a multi-parameter FACS staining.

Results: Tregs from T1D patients showed significantly decreased levels of pSTAT5 when compared to healthy controls after stimulation with 0.5 U IL-2 (mean fluorescence intensity MFI: 896 ± 502 vs 3435 ± 1035, p<0.001) and after stimulation with 1 U IL-2/ml (MFI: 1095 ± 942 vs 3835 ± 1415, p=0.004). Tregs from RA patients showed decreased levels after stimulation with 0.5 U IL-2/ml (p=0.043) but not after stimulation with 1U IL-2/ml when compared to healthy controls. No significant differences between cells from CRC patients and controls were found. Stimulation with 5 or 10 U IL-2/ml led to comparable levels of pSTAT5 in all investigated samples.

Conclusion: The response of Tregs towards low doses of IL-2 is highly significantly decreased in Tregs from T1D patients. This might contribute to the loss of tolerance in autoimmunity, such as T1D and RA, whereas low dose IL2 signaling seems not to be involved in the increased suppressive capacity of Tregs from CRC patients.

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Dsiclosure: **B. Prietl:** None.

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Inflammation mediates the deleterious effect of pancreatic ductal cells on human islet transplantation

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Background and aims: We have recently reported that pancreatic ductal cells have a negative impact on the metabolic evolution and grafted beta cell mass in experimental human islet transplantation. Ductal cells produce cytokines that may be detrimental to islet survival, but they also release angiogenic and growth factors that could improve islet survival and engraftment. The aim of this study was to investigate the mechanisms involved in the deleterious effect of pancreatic ductal cells on human islet transplantation.

Materials and methods: Pancreases of cadaveric organ donors were processed for islet isolation and ductal cells were purified from the exocrine fraction. Pancreatic ductal cells clustered into pancreatospheres (DPS) after 3 day-culture in suspension. Human islets were cultured with/without DPS. Glucose-stimulated insulin secretion (GSIS) (ELISA), β-cell apoptosis (TUNEL) and gene expression (RT-qPCR) of inflammation mediators (*il-1β*, *il1ra*, *nlrp3*, *cxcl11*), macrophages (*cd68*, *cd206*), angiogenic factors (*vegfa*), hypoxia (*hif1a*) and growth factors (*igf2*) was determined after 48 hours in culture. Supernatants were collected after 24, 48 and 72 hours. Eight-hundred human islets (Tx Group) or 800 human islets + 600 DPS (Co-Tx Group) were transplanted under the kidney capsule of immunodeficient mice and gene expression was determined in grafts harvested on day 3 after transplantation.

Results: After 48 hours in culture, β-cell apoptosis was similar in islets cultured with/without DPS (Islets: 0.74 ± 0.31%; Islets + DPS: 0.66 ± 0.22%). GSIS was significantly reduced in islets cultured with DPS (stimulation index, Islets: 6.41 ± 1.14; Islets + DPS: 4.40 ± 0.54; p<0.05). *il-1β* and *cxcl11* gene expression was increased in islets cultured with DPS (p<0.05). IL-1β was detected in 12% and 40% of samples after 48 and 72 hours in culture respectively in islets + DPS preparations, whereas it was not detected at any time in islets cultured in the absence of DPS. *il1ra* expression was increased in islets cultured with DPS although the difference did not reach statistical significance. *igf2* expression was almost undetectable in DPS. Macrophage markers, as well as *nlrp3* and *vegfa* expression were similar in islets cultured with/without DPS. Grafts showed similar gene expression profiles than islets cultured with/without DPS. *il-1β* and *il1ra* expression was increased in Co-Tx grafts (p<0.01), while gene expression of macrophages, angiogenic factors, hypoxia and growth factors was similar in Tx and Co-Tx grafts.

Conclusion: Enrichment of human islet cell preparations with ductal cells has a negative impact on beta cell function. The inflammation induced by pancreatic ductal cells may mediate the deleterious effect of ductal cells on islet transplantation outcome.

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Disclosure: **S. Marín:** None.

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The type 1 diabetes candidate gene DEXI modulates virus-induced beta cell dysfunction via regulation of type I interferons

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Background and aims: The chromosome 16p13 region has been associated with several autoimmune diseases, including type 1 diabetes (T1D), and CLEC16A has been proposed as the most likely candidate gene in the region. However recent findings suggest that autoimmune-disease associated SNPs in intron 19 of CLEC16A regulate expression of a neighboring gene, namely DEXI. This suggests that DEXI is the real etiologic gene in the region, potentially playing a role in the pathogenesis of T1D. Against this background, in this work we analyzed the role of DEXI in pancreatic β cell inflammation and death driven by viral infections.
Materials and methods: DEXI was inhibited or overexpressed in rat and human β cells by respectively transfection with specific siRNAs targeting DEXI (inhibition of >70 %) or with adenoviral vectors encoding DEXI (>200-fold increase). Subsequently cells were exposed to a synthetic analog of viral dsRNA (polyinosinic-polycytidylic acid; PIC) or infected with the coxsackie virus B5 (CVB5). Cell viability was evaluated by Hoechst-Propidium iodide staining. Expression of pro-inflammatory chemokines and type I interferons (IFNs) was determined by RT-PCR, and the STAT1 signaling pathway was evaluated by measuring ISRE reporter activity and STAT1 phosphorylation. Cellular localization of DEXI was analyzed in cell fractions by Western blot and interacting partners of DEXI were determined by co-immunoprecipitation experiments. Chemokine expression was also evaluated in DEXI-inhibited cells created by the CRISPR-Cas9 editing technique.
Results: DEXI knockdown protected β cells against PIC- or CVB5-induced apoptosis (30% and 70% protection, respectively; $p<0.001$; n=3-5). Inhibition of DEXI decreased PIC-induced CXCL9, CCL5 and CXCL1 expression in rat and human β cells (by 35-75%, $p<0.01$; n=4-5). In addition, DEXI inhibition decreased PIC-induced STAT1 signaling, as evidenced by decreased phosphorylation of STAT1 and reduced ISRE reporter activation (70% reduction; $p<0.001$; n=4). The decrease in STAT1 signaling may be explained by a significant decrease in PIC-induced IFNβ in DEXI-inhibited β cells. In a mirror image of these experiments, DEXI overexpression increased STAT1 and chemokine expression upon PIC exposure ($p<0.05$; n=3). The function of the protein encoded by DEXI is presently unknown, but bioinformatics analysis predicted the presence of transcription factor-related motifs in its sequence. In line with this, we observed that DEXI is apparently bound to the nuclear chromatin in pancreatic β cells, suggesting a role in transcriptional regulation.
Conclusion: Viral dsRNA up-regulates DEXI in pancreatic β cells, leading to activation of type I IFN signaling, and resulting in increased β cell inflammation and death via STAT1 upregulation. These observations suggest that DEXI, a potential candidate gene for T1D, may act via regulation of local cell innate immune responses in the pancreatic islets.
Disclosure: **I. Santin:** None.

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Therapeutic effects of co-transplanted angiogenic bone marrow-derived spheroids in the intraportal islet transplantation

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Background and aims: We have applied a strategy for co-transplanting accessory cells that possesses higher angiogenic or paracrine activities to islet transplantation. We recently reported that co-transplantation of bone marrow-derived spheroids (BM-spheroid) formed using 3-dimension culture from BM-derived mononuclear cells (BM-MNCs) enhanced therapeutic efficacy of islet in a marginal mass renal subcapsular islet transplantation model. In the present study, we investigated whether co-transplantation of islets with BM-spheroid can improve intraportal islet transplantation outcome in syngeneic mice and assessed the safety and the feasibility of co-transplantation of islets with BM-spheroid in nonhuman primate (NHP) intraportal islet allotransplantation.
Materials and methods: Mice was induced with streptozotocin (STZ) administered intraperitoneally at 180 mg/kg in a citrate buffer solution and cynomolgus monkeys received simultaneous subtotal pancreatectomy and 60-80 mg/kg streptozotocin (STZ) injections to induce diabetes. The efficacy of intraportally co-transplanted BM-spheroids was investigated using a syngeneic marginal mass (300 islets) transplantation in mice, and allogeneic marginal mass (20,000 IEQ per recipient's body weigh in kg) NHP islets were co-transplanted with NHP recipient's autologous BM-spheroids in parallel with immunosuppressive agents after islet infusion. The morphology of intraportally transplanted islet, revascularization of islets and iron-labeled BM-spheroids were examined by immunohistochemistry.
Results: In mice models, portal-spheroid co-transplantation with islets improved the post-transplant outcomes in terms of glucose tolerance, serum insulin levels, and diabetes reversal rate when compared with islet alone. The area of grafted endocrine tissue and vascularization of individual islets within the graft-bearing liver was significantly higher in the spheroid group compared to the islets alone. In NHPs model, post-transplant blood glucose levels were maintained within the target without administration of exogenous insulin only in BM-spheroid group. There were few islets in the recipient's liver in islet alone, whereas more islets were present in BM-spheroid. In both mice and NHPs models, iron-labeled BM-spheroids revealed visible hypointense spots in in vivo MRI, and those of tissue ex vivo MRI corresponded with Prussian blue-positive cells.
Conclusion: Our results suggest that intraportal co-transplantation of BM-spheroids presents a promising strategy for improving the efficacy of islet transplantation. In addition, NHP results suggest the clinical feasibility of intraportal co-transplantation of allogeneic islets and autologous BM-spheroids.
Supported by: NRF grant funded by the Korea government
Disclosure: **B. Oh:** None.

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The impact of pro-inflammatory cytokines on the regulatory landscape of the pancreatic beta cells

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Background and aims: Type 1 Diabetes (T1D) develops as a consequence of genetic predisposition and environmental factors that, combined, trigger an autoimmune assault against the pancreatic β-cells provoking local inflammation (insulitis) and progressive loss of β-cells due to apoptosis. During early insulitis, inflammation contributes to both the primary induction and secondary amplification of the immune assault with inflammatory mediators contributing to the functional suppression and apoptosis of β-cells. The precise mechanisms by which autoimmunity is triggered and aggravated in T1D remain to be clarified. We here seek to elucidate the role of epigenetic gene regulation and its interaction with T1D genetics in the context of the autoimmune mediators that may contribute β-cell death.

Materials and methods: Human insulin-producing EndoC-βH1 cells were exposed to proinflammatory cytokines (IL-1β and IFN-γ) for 48h. RNA-seq in 5 independent cell preparations was assessed to identify transcriptional changes; ATAC-seq in 3 paired replicates and ChIP-seq of H3K27ac were performed under similar experimental conditions to map chromatin remodelling and infer active regulatory elements in each condition.
Results: We found dramatic changes in both gene expression (~1,200 genes differentially regulated, log2FC> 1.5) and chromatin remodeling (~2,000 regions differentially opened, log2FC >1.5). Integration of changes in chromatin accessibility and H3K27ac enrichment unmasked ~4,000 cytokine-induced regulatory elements linked to differential expression of their target genes ($p < 0.001$). Analysis of the sequence composition of the dynamic regulatory landscape unmasked a central role of IRF (motif present in ~70% induced regulatory elements) together with islet-specific transcription factors (NeuroD1, Pdx1 and INSM1) pointing to the activation of specific regulatory networks. Finally, integration of our data with published GWAS studies revealed an enrichment ($P<0.05$) of cytokine induced chromatin remodelling sites at T1D loci.
Conclusion: The present data shows that exposure to proinflammatory cytokines causes profound changes in β-cells gene expression and regulatory landscape. Such changes unmask the activation of specific cis regulatory networks enriched in T1D associated loci, opening the avenue to the identification of new T1D candidate genes acting at the β-cell level.
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Disclosure: **M. Ramos-Rodríguez:** None.

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Everolimus suppress insulin secretion independently of its anti-proliferative or cytotoxic effects reveald by clinical case and in vitro study

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Background and aims: Everolimus, an orally administered mammalian target of rapamycin (mTOR) inhibitor, has been widely used as immunosuppressive and anti-cancer agents. Although it has been reported that everolimus can control hypoglycemia in patients with insulinoma, the underlying cellular mechanisms still remain to be elucidated.
Materials and methods: Here we report the case of a patient with metastatic insulinoma and refractory hypoglycemia that can be successfully controlled with everolimus. In addition, we established *in vitro* experimental models to investigate the effects of everolimus in insulinoma cell line MIN6 and human iPS cell-derived insulin-producing cells.
Results: A 53-year-old woman was admitted to the hospital for the control of refractory hypoglycemia. She had been diagnosed as primary pancreatic insulinoma at the age of 47 and a tumor resection was performed. Although there was no obvious metastatic lesion identified, she was treated with everolimus for prevention of recurrent severe hypoglycemia. In the first laboratory test, three days after the initial everolimus administration, substantial decrease in insulin secretion (IRI (μU/ml)/plasma glucose (mg/dl), 0.138 to 0.066) was observed and there was no hypoglycemic event after that. After the everolimus was administered for five weeks, it was discontinued since drug-induced lung injury was suspected. Notably, two days after the everolinus discontinuation, more than two-fold increase in serum insulin (IRI/PG 0.078→0.162) was observed. Thus, according to rapid decrease in insulin secretion after everolimus administration and reverse response just after its discontinuation, we hypothesized that everolimus can directly suppress insulin secretion on β cells, independently of its anti-tumor effects. To address this question, we examined glucose-stimulated insulin secretion (GSIS) in MIN6 cells treated with everolimus. Everolimus had no effect on basal insulin secretion with lower glucose concentration (2.8mM). On the other hand, insulin secretion at high glucose concentration (16.7mM) was significantly suppressed by everolimus (48% lower than control, $p<0.0001$). Since there was no significant changes in total insulin content and the number of EdU-positive cells, everolimus is likely to inhibit GSIS independently of its cytotoxic or anti-proliferative effects. Furthermore, everolimus suppressed insulin secretion in human iPS-derived insulin-producing cells.
Conclusion: Both the clinical case that we experienced and *in vitro* experiments with MIN6 cells and human iPS-derived insulin producing cells suggest that everolimus directly suppresses insulin secretion independently of its anti-proliferative or cytotoxic effects, which could reflect at least part of its therapeutic effect.
Disclosure: **L. Suzuki:** None.

PS 022 Pancreas and islet transplantation

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Impact of Prolylhydroxylases proteins on survival, function and angiogenesis in pancreatic islets: A target to improve islets graft?

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Background and aims: Islet number dramatically drops during the whole process of transplantation leading to high requirement in islets (>10,000 IEQ/kg) to reverse diabetes. For instance, 70% of the transplanted islets are destroyed within the first few days after transplantation, partly due to a poor revascularization. The aim of this project was to enhance angiogenesis from grafted islets targeting hypoxia pathway involving Hypoxia Inducible Factor-1α (HIF-1α). Angiogenesis is mainly triggered by the target genes of HIF-1α which require the prevention of its degradation to dimerize and translocate into the nucleus. The degradation being taken in charge by the Prolylhydroxylases, inhibition of those proteins could be an interesting approach to increase vascularization of the graft.
Materials and methods: The study was realized on rat pancreatic islets. After islet dissociation using Trypsin 0.5%, cells were transfected (Lipofectamine RNAiMax) with 50 nM of siRNA specific against PHD1, 2 or 3 for 4h. Then, after 3 days of islets reformation, viability was determined using FDA/PI staining. Functionality was assessed by glucose stimulation insulin and glucagon secretion (ELISA kit). PHDs and HIF-1α protein expressions were analyzed by western blotting. Finally, the secretion of VEGF was determined in the conditioned medium by Elisa test. Conditions were compared to following controls: untreated dissociated islets, undissociated islets, islets with lipovector alone and islets lipofected with siNUL. Statistics were performed using non parametric test (Mann Whitney). Data were reported as mean ± SEM for the indicated number of replicates and a p value of <0.05 was considered statistically significant.
Results: Islet viability was improved with siPHD1 or siPHD2 *versus* siNUL (siPHD1: 93 ± 1 %, siPHD2: 92 ± 2 % *vs* siNUL: 84 ± 2 %; $p<0.05$; n = 6). Regarding protein expression, PHD3 was up regulated by siPHD1 and siPHD2 (siPHD1: 230 ± 38 % and siPHD2: 250 ± 25 % of PHD3 expression *versus* CTL; $p<0.001$; n = 5), whereas no difference was observed with siPHD3 as compared to CTL. Moreover, siPHDs did not affect PHD1 expression in comparison to CTL and no PHD2 expression was detected. Furthermore, HIF-1α was significantly up regulated *versus* CTL using siPHD2 (254 ± 112 %; $p<0.01$; n = 7) and siPHD3 (188 ± 60 %; $p<0.01$; n = 12).VEGF secretion, major event for triggering angiogenesis, was increased by siPHD1 (siPHD1: 289 ± 121 % of secreted VEGF *versus* CTL; $p<0.05$; n = 15). Islet functionality was improved by siPHD1 with the restauration of glucose stimulation insulin response (2.8 mM glucose: 0.45 ± 0.12 %, 16.7 mM glucose: 1.60 ± 0.68 % of total insulin content; $p<0.05$; n = 9) *versus* siNUL (2.8 mM glucose: 2.65 ± 1 %, 16.7 mM glucose: 2.31 ± 0.78 % of total insulin content; n = 9). No significant results were obtained by the inhibition of both PHD2 and 3 isoforms. Finally, a physiological glucagon secretion in basal condition was observed with siPHD1, 2 and 3 (CTL: 0.78 ± 0.49 %; siPHD1: 0.56 ± 0.20 %, siPHD2:1.83 ± 0.81 %, siPHD3: 0.73 ± 0.21 % of total glucagon content) *versus* siNUL: 7.43 ± 3.37 % of total glucagon content; $p<0.05$; n = 6).
Conclusion: This work showed that PHDs, and more specifically isoform 1, are involved in glycemia regulation and angiogenesis. Consequently, targeting PHDs pathway could be a promising approach to improve islet survival, function and revascularization during transplantation.
Disclosure: **A. Langlois:** None.

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Progression of diabetic complications in patients with type 1 diabetes

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Background and aims: Evaluation of the effect of simultaneous transplantation of kidney and pancreas (SPK) on the dynamics of the manifestations of late complications of diabetes mellitus (DM).
Materials and methods: The study included 21 patients with T1DM after successful SPK. Posttransplantation period at the time of inclusion of patients was 11 months [8;18]. 14 people received standard triple immunosuppressive therapy during the study period, 7 patients canceled steroid therapy after 6 months. The average age was 34 years old [31;40], duration of T1DM 22 years [20,5;28], the duration of diabetic nephropathy10 years[8;14,5]. Donors of SPK were young 29 [25; 33] and transplant cold ischemia time was 8[7;10]hours. Follow-up period was 4,2 years [3,6;4,3]. Kidney transplant dysfunction markers: Cystatin C (serum, urine); NGAL, KIM-1, podocin, nephrin, IL-18, IP-10 (urine), TGF-β1, MMP-9, VEGF-A, OPN (serum) were defined.
Results: Patients with SPK functioning graft pancreas - 93.75% and kidney -100% of cases. According to a continuous glucose monitoring system using «iPRO2» euglycemia (glycemia(mmol/l)3,9-8,9-89%, lower than 3,9-11%, higher than 8,9-0%) was marked during the 6 days. HbA1c (before study 9.1% [8,7; 11] then decreased to 5.7% [5,55; 5,9], p<0.0001), insulin 12.5 mkE/ml [11,4; 15,3], basal C-peptide - 2.02 ng/ml [1,07; 2,77]; GFR was 87.6[70;94.4] ml/min/1.73 m^2; albuminuria - 2,6[0,6;5,9]mg/g, parathormone - 78[62,3;73,5] pg/ml, blood pressure 110[105; 122] mm Hg/70[64; 80] mm Hg, Hb - 116 g/l[109;128,5]. High level and a negative associated of blood cystatin C with GFR (r = - 0,36, p<0.05) and positive with albuminuria (r=0,40, p<0,05), as well as a direct link of podocin urine-with blood creatinine (r = 0,35, p<0.05) and NGAL with albuminuria (r = 0,35, p<0.05) in patients after transplantation were defined. Association between podocin with MMP-9 (r = 0,46, p<0,05) and NGAL (r = 0,33, p<0,05) indicated correlation of renal microstructures stress factors . In control terms in 10% of cases noted the need for vitrectomy and additional sessions of photocoagulation of the retina about diabetic retinopathy (20%); newly diagnosed cataract (81.25%), secondary cataracts (25%), glaucoma (25%), macular edema (12.5 %). 35% have ulcers of the lower extremities, 4 patients - manifestation of Charcot's osteoarthropathy, 2 patients underwent amputation with resection of the metatarsal bones, the osteomyelitis in the framework of the diabetic foot syndrome. In connection with the development of stenosing atherosclerosis of vessels of lower extremities, one patient underwent balloon angioplasty and stenting.
Conclusion: Despite the euglycemia and renal function normalization after SPK the progression of diabetic complications and high levels of renal graft dysfunction biomarkers were observed. This fact indicates the need of further monitoring and treatment in this category of patients.
Disclosure: **A. Glazunova:** None.

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Cardiovascular autonomic function after simultaneous pancreas-kidney and kidney-alone transplantation in patients with diabetes

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Background and aims: Cardiovascular autonomic dysfunction (CAD) is a common and severe complication of diabetes. Likewise, end-stage renal disease (ESRD) can lead to the development of CAD as dysglycemia as well as uremia both are contributing factors in the etiology of CAD. Moreover, CAD can also be caused by tissue hypoxia. CAD leads to sympathetic nervous system overactivity as well as to a decrease in baroreflex sensitivity (BRS) and heart rate variability (HRV). Kidney-alone (KA) transplantation corrects uremia but simultaneous pancreas-kidney (SPK) transplantation also leads to normalization of blood glucose. The aim of the study was to examine whether CAD is improved at an early stage after KA or after SPK transplantation in patients with diabetes and ESRD. In addition, we wanted to investigate whether tissue hypoxia is improved after transplantation.
Materials and methods: We studied cardiovascular autonomic function in 56 ESRD patients, but only those with diabetes were included in this analysis. Thus 10 patients undergoing KA (59.9 [57.6-67.4] years, median (IQR); 7 men) and 13 undergoing SPK transplantation (36.5 [34.2-49.3] years; 11 men) were selected. BRS, HRV (standard deviation of heart period) and tissue oxygenation (NIRS) were measured before and after (KA 5.9 [3.9-7.2], SPK 3.9 [2.9-7.5] months) transplantation. All SPK and 3 KA patients had type 1 diabetes, while 7 KA patients had type 2 diabetes. In addition, 55 healthy controls (38.3 [32.9-51.0] years, 33 men) were studied.
Results: Before transplantation the autonomic function tests BRS and HRV were reduced but tissue oxygenation was similar in the KA group (BRS 2.1 [1.5-3.2], HRV 4.9 [3.8-5.9], NIRS 62.9 [62.5-62.9]) compared to the SPK group (BRS 2.6 [1.3-4.7], HRV 8.3 [4.7-10.4], NIRS 62.3 [61.9-62.8]). Compared to controls, all values were lower in the KA and SPK groups (controls: BRS 11.7 [8.7-19.3], HRV 28.5 [21.8-39.9], NIRS 65.6 [64.7-66.7]; $p<0.005$). Age-adjusted HRV improved in KA patients after transplantation (7.1 [5.1-8.1]; $p<0.05$). Both BRS and HRV improved in the type 2 diabetic KA patients after transplantation (BRS from 2.1 [1.4-3.0] to 2.6 [1.9-4.0], HRV from 5.2 [2.9-5.8] to 5.3 [4.3-5.9]; $p<0.05$). Furthermore, there was a trend of improvement of age-adjusted BRS, age-adjusted HRV and NIRS in the SPK group after transplantation (BRS 3.9 [2.6-7.6], HRV 9.4 [4.7-11.2], NIRS 64.2 [62.6-65.5]; $p\leq0.1$). Although the improvement in tissue oxygenation after transplantation was not significant between the different groups, it was significant when the data were pooled (NIRS, [n=20], from 62.3 [61.8-62.8] to 64.2 [62.4-66.0]; $p<0.05$).
Conclusion: Diabetic cardiovascular autonomic dysfunction improved early after KA transplantation and after SPK there was a trend of improvement. The data suggest that change in tissue oxygenation and thus relief of tissue hypoxia could play a role in the improvement of cardiovascular autonomic function after transplantation.
Supported by: Folkhälsan and Stockmann grant
Disclosure: **H. Paajanen:** None.

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Comparaison of metabolic results and complications of procedures for pancreas or islet grafts in the two years following transplantation: a single centre experience

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Background and aims: The restoration of pancreatic beta-cell function, through allogeneic transplantation of pancreas (PT) or islets (IT), can be proposed for selected patients with type 1 diabetes. Results of IT continuously improved from the Edmonton protocol and are almost at the level of those of PT. We compared metabolic results and complications of these 2 techniques, obtained in the 2 years following the first transplantation in patients grafted by one of these techniques in our center.
Materials and methods: Twenty PT (10F/10M) patients (17 received simultaneously a renal transplant) were compared to 14 IT (9F/5M): 3 after a pancreatic transplant failure, 2 after a renal transplant and 9 with an IT alone. Islets transplanted patients' data were taken from GRAGIL and TRIMECO trials and pancreatic transplanted patients were followed at 3, 6 and 12 months by the diabetologist in our center. Data were collected prospectively and expressed as mean. Comparison were made using t-test.
Results: At the time of transplantation, PT recipients were younger than IT (respectively mean age 39 and 49 yrs, $p=0.01$). The 2 groups were similar according to duration of diabetes, BMI, HbA1c and insulin requirements before graft. Nine PT and 12 IT recipients were followed up at 2years. Both grafts significantly reduced HbA1c: median with IQ of 5.3% [5.0; 5.6] and 6% [5.6;6.3] at 1year post-graft, and 6.0% [5.7;7.5] and 6.6% [5.9;7.3] at 2 years, for PT and IT respectively. Insulin-independence in PT often occurs very early after surgery and is estimated at 94%, 81% and 67% respectively at 6 months, 1 and 2 years post-graft. In IT, insulin-independence is estimated at 62%, 69% and 75% after completion of the second or even third islet infusion. Among 31 islet infusions performed, 8 perihepatic hematomas (7 patients), 1 transfusion and 1 radiological embolization occured. Complications occurred in 18 out of 20 PT patients: 6 hemodynamic shocks, 4 pulmonary embolisms, 17 anemias with need of transfusion and 6 sepsis. Early re-surgery (in first 15 days) was necessary in 7 PT (35%): 2 pancreatic venous thrombosis, 1 renal transplantectomy, 4 peri-anastomotic hemorrhages. We report 3 later surgeries: 2 drainages of pancreatic abscess and 1 evisceration. A loss of graft was observed in 4 PT and 3 IT. The iatrogenicity of immunosuppressive drug is comparable between the 2 groups.
Conclusion: Both graft types improved all metabolic parameters in all patients at 2 years post transplantation. These results are observed very early in PT, delayed at 6 months in IT to finally be comparable on the rate of insulin-independence at 2 years. IT remains minimally invasive. PT is associated with severe morbidity with 30% life threatening complications, all favorable at 3 months. Each procedure therefore retains its indications, which must be strictly reserved for patients for whom the benefit/risk balance remains positive.
Disclosure: **O. Villard:** None.

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One world inside another: co-encapsulation of beta cells and nanoparticles containing GLP-1 improves insulin secretion in alginate-based bioartificial pancreas

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Background and aims: The bioartificial pancreas presents a promising methodology to treat diabetes: cells are commonly entrapped in alginate hydrogels, being able to secrete insulin properly while being protected from the immune system. However, this method is still associated with a high rate of graft failure caused by poorly functioning cells. In the present work, we developed a novel model based on the co-encapsulation of beta cells with nanoparticles containing glucagon like peptide-1 (GLP-1) on alginate hydrogels, allowing its delivery and action in the specific target, the beta cells.
Materials and methods: Poly(lactide-co-glycolide) (PLGA) was used to produce nanoparticles containing GLP-1 through a modified solvent emulsification-evaporation method, based on the water-in-oil-in-water

(w/o/w) double emulsion technique. Nanoparticles were characterized by dynamic light scattering (DLS) and transmission electron microscopy (TEM). GLP-1 association and release were calculated using a GLP-1 EIA commercial kit. INS-1E beta cells were maintained in culture and, for encapsulation, $5x10^6$/mL were mixed only with 2wt% alginate (Alg 0) or also with nanoparticles (Alg GLP-1) and extruded under a coaxial nitrogen flow using a VarJ1 Encapsulation Unit to a calcium bath for crosslinking. Cellular metabolic activity was assessed by Resazurin and levels of insulin were measured by an insulin ELISA commercial kit after glucose-stimulated insulin secretion assay.

Results: The produced PLGA nanoparticles had an average size of 169.3 ± 3.24 nm, a polydispersity index of 0.05 ± 0.01 and an average zeta-potential of -24.3 ± 3.94 mV. PLGA nanoparticles induced a very low grade of cytotoxicity, decreasing 12% of INS-1E metabolic activity ($p<0.05$) only at a PLGA concentration of 10 mg/ml after 48h of exposure. The average association efficiency of GLP-1 was 65.4% ± 11.5 and the in vitro release test showed a gradual increase of GLP-1 release during time, reaching 71% ± 0.0 after 7 days. Comparing to cells encapsulated in the absence of nanoparticles (Alg 0), the co-encapsulation of nanoparticles containing GLP-1 with INS-1E cells in alginate hydrogels (Alg GLP-1) increased cellular metabolic activity at all timepoints ($p<0.001$ at days 1 and 4 and $p<0.05$ at day 7). The capacity of beta cells to secrete insulin also increased in the presence of nanoparticles: comparing 2mM and 20mM of glucose stimuli, insulin secretion increased 1.8-fold and 5.7-fold ($p<0.001$) in conditions Alg 0 and Alg GLP-1, respectively.

Conclusion: Our results reveal a promising approach to ensure the proper delivery and action of GLP-1 in encapsulated beta cells, leading to a marked increase of insulin secretion. More experiments are required to better characterize this novel model, which can effectively contribute to bioartificial pancreas success.

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Disclosure: **J. Crisóstomo:** None.

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Continuous glucose monitoring in a mouse model of islet transplantation

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Background and aims: Blood glucose concentrations are an important end-point in diabetes research utilising mouse models of diabetes. Typically glucose is measured using a glucometer at a frequency of once a day or less often. However, the accuracy of this measurement could potentially be affected by blood glucose fluctuations. Using continuous blood glucose monitoring in unrestrained mice we measured blood glucose fluctuations in mice in the normoglycaemic condition, after streptozotocin injection to induce hyperglycaemia and after islet transplantation. The aim of this study was to measure fluctuations in blood glucose in mice in different states of hyperglycaemia to determine the extent and timings of blood glucose fluctuations.

Materials and methods: Five male C57Bl/6 mice were implanted with continuous glucose telemetry devices with blood glucose measured in the carotid artery in accordance with manufacturer's instructions. Continuous blood glucose monitoring started seven days after probe implantation, with averages reported every 10 seconds. After five days of baseline measurements the mice were injected with 180mg/kg streptozotocin (STZ) to induce diabetes. Five days later, 200 C57Bl/6 islets were implanted under the kidney capsule and animals were maintained for a further 14 days. The light/dark cycle was 12 hours with lights on at 7am and off at 7pm.

Results: In normoglycaemic mice, average day time blood glucose was lower than average night time blood glucose (8.2±0.2 mmol/l vs 8.6 ±0.1mmol/l; $p<0.05$, paired t-test, n=5). Average daily excursions (from lowest to highest measurement) were 5.0±1.2mmol/l. STZ injection led to a rapid peak in blood glucose within 3.0±0.6 hours (18.6±0.4 mmol/l), which was followed by a nadir at an average time of 10.5±0.7 hours after injection (3.6±0.2 mmol/l). Blood glucose concentrations then increased and persistent hyperglycaemia (>16.7 mmol/l) was reached at an average of 40.2±9.0 hours after STZ injection. After islet transplantation, all mice had reduced blood glucose concentrations ($p<0.01$, paired t-test, n=5). Average day time blood glucose was lower than night time blood glucose (12.6±0.6 mmol/l vs 15.5±0.6 mmol/l, P=0.0003, two way repeated measure ANOVA, n=5). Considerable glucose excursions were present throughout the day and night with larger fluctuations at night (median: 9.2mmol/l vs 10.1mmol/l respectively, $p<0.05$, Wilcoxon Rank Sum Test, n=5). Nonetheless, daytime fluctuations were considerable with blood glucose changing rapidly between 7am and 10am (Figure 1), which is a common time to measure blood glucose in mice.

Conclusion: Using continuous glucose monitoring it is clear that blood glucose excursions are considerable in mice. While average day time blood glucose concentrations are lower than night time, fluctuations throughout the day could mean that a single blood glucose measurement may misrepresent the overall glycaemic control of the mouse.

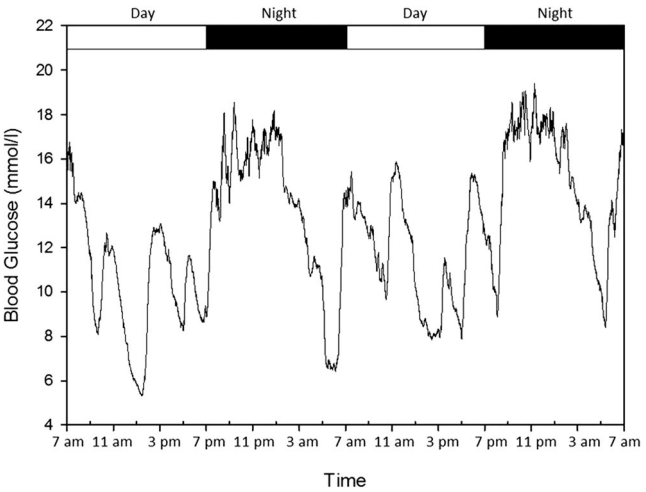

Figure 1. 48h glucose monitoring trace from a diabetic mouse following islet transplantation.

Supported by: DSI

Disclosure: **A.L.F. Austin:** None.

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Pancreatic extracellular matrix: novel microencapsulation platform for diabetes cell-based therapy

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Background and aims: Encapsulation of pancreatic islets has been investigated for over three decades, aiming to improve islet transplantation outcomes in diabetic patients. Nevertheless, major hurdles impede this approach from reaching the clinic, mainly the limited longevity of the isolated pancreatic islets. Growing understanding of the interactions between cells and their surrounding tissue has led, in the recent years, to componential and structural mimicking of the islets' natural microenvironment, their extracellular matrix (ECM). We previously demonstrated ECM-based encapsulation of human mesenchymal stem cells and liver cells, which were induced to transdifferentiate into insulin producing cells. While this platform resulted in significant amelioration in hyperglycemia and provided a proof of concept for ECM-microencapsulation, we believe the full potential of our platform can be revealed upon addressing

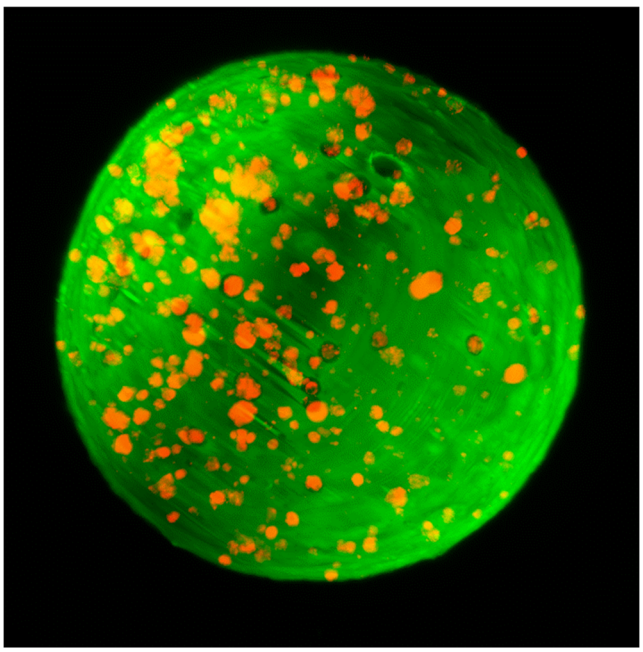
the ECM-microencapsulation of pancreatic islets, naturally secreting substantial doses of insulin in a glucose regulated manner. The overall aim of our study was, therefore, to develop an ECM-based microencapsulation platform for the entrapment of pancreatic islets, to maximize their long term survival and function, while isolating and protecting them from the host immune system.

Materials and methods: We used porcine pancreatic ECM which was decellularized using unique physical, enzymatic and chemical process, and enzymatically solubilized. This solubilized ECM was reassembled into microcapsules of ECM hydrogel, entrapping freshly isolated murine islets.

Results: The unique ECM-microencapsulation platform was optimized to support the long-term viability and function of islets (Fig. 1). Microcapsule size, ECM concentration and islets density were addressed. Hence, upon ECM-microencapsulation the islets remained viable and secreted insulin in a glucose-regulated manner for more than four weeks, indicating at the potential of this platform in overcoming islets' natural poor survival rates post isolation. Islets cultured on ECM gel, adhered to its fibers and remodeled it, revealing a crosstalk between the cells and the ECM, and proving the gel's bioactivity.

Conclusion: Overall, our unique ECM-microencapsulation platform provides pancreatic islets a microenvironment that is permissive for long term survival and insulin secretion, and can therefore, be considered as a prospective platform for beta cell replacement therapy.

Fig. 1. ECM-based microencapsulation.



Supported by: the Israel Innovation Authority

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RAGE-binding peptide (RBP) as a novel anti-HMGB1 therapy for improving islet transplant survival

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Background and aims: The receptor for advanced glycation end products (RAGE) is a multi-ligand cell surface receptor and is involved in a wide range of inflammatory and degenerative disorders by engaging diverse ligands, including high-mobility group protein B1 (HMGB1). HMGB1 protein released from transplanted islets can trigger innate immune rejection and accelerate the injury to transplanted islets. Therefore, anti-HMGB1 therapy might be a candidate for further improving the outcomes of islet transplantation. In this study, we evaluated whether the blocking of HMGB1/RAGE signaling pathways with novel RAGE-binding peptide (RBP), which is derived from RAGE binding domain (amino-acids 150-183 segment) of HMGB1, can protect islet cells from HMGB1-induced damage in vitro and enhance survival of mouse islet grafts in vivo.

Materials and methods: RBP was overexpressed in bacteria and purified by consecutive chromatographies. INS-1 cells or isolated mouse islets were treated with RBP (5 μg/mL) added in culture media and then treated with HMGB1 (2.5 μg/mL). Intracellular ROS generation in INS-1 cells was measured with H_2DCFDA staining. To assess RAGE expression, INS-1 cells were incubated with rabbit anti-RAGE antibody and FITC-conjugated secondary antibody. Expression of inflammatory cytokine genes was assessed by RT-PCR. Apoptosis was measured in INS-1 and islet cells using Cell-APOPercentage™ Apoptosis Assay. A suboptimal mass of control or RBP-treated syngeneic islets were transplanted intraportally into the liver of streptozotocin-induced diabetic mice. After transplantation, non-fasting blood glucose levels were measured 3 days per week for 30 days.

Results: In INS-1 cells, RBP treatment significantly reduced HMGB1-induced intracellular ROS production, RAGE expression, and apoptotic cell death. In mouse islets, IL-1β and IFN-γ gene expression was highly upregulated after exposure to HMGB1 and RBP-pretreatment reduced IL-1β and IFN-γ gene expression. Islet cells were also protected from HMGB1-induced apoptosis with RBP treatment. Diabetic mice transplanted with a suboptimal mass of RBP-treated islets into the liver became normoglycemic in 50% of the recipients at 30 days post-transplant, whereas none of the mice transplanted with control islets was normoglycemic ($P < 0.05$).

Conclusion: This study shows that RBP suppresses HMGB1/RAGE pathway-dependent proinflammatory and proapototic activation of islets and enhances islet cell survival during the early post-transplant period, and preserved islet mass and functions over time in the transplants. In conclusion, RBP treatment might be a novel anti-HMGB1 therapy to improve the outcome of clinical islet transplantation.

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Disclosure: **S. Ihm:** None.

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Dynamic molecular changes of porcine neonatal pancreatic cell clusters in culture and after transplantation

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Background and aims: It takes more than 8 weeks to achieve normoglycemia in diabetic nude mice after transplantation (Tx) of isolated porcine neonatal pancreatic cell clusters (NPCCs). The ductal epithelium is primarily detected in NPCC grafts suggesting that initial grafts exhibit progenitor-like phenotype. To further clarify the cell identity of NPCCs grafts, we examined the molecular profile of NPCCs from 1-day neonatal pigs before and after Tx.

Materials and methods: Pancreases obtained from 1-day-old neonatal pigs were cut into fragments, digested by collagenase and maintained in culture. Six and twelve hundred NPCCs were transplanted under the kidney capsule of nondiabetic and diabetic nude mice, respectively. The

mRNA expression of insulin, glucagon and carboxypeptidase B (CPB) in isolated NPCCs were detected by semi-quantitative RT-PCR. NPCC cultures and grafts were fixed, sectioned and quantitatively stained for insulin, glucagon, somatostatin, pancreatic polypeptide, PDX-1, SOX-9 and Ki67.

Results: During 4-day NPCC culture, endocrine insulin and glucagon mRNAs increased while mRNA expression of exocrine amylase and CPB decreased gradually. Soon after NPCC isolation, $Pdx1^{+}$/$Insulin^{-}$ (78.7±1.7% after isolation vs. 51.9±3.1% in 1-day-old pig pancreas) and $Sox9^{+}$ (67.1±3.5% after isolation vs. 6.8±0.7% in 1-day-old pig pancreas) pancreatic progenitors dramatically increased. Moreover, dual-hormonal progenitor-like cells including $insulin^{+}$/$glucagon^{+}$, $insulin^{+}$/$somatostatin^{+}$ and $insulin^{+}$/pancreatic $polypeptide^{+}$ were observed. After Tx, $insulin^{+}$ cells increased whereas $Pdx1^{+}$ and $Sox9^{+}$ progenitors were downregulated in both non-diabetic and streptozotocin-induced diabetic recipient mice over 2 months. Strikingly, it was found that significant higher percentage of $insulin^{+}$ cells were only detected in 9-day (5.24 ±0.42% in diabetic grafts vs. 3.48±0.39% in nondiabetic grafts) and 16-day grafts (5.84±0.68% in diabetic grafts vs. 4.48±0.57% in nondiabetic grafts), but not in 23-day, 30-day and 60-day grafts, indicating that hyperglycemia could facilitated NPCC-derived β cells early post-Tx. Further mechanistic analysis showed that increased diabetes mediated NPCC derived β cells post-Tx is not through an enhanced replication of β cells but via upregulated neogeneic differentiation based on the detection of $insulin^{+}$ cells budding out from $Pdx1^{+}$/$Sox9^{+}$ progenitors in NPCCs grafts. Interestingly, significant decrease of $Sox9^{+}$ progenitors was detected 16 days post-Tx in diabetic grafts (14.14±2.27% in diabetic grafts vs. 49.67±2.63% in non-diabetic grafts); unlike $Sox9^{+}$ cells, $Pdx1^{+}$ precursors between diabetic and nondiabetic grafts do not show significant difference until 60 days post-Tx (33.72±4.52% in diabetic grafts vs. 54.59±6.21% in nondiabetic grafts) implying that distinct NPCC-derived progenitor populations differentially fuel new β cells post-Tx.

Conclusion: In conclusion, our results demonstrated that islet precursors could be activated during NPCC isolation while NPCC progenitors maintained their pluripotency in culture and in early stage post-Tx while neogenesis underlies hyperglycemia mediated graft β cell maturation.

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Disclosure: **W. Li:** None.

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Impact of diabetes on human pancreatic and bone marrow vascular network

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Background and aims: The variability in tissue response to chronic hyperglycaemia makes it difficult to sustain a unified hypothesis on diabetes associated multiorgan failure. A significant literature exists on the functional impairment of circulating and bone marrow endothelial progenitor cells as a feature of diabetes. However, whether this phenomenon results in an unbalance between injury and repair s is poorly described. Thus, a morphometric analysis of the vascular network on pancreatic and bone marrow tissues in response to diabetes was carried out.

Materials and methods: The effects of type 2 diabetes on different vascular compartments was investigated in 20 patients and compared to 20 normoglycemic subjects. The number and distribution of capillaries, venules, arterioles and lymphatics were determined by immunohistochemistry in pancreatic and bone marrow tissues obtained by the same patients.

Results: Capillary and venules density were significantly reduced in pancreatic insulae, whereas no changes were observed in exocrine parenchyma. However, the reduction in functional parenchyma as a result of diabetes tended to decrease vessels/cells ratio compared to control parenchyma. Similarly, capillary and sinusoids density was significantly reduced in diabetic bone marrow parenchymal and paratrabecular areas when compared to non diabetic cases ($p<0.05$). Compared to controls, lymphatic vessels were also significantly reduced in diabetic pancreas ($p<0.05$) while arteriolar density was unaffected. Interestingly, $CD34^{pos}$ progenitor cells were significantly reduced ($p<0.01$) in both bone marrow and pancreas of diabetic patients compared to controls.

Conclusion: Rearrangement of the blood and lymphatic network and reduction in $CD34^{pos}$ progenitors concur in multiple tissues with diabetes. Although we did not established whether this was a consequence or a cause of diabetes associated multiorgan damage, our approach may offer new insights on the understanding of the diabetic paradox of a tissue specific angiopathy.

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Disclosure: **G. Graiani:** Grants; Italian Ministry of Health Ricerca finalizzata GR2011-02347600.

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Dietary influence on GLP-1 dependent effects in the liver and adipose tissue

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Background and aims: Besides its well-known systemic effects on glucose homeostasis by modulating insulin and glucagon secretion glucagon-like-peptide 1 (GLP-1) also affects energy metabolism in various tissues such as the myocardium, liver or adipose tissue. In the pancreatic β-cells GLP-1 exerts its metabolic effects by activating GLP-1 receptor (GLP1R). In other tissues mode of action is less clear: both, GLP1R dependent and GLP1R independent mechanisms have been proposed. In previous studies, incretin mimetic exendin-4 was found to increase sirtuin 1 (Sirt1) expression via nicotinamide phosphoribosyltransferase (Nampt) in hepatocytes thereby improving fatty liver disease in high fat diet fed mice. Aim of this study was to investigate whether GLP-1 dependent effects in the liver and the adipose tissue are influenced by dietary intake.
Materials and methods: Six weeks old male C57BL/6J mice were fed either a fructose-rich (HFru), high fat diet (HFD), mixed high sucrose and high fat diet (HSHFD) or standard diet (SD) for 10 weeks. Glucose tolerance and insulin sensitivity was estimated by oral glucose tolerance test and intraperitoneal insulin sensitivity test. NAMPT and SIRT1 mRNA expressions were determined in livers and visceral adipose tissue samples by fluorescence based real time PCR.
Results: After 10 weeks of feeding HFD (45.12g) and HSHFD (40.13g) but nor HFru (28.76g) fed mice gained significantly more weight than SD (29.54g) mice. The Glucose tolerance as well as insulin sensitivity was significantly impaired in HFD and HSHFD and worsened in HFru mice compared with SD mice. In the liver NAMPT expression was highly upregulated in mice fed a HFD and downregulated in mice fed with HFruD or HSHFD when compared to SD. Hepatic SIRT1 expression tended to be lower in mice fed any carbohydrate-rich diet and was tendentially higher in HFD fed mice. Conversely, NAMPT expression was not relevantly influenced by dietary intake in the visceral adipose tissue, while SIRT1 expression was significantly reduced in HFD and HSHFD fed mice.
Conclusion: In summary we found that dietary intake influences GLP-1 effector proteins and enzymes in a tissue-specific manner. From our data we hypothesize that GLP-1 receptor agonist therapy might be especially beneficial in patients with high dietary carbohydrate intake due to marked downregulation of hepatic NAMPT expression in this setting.
Disclosure: **S. Folie:** None.

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The anti-diabetic role of heat shock protein 72 in mouse model of type 2 diabetes

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Background and aims: Cell stresses, such as endoplasmic reticulum stress or oxidative stress is one of the key mediators in pathophysiology of type 2 diabetes. Molecular chaperone, which modulate protein folding and/or assembly and protect cells from those stresses, may be a favorable target for diabetic treatment. Heat shock protein (HSP) 72 is a major inducible heat shock protein against heat, ultraviolet, heavy metals or infection, and serves to protect cells from those cellular stress signals. Induction of HSP72 by pharmacologic agent or mild electrical stimulation with heat shock improves glucose intolerance in diabetic model mice. In this study, we investigated glucose metabolism in whole body HSP72 deficient (KO) mice to explore the roles of HSP72 in diabetes.
Materials and methods: Male HSP72 KO mice or control mice were subjected to a high-fat diet (HFD) regimen for 16 weeks. Metabolic parameter and pathophysiological examination were performed. HSP72 was overexpressed in HSP72 whole body knockout by lenti-virus system to investigate the role of HSP72 in liver.
Results: KO mice showed significantly higher body weight after 10 weeks of HFD (KO: 36.9 g v.s. control: 33.4 g). Fasting blood glucose was significantly elevated after 11 weeks of HFD (KO: 143.3 mg/dL v.s. control: 115.5 mg/dL). Random fed blood glucose and food intake were comparable. Upon glucose challenge test, blood glucose levels at any time points measured were higher in KO mice. On insulin tolerance test, KO mice exhibited insulin resistant phenotype. Visceral fat mass was increased and hepatic steatosis was obvious in KO mice. Upon insulin stimulation, phosphorylation of Akt was decreased by approximately 50% in KO liver, with increased activation of c-jun N-terminal kinase. Hepatic gluconeogenesis was not suppressed in KO mice. When adding back to HSP72 expression in liver by lenti-viral system on HFD fed KO mice, fasting blood glucose was reversed compared to control mice on HFD.
Conclusion: Deficiency of HSP72 leads to increased visceral adiposity, hepatic insulin resistance, glucose intolerance and fatty liver. As induction of HSP72 in liver is beneficial to treat diabetes, our observations strongly indicate the abundance of HSP72 in liver is critical in diabetic pathophysiology.
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Disclosure: **T. Kondo:** None.

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From mouse and man: the insulin-degrading enzyme gene governs insulin clearance and drives glucose intolerance and NAFLD development

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Background and aims: The liver removes 60 to 40% of the circulating insulin that is secreted by the pancreas and reaches the portal vein, a process called Insulin Clearance (IC). IC is a key regulator of peripheral levels of insulin, insulin sensitivity and is associated to metabolic conditions such as Type 2 Diabetes (T2D) and non-alcoholic fatty liver disease (NAFLD). We have previously found that IC impairments in prediabetic individuals were related to a dysmetabolic condition that suggests an inhibition of Insulin Degrading Enzyme (IDE) by increased levels of nitric oxide produced at the post prandial state. Using a human cohort and IDE knockout (IDE-KO) mice we aimed to ascertain if IC is dependent on the IDE gene and test if genetically determined IC impairments contribute to metabolic impairments such as NAFLD and glucose intolerance induced by hypercaloric diets.
Materials and methods: IDE-KO mice with six weeks of age were exposed to a hypercaloric regimen or to standard chow diet and compared to age and sex matched wild type controls. The mice were monitored during 12 weeks of diet exposure by an Oral Glucose Tolerance Tests (OGTT) performed at 4, 8 and 12 weeks. Glucose levels were measured at fasting and at 15, 30, 60 and 120 minutes after a glucose challenge. Serum was obtained at the same time points for IC estimation (Area under the curve (AUC) c-peptide/AUC insulin). In parallel, we evaluated glucose tolerance and IC in the PREVDIAB2 cohort, a collection of 1,088 individuals from the Portuguese population that underwent OGTT analyzed at 0, 30 and 120 min after the glucose challenge. Fatty liver index (FLI) was estimated using validated methodology. Studies of genetic

association in IDE-KIF11-HHEX genomic region were performed using quantitative trait locus (QTL) analysis.
Results: We found that IDE-KO mice show an IC reduction, supporting the notion that the IDE gene is a key regulator of IC. At all time points analyzed and regardless of sex and diet regimen being aggravated with the diet, the IDE-KO mice presented higher levels of glucose intolerance when compared with wild type controls as measured by increased area under the curve of the glucose excursions during OGTT. Genetic association analysis in the PREVADIAB2 cohort identified that 3 SNP variants in the IDE-KIFF11 intergenic region and a SNP located in the upstream region of the IDE gene that are associated with reduced IC and increased liver fibrosis index (NAFLD Fibrosis Score) (0.008<P-value<0.030). Interestingly, subjects with high FLI (FLI>60) showed reduced IC during the OGTT.
Conclusion: Together these results revealed that the IDE gene is critical for the IC mechanism and support the notion that IC impairments governed by the IDE gene are associated with glucose intolerance and NAFLD development. Furthermore, the finding that the 3 SNP variants in the IDE-KIFF11 intergenic region and a SNP located in the upstream region of the IDE gene controls IC provide an explanation for the involvement of this genomic region in Prediabetes/T2D genetic susceptibility.
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Alterations in adenosine metabolism and adenosine receptor expression are associated with insulin resistance

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Background and aims: Type 2 diabetes *mellitus* (T2D) is a metabolic chronic disease that is characterized by hyperglycemia concomitantly with insulin resistance and abnormal insulin secretion. Adenosine is a key extracellular signaling molecule that regulates tissue homeostasis through its action in A_1, A_{2A}, A_{2B} and A_3 receptors. It is consensual that in the liver, skeletal muscle and adipose tissue the adenosinergic system is crucial in the regulation of glucose homeostasis/insulin action and in the pathophysiology of T2D. However, remains to clarify which modifications in the adenosinergic system are associated with insulin resistance and with glucose intolerance. Herein we have investigated if alterations in adenosine metabolism and in the expression of adenosine receptors in insulin sensitive tissues, namely liver, adipose tissue and skeletal muscle, are associated with insulin resistance.
Materials and methods: The experiments were performed in 9 - 20 weeks male *Wistar* rats (200-450g). Two groups of rats were used: a control group and the high sucrose (HSu) group. The Hsu group was obtained by submitting the animals to 35% sucrose in drinking water for 4 weeks. Animals were anesthetized and the insulin sensitive-tissues, liver, visceral adipose tissue and soleus muscle were collected. For adenosine release and production, the tissues were incubated during 10, 30 and 60 minutes, in the presence of EHNA (adenosine desaminase inhibitor, 25 μM) and NBTI (equilibrative nucleoside transport inhibitor, 5 μM). The adenosine present in the tissues and in the incubation medium was measured by HPLC. Also, the expression of A_1, A_{2A} and A_{2B} adenosine receptors expression in these tissues was investigated by Western-blot.
Results: In *Soleus* muscle HSu diet significantly increased adenosine production by 102, 141 and 56% and adenosine release by 40, 53 and 80% (for 10, 30 and 60 min of incubation, respectively). In the adipose tissue Hsu diet increased significantly adenosine release by 281.33%, 163.64% and 220.22% after 10, 30 and 60 minutes of incubation, without changing significantly adenosine production. In the liver, Hsu diet did not modify nor the release or the production of adenosine in any of the times of incubation tested. In *Soleus* muscle, HSu diet increased by 40%, 48% and 20%, respectively, the expression of A_1, A_{2A} and A_{2B} receptors. On the other hand, Hsu diet in the adipose tissue, A_1 receptors decreased by 28%, although without statistical significance and A2A receptors decreased by 45%, without altering the expression of A2B receptors. In the liver, Hsu diet decreased by 29.29% A_{2A} receptor expression and increased by 73% A_{2B} expression, without affecting the expression of A_1 receptors.
Conclusion: We can conclude that alterations in the metabolism of adenosine and in the expression of adenosine receptors mainly in the skeletal muscle and the adipose tissue are associated with insulin resistance.
Disclosure: **I.B. Martins:** None.

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Obesity induced microRNA-222 impairs insulin signalling through the repression of IRS-1 expression in hepatocytes

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Background and aims: MicroRNAs (miRNAs) are short non-coding RNAs that post-transcriptionally regulate gene expression, and have been shown to participate in various cellular processes. Several miRNAs have recently been reported to implicate in glucose metabolism, but roles of miRNAs in insulin resistant states, such as obesity or type 2 diabetes, are largely unknown. In this study, we focused on miR-222, whose expression was increased in the livers of high-fat diet fed mice, and investigated the effect of miR-222 on gene expression and insulin signaling in liver.
Materials and methods: MiR-222 expression levels were measured in the livers of HFD-fed mice by real-time quantitative PCR. To examine the role of miR-222 on insulin signaling, hepatocytes were overexpressed with miR-222 and stimulated with insulin. Insulin-stimulated Akt phosphorylation and expression of gluconeogenuc genes were evaluated. In silico analysis, miR-222 potentially binds to the 3' UTR of the IRS-1 gene, a key insulin signaling molecule. To confirm the direct interaction between miR-222 and 3' UTR of IRS-1 gene, a dual-luciferase assay was perfomed.
Results: MiR-222 expressions were upregulated in the livers of HFD-fed mice. Overexpression of miR-222 in primary mouse hepatocytes attenuated protein levels of IRS1 and insulin-induced Akt phosphorylation. In accordance with reduced Akt phosphorylation, Pck1 mRNA was increased. Direct interaction between miR-222 and 3'UTR of IRS-1 gene was confirmed by luciferase assay.
Conclusion: This study demonstrates that hepatic miR-222 is upregulated in an insulin resistant state, which in turn impairs insulin signaling through the repression of IRS-1 expression. These findings suggests that miR-222 could be a novel target for the treatment of obesity associated metabolic disorders.
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Metabolic effects of a mixture of low-dose pollutants in a mouse model of estrogen deficiency

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Background and aims: Solid evidences have demonstrated that environmental pollutants contribute to the etiology of obesity and related metabolic disorders. Using a model of lifelong exposure to a mixture of low-dose pollutants, we recently observed aggravation of glucose intolerance

and hepatic insulin resistance of adult females but not of males fed a high-fat high-sucrose diet (HFSD). Conversely, lifelong exposure to the pollutant mixture induced alleviation of glucose intolerance and enhanced insulin sensitivity of immature female mice also HFSD-fed (all occurring in the absence of body weight changes). Thus, we hypothesized that the mixture of pollutants may induce estrogeno-mimetic activities. Indeed, it is well established that estrogens protect females against metabolic disorders at physiological levels but that insulin resistance develops following overstimulation of estrogen receptors or in conditions of estrogen deficiency. To further validate our hypothesis, we evaluated if the mixture of pollutants could reduce the deleterious metabolic effects induced by ovariectomy.

Materials and methods: This study has been carried out along the "Principles of laboratory animal care" (NIH Publication no. 85-23, revised 1985). C57BL/6J female mice were exposed from preconception until adulthood to a HFSD containing (HFp) or not (HF0) a mixture of persistent organic (TCDD, PCB 153) and short-lived (BPA, DEHP) pollutants, each used in the range of its tolerable daily intake reference dose. At 5 weeks of age, mice underwent ovariectomy (OVX) or were sham-operated, generating a total of 4 groups (HF0-sham/HF0-OVX/HFp-sham/HFp-OVX). Weight and food intake were weekly recorded. Several blood parameters, glucose tolerance, plasma lipids and hepatic expression of estrogen receptor α (Esr1) were measured.

Results: OVX resulted in body weight increase with no modification of food intake. Mice fed HFSD showed glucose intolerance and high plasma insulin levels which worsened in OVX mice. Pollutant exposure did not impact body weight, food intake or plasma insulin levels. However, it resulted in a decrease in plasma triglyceride level (0.71 ±0.019 mM *vs* 0.83±0.033 mM; p=0.008) and improvement of glucose tolerance in OVX females. In addition, fasting plasma insulin levels measured at the peak of response after the injection of glucose during the GTT (i.e. 15 minutes) were 1.6-fold higher in HFp-OVX mice than in HF0-OVX mice. This effect was not observed with sham mice. Thus, and although the *p*value=0.06, it may explain alleviation of glucose tolerance by pollutants in OVX conditions. Furthermore, in HFp-OVX mice, the mixture of pollutants led to a 1.4-fold increase in the expression of Esr1 gene (p=0.038) as compared to non-exposed mice that underwent OVX.

Conclusion: Collectively, these results illustrate the cocktail effect resulting from exposure to a mixture of environmental pollutants, each used at a dose supposedly without effect. Consistently with our working hypothesis on the estrogeno-mimetic activity of the mixture of pollutants, we observed beneficial metabolic effects of the pollutant mixture in conditions of estrogen deficiency. Because of the very low doses of pollutants used in mixture, these findings may have strong implications in terms of understanding the potential role of environmental contaminants in the development of metabolic diseases.

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Estradiol enhances insulin sensitivity in malnourished mice through estrogen receptor alpha

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Background and aims: Malnourished mice develop increased insulin sensitivity in peripheral tissues and a low insulin secretion by beta cells. Estradiol is a steroid hormone involved in the control of energy balance and glucose homeostasis. The aim of this study was to assess the role of estradiol on glucose homeostasis of malnourished mice.

Materials and methods: To develop the malnutrition model, post-weaned Swiss male mice were fed with low protein diet (6%) during 8 weeks. The insulin hypersensitivity is a characteristic of protein malnutrition. We characterized the malnourishment phenotype through analyses of the body weight, insulin, protein and albumin plasma levels. We analyzed the insulin sensitivity and the effect of 10μg/Kg E2 on it by hyperinsulinemic-euglycemic clamp assay. Western blot was performed to study the insulin pathway in the liver, adipose tissue and, skeletal muscle. Data are expressed as mean ± SEM and statistical significance was determined using two-tailed T-test.

Results: Malnourishment phenotype was confirmed by a lower body weight (control: 41.23±30.64g; LP: 36.30.83±0.38g; p-value: 0.0001), lower plasma levels of insulin (control: 2.94±0.51ng/mL; LP:1.20 ±0.18ng/mL; p-value: 0.002), albumin (control: 2.16±0.07g/dL; LP: 1.92±0.07g/dL; p-value: 0.02) and protein (control: 5.61±0.23g/dL; LP: 4.86±0. 19g/dL; p-value: 0.02). The hypersensitivity to insulin was demonstrated by an increased in glucose infusion in LP group compared with control (control 0.57±0.08mL/min; LP 0.89±0.1mL/min; p-value: 0.005) by a hyperinsulinemic-euglycemic clamp. The stimulation with of 10μg/Kg E2, in the clamp, showed a statistically significant increase in glucose infusion rate in LP group compared with control (control: 0.65 ±0.06mL/min; LP: 1.25±0.12mL/min; p-value: 0.005). This date indicates enhanced insulin sensitivity in LP mice. In addition, estradiol increased the glucose uptake on peripheral tissues (control: 16.33±1.6mg/Kg/min; LP: 36.18±4.2mg/Kg/min; p-value: 0.006). To verify whether these effects were produced by estrogen receptors (ER), mice were treated with ICI 182,780 and MPP, estrogen receptor antagonists, during 4 days. In these mice, the effect of estradiol on glucose infusion and uptake were absent (glucose infusion rate: $LP_{vehicle}$:0.78±0.16mL/min; $LP_{estradiol}$: 0.84 ±0.23mgL/min; glucose uptake: $LP_{vehicle}$: 21.6±4.6mg/Kg/min; $LP_{estradiol}$: 23.4±6.4mg/Kg/min). MPP, an ER alpha antagonist, also prevented the effect of estradiol to enhance insulin sensitivity (glucose infusion rate: $LP_{vehicle}$:1.63±0.19mL/min; $LP_{estradiol}$: 1.05±0.14mgL/min; glucose uptake: $LP_{vehicle}$: 37.94±6.62mg/Kg/min; $LP_{estradiol}$: 26.48 ±2.58mg/Kg/min). We observed an increased activity of insulin pathway, represented by a higher AKT phosphorylation in muscle tissue.

Conclusion: We conclude that the effect of estradiol enhanced the insulin sensitivity on malnourished mice was produced by estrogen receptor alpha, through an increased AKT phosphorylation in muscle.

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Beta cell insulin resistance dynamics are different in various animal models

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Background and aims: β-cell failure is central to the development of type 2 diabetes (T2DM). Since the pancreatic β-cell itself is a target for insulin action, β-cell insulin resistance can contribute to β-cell dysfunction. So far β-cell insulin resistance has not been studied in vivo in the context of insulin resistance in other tissues. Here, we used a recently by us developed method that allows specific monitoring of β-cell insulin resistance dynamics in vivo in various genetic and dietary animal models in the context of glucose tolerance, whole body and liver insulin resistance.

Materials and methods: As genetic models for insulin resistance we used ob/ob mice and NZO mice. For diet-induced insulin resistance, C57BL/6J mice were used. Here, mice were treated for 8 weeks with one of the following diets: High-Sucrose-Diet (HSD), High-Fat-Diet (HFD), High-Fat-High-Sucrose Diet (HFHSD) or High-Fat-High-Fructose-Diet (HFHFrD). To monitor β-cell insulin resistance isolated islets of Langerhans were transduced with the β-cell insulin resistance biosensor. This biosensor is based on GFP labelled FoxO1 that changes its position from the cytoplasm to the nucleus in case of insulin resistance. For each model and imaging time point the biosensor allows us to

calculate a relative β-cell insulin resistance index (rβIRI). This index represents the ratio of nuclear to cytoplasmic FoxO1-GFP localization normalized to the control (ob-control or C57BL/6J fed a control diet). Transduced islets were transplanted into the anterior chamber of the eye of mice and imaged by confocal microscopy at distinct time points from 4 weeks after transplantation on. Additionally glucose-, insulin and pyruvate-tolerance tests were performed.

Results: Ob/ob mice are β-cell-, whole-body- and liver insulin resistant at young age (3 months of age (moa); ob-control: rβIRI = 1 ± 0.02; obob: rβIRI = 1.24 ± 0.03; p<0.001). These mice recover from their insulin resistance at older age (10 moa; ob-control: rβIRI = 1.11 ± 0.02, obob: rβIRI = 1.12 ± 0.02; n.s.). NZO mice are β-cell and whole-body insulin resistant from 3 moa (NZO: rβIR = 1.2 ± 0.02; ob-control: rβIRI = 1 ± 0.02; p<0.001) to 6 moa, but did not develop liver insulin resistance. Differences were also seen in diet-induced animal models. While a HSD did not change any of the measured parameters, both a HFD and HFHFrD led to the development of whole-body and liver insulin resistance. Only feeding of a HFHSD resulted in the development of β-cell insulin resistance (after 8 weeks of treatment: Control: rβIRI = 1.04 ± 0.02; HFHSD: rβIRI = 1.18 ± 0.04 p<0.01). This occurred together with whole-body insulin resistance, but not liver insulin resistance. All types of insulin resistances were paralleled by impaired glucose tolerance.

Conclusion: This study shows that different insulin resistance dynamics exist in different models of insulin resistance. Furthermore, we provide evidence for diet-depend differences in T2DM development. Thus, the presented mouse models can be used to study different insulin resistance and T2DM development patterns and lay the ground for the investigation of the underlying molecular mechanisms. This will help to develop novel treatment and prevention strategies to counteract diabetes.

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Activation of pregnane X receptor modulates glucose homeostasis in healthy and obese mice

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Background and aims: The pregnane X receptor (PXR; gene name NR1I2) is a ligand-activated nuclear receptor expressed mainly in the liver and intestine. Its activation leads to induction of drug-metabolism related genes as it acts as a regulator of xenobiotic metabolism. Multiple prescription drugs, environmental pollutants and pesticides are PXR ligands. Recent studies have revealed an additional role of PXR as a regulator of glucose metabolism. In clinical studies, PXR agonists have been shown to impair postprandial glucose tolerance. Also, statins with PXR-activating properties are known to induce hyperglycemia and they have been linked to the prevalence of type 2 diabetes (T2D). The link between exposure to environmental pollutants and T2D is also well established. In mouse experiments, PXR knock-out has been shown to protect from high fat diet-induced impairment of glucose metabolism but PXR activation has also been reported to have a protective effect against high fat diet. To further evaluate and characterize the effect of PXR activation, we administered a selective PXR agonist to both healthy and obese wildtype mice and also to PXR knockout (PXR-KO) mice, and evaluated glucose and insulin tolerance.

Materials and methods: C57BL/6 mice and PXR-KO mice were administered selective murine PXR agonist, pregnenolone-16α-carbonitrile (PCN), 50 mg/kg or vehicle control once a day i.p. for 4 days. Then, the mice were fasted for 12 hours for OGTT or 6 hours for insulin tolerance test (ITT). For OGTT mice were given orally 2 g/kg D-glucose. For ITT, mice were administered 1 U/kg human insulin i.p. The mice were anesthetized with fentanyl-fluanisone and midazolam and blood glucose was monitored for 2 hours. For high fat diet (HFD) experiment, mice were fed high fat diet (Envigo td.06414, 60% of calories from fat) for 15 weeks and weight gain was followed weekly. On week 15, the mice were treated with PCN (n=8/group) or vehicle control and OGTT was conducted. Student's T test was used for statistical analysis for glucose AUC values.

Results: PXR activation by 4-day administration of PCN impaired postprandial glucose tolerance in wildtype mice (P=0.015; n=6) compared to vehicle group (n=6) but not in PXR-KO mice (n=10/group), clearly indicating that the effect of PCN on glucose tolerance is mediated by PXR. However, ITT performed with wildtype mice did not show impairment in insulin sensitivity after PXR-activation, suggesting that detrimental effect of PXR activation arise from diminished insulin secretion. After blood glucose started to rise after insulin administration, from 30 to 120 minutes, PCN treated mice had significantly lower blood glucose level compared to vehicle controls (P=0.001) suggesting inhibition of gluconeogenesis, which has been reported earlier. Interestingly, in obese wildtype mice fed with high-fat diet, the acute PXR-activation improved oral glucose tolerance (P=0.026) showing that PXR-activation possess multifaceted functions on glucose metabolism.

Conclusion: Our results demonstrate that PXR activation impaired postprandial glucose tolerance in healthy mice but improved glucose tolerance after high fat diet, and insulin sensitivity was not affected in healthy mice. PXR activation exhibit clear but complex roles in glucose metabolism possibly providing mechanical link between prevalence of T2D, drugs and environmental chemicals.

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Method considerations when estimating insulin sensitivity in streptozotocin-induced diabetic and normal Göttingen minipigs

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Background and aims: The gold standard for assessing insulin sensitivity in both pre-clinical and clinical studies is the euglycemic hyperinsulinemic clamp. Indices of insulin resistance can also be obtained from glucose tolerance tests, such as the frequently sampled intravenous glucose tolerance test (FSIVGTT). Comparing normal and insulin-depleted individuals is not trivial and the aim of the present study was to evaluate methods for estimating insulin sensitivity in normal and insulin-dependent STZ-induced diabetic minipigs, using different *in vivo* methods.

Materials and methods: Female Göttingen minipigs with (DIA, *n*=6) or without (NORM, *n*=6) STZ-induced insulin-dependent diabetes, were clamped at euglycemia, using a constant i.v. infusion rate of insulin aspart at 0.5, 2, 8 or 16 pmol/kg/min, with or without i.v. insulin bolus or an insulin pre-infusion. Animals were clamped up to 300 min with glucose infusion rates (GIR) adjusted every 15 min from plasma glucose (PG) measurements. In addition, a 2 hour FSIVGTT was performed in both groups dosing a porcine insulin bolus in DIA (0.4 nmol/kg) at t=20 min. PG, insulin exposure, C-peptide, glucagon and endogenous insulin were assessed regularly during both tests. For the clamp, steady state (SS) GIR, insulin infusion rates and plasma insulin exposure across groups were used to fit sigmoidal dose response curves with maximum GIR fixed at 30 mg/kg/min. From the FSIVGTT, insulin sensitivity was estimated by minimal modelling (S_I).

Results: DIA had lower bodyweight (25.2 kg±0.4 (mean±SD) vs. 27.4 ±0.4, p<0.05) and higher fasting PG (16.4 mmol/L±1.4 vs. 3.2±0.4, p<0.05) compared to NORM. In the clamp, a pre-infusion of 180 min

(8 pmol/kg/min) and a dose level of 16 pmol/kg/min were necessary to ensure PG target at SS. An i.v. insulin bolus and i.v. infusion rates of 8 pmol/kg/min or less, was not consistently sufficient for all animals to reach SS and target PG. At comparable insulin infusion rates (8 pmol/kg/min), GIR in DIA was significantly different ($p<0.05$) compared to NORM (1.9 mg/kg/min±0.4 vs 11.9±1.8). In a fitted dose-response curve of GIR to infusion rate, the ED_{50} in DIA was 29 pmol/kg/min compared to NORM ED_{50} of 9.8 pmol/kg/min. S_I was $1.50*10^{-04}$ mL/(min*μU)±$1.02*10^{-04}$ in DIA and $4.75*10^{-04}$±$2.22*10^{-04}$ in NORM.

Conclusion: In this study, optimization of the euglycemic hyperinsulinemic clamp protocol in STZ-induced insulin-dependent diabetic minipigs compared to normal animals was explored. An insulin pre-infusion rate for 180 min (at 8 pmol/kg/min) and an infusion rate of 16 pmol/kg/min were necessary to ensure PG target at SS. Also, data from both tests indicated that DIA had a different response to insulin compared to NORM. However, the pre-hepatic contributions in NORM and the difference in FSIVGTT insulin-profiles exemplifies the challenges in comparing NORM and DIA and this should be taken into account when evaluating insulin sensitivity across the groups. Overall, characterizing the insulin sensitivity and potentially understanding the mechanisms behind the difference of DIA and NORM is an important step towards a better study design and use of the models in the pre-clinical part of drug development.

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Absence of Cannabinoid 1 receptor (CB1) in skeletal muscle in mice causes increased lean muscle mass and enhanced insulin action

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Background and aims: Type 2 diabetes mellitus is characterized by deficient insulin secretion and reduced insulin action. We previously reported that inhibiting the cannabinoid 1 receptor (CB1) improves both insulin secretion and insulin action in pancreatic beta cells. Skeletal muscle plays an important role in metabolism because it is responsible for the majority of glucose uptake in the fed state, especially in humans. Therefore, we developed a specific skeletal muscle CB1 knockout (Skm-$CB1^{-/-}$) mouse to determine what role, if any, CB1 plays in skeletal muscle.

Materials and methods: We infused 8-week old Skm-$CB1^{-/-}$ and control Skm-$CB1^{+/+}$ littermates with S961 (insulin receptor blocker) for 6 days to induce acute insulin resistance during which we monitored blood glucose and plasma insulin. We also fed 6-to-8-week old Skm-$CB1^{-/-}$ and Skm-$CB1^{+/+}$ male mice either a standard diet (SD), or a high fat/high sugar diet (HFHS) for 15 weeks to induce chronic insulin resistance. We measured body weight and blood glucose levels throughout the study, and performed an intraperitoneal insulin tolerance tests (ITT) prior to sacrifice. We measured metabolic parameters utilizing CLAMS system, and body composition by nuclear magnetic resonance (NMR) at the end of the study. We evaluated physical performance by placing the mice on a rotarod and a treadmill, and by grip strength test prior to sacrifice. At the end of the study, we cryopreserved muscles for protein extraction and for histological analysis.

Results: S961 induced a 2.8-fold increase in blood glucose (BG) and 30-fold increase in plasma insulin (PI) in Skm-$CB1^{+/+}$ mice, but 1.7-fold and 15-fold respectively in Skm-$CB1^{-/-}$ mice. SD-Skm-$CB1^{-/-}$ had a 1.6-fold increase in endurance only in the rotarod, compared to SD-Skm-$CB1^{+/+}$ mice. After 15 weeks on HFHS body weight and BG were similar in both strains, but circulating PI levels were 25% lower in HFHS-Skm-$CB1^{-/-}$ mice and they were more insulin sensitive based on ITT than HFHS-Skm-$CB1^{+/+}$ mice. Additionally, the respiratory exchange ratio of HFHS-Skm-$CB1^{-/-}$ was significantly lower than in HFHS-Skm-$CB1^{+/+}$ mice, suggesting that Skm-$CB1^{-/-}$ mice are more reliant on fatty acid than glucose metabolism. The lean/fat ratio was significantly higher and the mass of gastrocnemius, soleus, and quadriceps was greater in HFHS-Skm-$CB1^{-/-}$ mice compared to HFHS-Skm-$CB1^{+/+}$ mice. Physical performance tests showed that HFHS-Skm-$CB1^{-/-}$ had greater endurance than HFHS-Skm-$CB1^{+/+}$ mice. Protein array performed on gastrocnemius protein extracts revealed a change in phosphorylation of downstream targets of the insulin receptor pathway.

Conclusion: Absence of CB1 specifically in skeletal muscle increases insulin sensitivity in mouse models of both acute and chronic insulin resistance. Absence of CB1 in skeletal muscle also leads to increased lean/fat ratio, increased muscle mass and improved mouse endurance in physical performance tests. These results collectively show that CB1 is involved in regulating muscle mass and insulin sensitivity, and consequently physical performance.

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KBP-089 improves glucose control and reduces liver steatosis, inflammation and fibrosis stage in high fat, high cholesterol fed rats

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Background and aims: Insulin sensitizers such as the different PPAR agonists have shown promise as therapies for nonalcoholic steatohepatitis

(NASH), and while these agents have proven efficacious with regards to hepatic histology and glucose control - their use is limited by the conservative beneficial effects and the undesired side effects associated with their use, namely weight gain. Here we present a highly potent dual amylin- and calcitonin receptor agonist (DACRA), KBP-089, which is able to induce substantial weight loss and improve glucose control and insulin sensitivity. Here we evaluate the effect on body weight, glucose control as well as different hepatic features of NASH in rats.

Materials and methods: 6 week old rats received a high fat diet (HFD) for 8 weeks to induce obesity followed by a high fat, high cholesterol and cholate diet (HFCC) for 56 days. After HFD, the rats were significantly obese ($p<0.01$). The rats were assigned into treatment groups receiving either vehicle (saline) or KBP-089 in four doses. All the rats were escalated weekly starting from the lowest dose 0.625 μg/kg and to 1.25, 2.5 and 5.0 $\mu g \cdot kg^{-1}$, respectively. We evaluated the livers using the NAFLD activity score (NAS). The blinded histological assessment of NAS was performed on Masson's Trichrome and Sirius Red stained terminal hepatic tissue from vehicle and KBP-089 E5 μg/kg treated HFCC rats.

Results: KBP-089 induced and sustained a significant vehicle-corrected weight loss - 16.5% in the group receiving the highest dose - and reduced overall adiposity. Importantly, treatment with KBP-089 improved glucose tolerance and enhanced insulin action during and oral glucose tolerance test, resulting in significantly reduced AUC values for both glucose and insulin. Additionally, KBP-089 treatment reduced total triglyceride and AST levels, albeit no difference was observed in ALT levels. At the histological level, KBP-089 impressively reduced both the combined NAFLD activity score and HFCC induced fibrosis stage. Finally, KBP-089 improved insulin action during an oral glucose tolerance test. After 8 weeks of treatment, the HFCC diet induced hepatomegaly was dose-dependently reduced by KBP-089; a reduction, which was equalized when normalized to the individual body weight. The HFCC feeding induced massive lipid accumulation, ballooning and inflammation in the vehicle livers. Notably, after treatment with KBP-089 for 8 weeks, this inappropriate storage of lipids, ballooning, and inflammation were reduced by treatment with KBP-089, hence significantly reducing NAS. Finally, KBP-089 significantly reduced the fibrosis stage in HFCC rats by approximately 60%.

Conclusion: In conclusion, KBP-089 is a weight reducing agent that is well tolerated when introduced by dose escalation. Importantly, KBP-089 enhances glucose tolerance and insulin action and improves hepatic features of NASH, hence revealing the potential of KBP-089 as a therapeutic target in the treatment of T2D and NASH.

Disclosure: **S. Gydesen:** Employment/Consultancy; Nordic Bioscience.

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Bromocriptine changes adipocyte lipid metabolism improving insulin sensitivity in obese diabetic rats

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Background and aims: Alterations of adipocyte lipid metabolism in obesity is associated with the development of adipose tissue dysfunction and insulin resistance, ultimately leading to type 2 diabetes (T2D). Bromocriptine, a D2 dopamine receptor agonist, has been shown to be a promising therapeutic strategy in the treatment of T2D, leading to a significant reduction of glycemia. However, its role in modulating the mechanisms of insulin resistance is not known. Thus, our main goal was to assess the therapeutic usefulness of bromocriptine in modulating the mechanisms of lipid and glucose metabolism in periepididymal adipose tissue, as well as the insulin sensitivity in obese type 2 diabetic rats.

Materials and methods: We studied male Wistar rats fed a standard diet (group 1) and non-obese type 2 diabetic Goto-Kakizaki (GK) rats divided into 4 groups: a group fed the standard diet (group 2), a group of obese GK rats induced by a high-fat high-sucrose diet (group 3), a group of obese GK rats treated with bromocriptine 10mg/Kg/day for 30 days (group 4) and a group of obese GK rats treated with the vehicle (group 5) (n=8/group). The glycemic and lipid profiles were assessed and an insulin tolerance test was performed. Moreover, insulin receptor pathway and lipid oxidation, synthesis and storage pathways were also assessed.

Results: Obese GK rats had impaired insulin tolerance, fasting hyperglycemia and higher cholesterol and triglycerides levels than their lean controls. Bromocriptine treatment improved insulin sensitivity and fasting glycemia. Furthermore, despite no alteration were observed in insulin receptor activation, bromocriptine dramatically increased dopaminergic signaling and GLUT4 levels in periepididymal adipose tissue. Regarding the mechanisms involved in lipid metabolism, bromocriptine increased the activation of AMPK, important for lipid oxidation, and caused a downregulation of ACC, FAS and HSL, key enzymes in fatty acid synthesis pathways.

Conclusion: Although the mechanisms still need future investigation, our results show that bromocriptine changes adipose tissue lipid metabolism, also increasing GLUT4 levels. Such events are involved in improving fasting glycemia and insulin sensitivity, suggesting that bromocriptine may be a promising therapeutic strategy in T2D.

Supported by: FCT; SPD

Disclosure: **P. Matafome:** None.

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Bilateral electrical modulation of carotid sinus nerve improves glucose homeostasis in rodent type 2 diabetes model

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Background and aims: Bilateral abolishment of carotid body (CB) activity, through surgical resection of the carotid sinus nerve (CSN), restores insulin sensitivity and glucose tolerance in prediabetes and early-phase type 2 diabetes animal models by positively impacting on increased liver and visceral adipose tissue glucose uptake. Application of a high frequency alternating current (HFAC) onto the CSN inhibits action potential propagation through the nerve and was used to study the effect of an electrical, reversible, CSN neuromodulation upon glucose metabolism in a diet-induced early stage type 2 diabetes animal model.

Materials and methods: Rats were fed either a chow or high-fat-high sucrose (HFHSu) diet (60% lipid-rich diet plus 35% sucrose drinking water) during 14 weeks. HFHSu fed rats developed insulin and glucose intolerance. Neural interfaces were bilaterally implanted in the CSNs and attached through a head cap and tether to an external pulse generator. The rats were then randomized to electrical neuromodulation or no neuromodulation groups. Blocking parameters were defined by testing the effect of acute CSN blocking in the response to hypoxia (10% O_2+ 90% N_2, 1 min). Insulin sensitivity was evaluated through an insulin tolerance test and glucose tolerance by an oral glucose tolerance test throughout the duration of the study. Fasting glycemia, insulinemia, ventilatory responses and behavior were also monitored.

Results: HFAC neuromodulation of the CSN, applied continuously over 9 weeks restored insulin sensitivity (K_{ITT} HFHSu sham=2.56±0.41 %glucose /min; K_{ITT} HFHSu CSN electrical block= 5.01±0.52 %glucose/min) and glucose tolerance (AUC HFHSu sham=1278±20.36 mmol/lxmin; AUC HFHSu CSN electrical block=1054.15±62.64 mmol/lxmin) in early-stage type 2 diabetes rat models. Upon cessation of the electrical stimulus, the insulin resistance and glucose intolerance returned within 5 weeks. HFAC neuromodulation of the CSN in chow fed rats decreased basal ventilation and the ventilatory response to hypoxia but behavioral

alterations were not observed. The ventilatory responses were reversible upon cessation of the electrical stimulus.
Conclusion: The present study shows that high frequency block of the CSN improves metabolic control in early-stage type 2 diabetes animal models representing a potential therapeutic tool for the treatment of type 2 diabetes.
Supported by: Galvani Biolectronics
Disclosure: **S.V. Conde:** Grants; Received Research support from Galvani Biolectronics, Galvani Biolectronics.

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Diet-specific effects of apolipoprotein A5 on glucose metabolim

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Background and aims: Apolipoprotein A5 (ApoA5) is a liver-derived protein with major impact on triglyceride metabolism that has also been found to determine ectopic lipid accumulation in high fat diet fed mice. While its extracellular effects on lipoprotein lipase activity are well understood, cellular effects have not been clearly identified yet. In this study we set out to clearly define diet-specific effects of apoA5 in glucose metabolism.
Materials and methods: Male, apoA5 knockout (apoA5$^{(-/-)}$) and wildtype (wt) mice were fed with different metabolically harmful diets (high fat diet (HFD), high fructose diet (HFruD), mixed high sucrose and high fat diet (HSHFD) or standard diet (STD) for 10 weeks. Characterization of all mice included performance of oral glucose tolerance test (GTT) and intraperitoneal insulin tolerance test (ITT) calculated as glucose area under the curve.
Results: Body weight was comparable in apoA5$^{(-/-)}$ and wt mice in any diet group. Glucose area under the curve during oral glucose tolerance test was significantly lower in HFruD fed apoA5$^{(-/-)}$ mice when compared to HFruD fed wt mice. In any other diet group, glucose tolerance was similar in apoA5 deficient and wildtype mice. In accordance with results from the oral glucose tolerance test, insulin sensitivity estimated by an intraperitoneal insulin tolerance test was significantly higher in HFruD fed apoA5$^{(-/-)}$ mice than in wt littermates, while insulin sensitivity was comparable in apoA5 deficient and wt mice after HFD, HSHFD or SD feeding. As expected, serum triglyceride levels were significantly higher in all apoA5$^{(-/-)}$ mice than in wt mice.
Conclusion: In our studies apoA5 deficiency was associated with improved glucose tolerance and insulin sensitivity in high fructose but not in high fat or mixed high sucrose and high fat diet fed mice. From these results we hypothesize that apoA5 is critically involved in pathogenesis of metabolic disturbances caused by high fructose intake and thus warrants further investigation as novel treatment target in diet-induced metabolic disease.
Supported by: CDG
Disclosure: **C. Ress:** None.

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Hepatocyte-specific Shp1 deletion increases the beneficial effect of rosiglitazone in diet-induced obese mice

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Background and aims: The protein-tyrosine phosphatase Shp1 plays a critical role in the development of obesity-linked insulin resistance. Hepatocyte-specific deletion of Shp1 in mice ameliorates liver glucose homeostasis and hepatic lipid metabolism. While Shp1 hepatocyte-specific knockout mice (*Ptpn6*$^{H\text{-}KO}$) fed a high-fat diet (HFD) develop increased hepatic steatosis compared to their wild-type littermates (*Ptpn6*$^{fl/fl}$) on the same diet their livers exhibit a reduced inflammatory profile and less hepatocellular damage. Interestingly, we found that peroxisome proliferator-activated receptor gamma (PPARγ) expression and activity was increased in the livers of *Ptpn6*$^{H\text{-}KO}$ mice suggesting a new role for Shp1 in the regulation of PPARγ and the control of hepatic lipid metabolism. Thus, the aim of the present study was to determine whether increasing hepatic PPARγ activity in the liver of the *Ptpn6*$^{H\text{-}KO}$ mice using rosiglitazone (RSG) treatment could further improve hepatic glucose and lipid homeostasis as well as the inflammatory profile in these mice lacking hepatocyte Shp1.
Materials and methods: *Ptpn6*$^{H\text{-}KO}$ and *Ptpn6*$^{fl/fl}$ mice were kept on a standard or HFD (55% kcal fat) for 18 weeks starting at 8 weeks of age. During the last 4 weeks animals were treated with a very low dose of RSG (0.3 mg/kg/day), which aims at mainly targeting the liver to avoid confounding effects on the liver due to rosiglitazone acting on adipose tissue. Oral glucose tolerance tests (oGTT) were performed before and at the end of RSG treatment. At 18 weeks mice were sacrificed for further plasma and tissue analyses.
Results: RSG treatment improved glucose tolerance more in high-fat fed *Ptpn6*$^{H\text{-}KO}$ mice as compared to *Ptpn6*$^{fl/fl}$ littermates. Glucose-induced insulin response during oGTT was not affected. Insulin-induced Akt phosphorylation was increased in RSG-treated *Ptpn6*$^{H\text{-}KO}$ and *Ptpn6*$^{fl/fl}$ mice on HFD compared to untreated HFD mice. Liver weight and hepatic triglyceride levels were increased by RSG in both *Ptpn6*$^{fl/fl}$ and *Ptpn6*$^{H\text{-}KO}$ mice on HFD. Despite this increase in hepatic steatosis, we observed a stronger increase in IL-10 expression in RSG treated *Ptpn6*$^{H\text{-}KO}$ mice than in the *Ptpn6*$^{fl/fl}$ mice without any changes in TNFα or IL-1β levels or macrophage infiltration based on determination of F4/80 levels. Interestingly, RSG treatment did not affect any of the fat pad weights nor did it alter adiponectin level in mice of both genotypes on HFD confirming that the low dose of RSG mainly affected the liver.
Conclusion: Our data suggest that hepatic invalidation of Shp1 increased the beneficial effect of RSG on glucose tolerance, which is associated with increased production of anti-inflammatory IL-10 in the liver of HFD-fed obese mice.
Supported by: CIHR
Disclosure: **K. Bellmann:** None.

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Liver-specific ablation of insulin-degrading enzyme causes hepatic insulin resistance and glucose intolerance, without effect on insulin clearance in mice

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Background and aims: Insulin-degrading enzyme (IDE) is a ubiquitously expressed zinc-metalloprotease that is one of the principal enzymes implicated in the degradation and clearance of insulin, in addition to glucagon, amylin and the amyloid-ß protein (Aß). Several polymorphisms of IDE are associated with risk for type 2 diabetes (T2D) in humans, whereas hepatic insulin clearance is reduced in T2D patients. Although strongly implicated in insulin clearance in vivo, the role of IDE specifically in the liver—the principal site of plasma insulin clearance—remains unclear. The aim of this study is to decipher the role of IDE on hepatic insulin signaling and its impact on whole-body glucose metabolism and insulin clearance.
Materials and methods: To ablate IDE selectively in hepatocytes, mice homozygous for a floxed *IDE* allele were intercrossed with albumin-Cre

mice (hereafter L-IDE-KO). Metabolic studies were performed in 3-month-old male mice for fasting and non-fasting glucose, insulin, triglycerides, and body weight. Likewise, intraperitoneal glucose and insulin tolerance tests, and hepatic plasma insulin clearance were assessed. To analyze hepatic insulin signaling, fasted mice were injected intraperitoneally with insulin (0.75U/Kg) for 10 min. Afterwards, mice were sacrificed and liver (or skeletal muscle) tissues analyzed by western blot for total and phosphorylated levels of the insulin receptor (IR), and protein kinase B (AKT1 and AKT2). Glucagon, Aβ40 and amylin levels were assessed by ELISA.

Results: L-IDE-KO fasting and non-fasting plasma glucose levels were higher (~20%) than control mice. These changes were not associated with augmented food intake or body weight. Of note, fasting and non-fasting plasma insulin levels were similar between control and L-IDE-KO mice. In parallel, L-IDE-KO showed glucose intolerance and insulin resistance. Surprisingly, hepatic plasma insulin clearance was similar between L-IDE-KO and control mice. Plasma amylin and Aβ40 levels remained unchanged between control and L-IDE-KO mice, whereas non-fasting plasma glucagon levels showed a ~50% non-statistically significant increase in L-IDE-KO mice compared to control mice. Hepatic insulin resistance in the L-IDE-KO mouse was associated with reduced plasma membrane IR levels (~30%), as well as reduced phosphorylation levels (~55%). Consistently, phosphorylation of AKT1 and AKT2 were reduced by ~45% at both Ser473 and Thr308 residues. Finally, muscle insulin signaling (phosphorylation of AKT) was unaffected by hepatic ablation of IDE.

Conclusion: We have revealed a new role for IDE in the regulation of hepatic insulin signaling. Our data suggest that IDE might be a key player in hepatic insulin resistance. We hypothesize that IDE regulates IR recycling to plasma membrane, and its activity, in response to insulin stimulation in hepatocytes. Finally, our data demonstrate that IDE is not necessary for hepatic plasma insulin clearance.

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Disclosure: **P. Villa-Perez:** None.

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Febuxostat improves skeletal muscle insulin resistance via upregulating skeletal muscle PGC-1α expression in high fat diet fed male Wistar rats

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Background and aims: Hyperuricemia is closely associated with various metabolic disorders such as diabetes, hypertension, dyslipidemia, and cardiovascular diseases. Xanthine oxidoreductase (XOR) is a key enzyme known to catalyze purines to uric acid. Febuxostat is an orally-active, potent, non-purine, selective XOR inhibitor. However, the effect of febuxostat on glucose and insulin metabolism has not been fully elucidated. In current study, we investigated the effect of febuxostat on insulin sensitivity in male Wistar rats, whereby insulin sensitivity was measured directly using the hyperinsulinemic-euglycemic glucose clamp studies (at 25 mU/kg/min insulin infusion rate) after an 8 hour fast.

Materials and methods: Male Wistar rats were fed normal chow diet (NCD), or 60% high fat diet (HFD) containing with either febuxostat (~4 mg/kg/day) or not, for 4 weeks. Euglycemic-hyperinsulinemic clamp studies were performed after an 8-hour fast. All procedures were performed in accordance with the Guide for Care and Use of Laboratory Animals of the NIH and were approved by the Animal Subjects Committee of our institute.

Results: In the NCD fed rats, during the clamp studies, the glucose infusion rate (GIR), the clamp hepatic glucose output (cHGO) and insulin-stimulated glucose disposal rate (IS-GDR) were no significant changes between two groups. On the other hand, in the HFD fed rats, the GIR and IS-GDR were significantly increased by 12% and 17%, respectively, in febuxostat group compared to in the HFD fed control group. But cHGO was no significant change between two groups. Consistent with the clamp data, the insulin-stimulated phosphorylation of Akt and AMPK were significantly increased by 90% and 53%, respectively, in skeletal muscle of HFD fed febuxostat treated HFD fed rats. Furthermore, peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α expression in skeletal muscle, which is a key regulator of mitochondrial content and function, was significantly increased by 2 folds in HFD fed febuxostat treated rats compared to the HFD fed control rats, as assayed by real-time qRT-PCR.

Conclusion: Our findings demonstrated that febuxostat had beneficial effects on skeletal muscle insulin sensitivity in an insulin resistant state.

Disclosure: **C. Moriya:** None.

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A_2 adenosine receptors mediate chronic caffeine effects on insulin sensitivity

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Background and aims: Several epidemiological studies showed that chronic caffeine intake decreased the risk of type 2 diabetes, and our group demonstrated that chronic caffeine intake prevents the development of insulin resistance in diet-induced insulin resistance rats. Caffeine has several cellular mechanisms of action, being the antagonist of adenosine receptors the only attained with human coffee consumption. The aim of this work is to unravel the subtypes of adenosine receptors involved on the effects of chronic caffeine intake on insulin sensitivity and the mechanism behind this effect.

Materials and methods: Two groups of Wistar Rats of both sexes (9-12weeks) were used. The control group fed a sham diet and the high-sucrose (HSu) group fed 35% sucrose in drinking water during 28 days. The control and HSu animals were randomly divided into three subgroups that were submitted to the i.p. administration of DPCPX (A_1 antagonist, 0.4mg/kg), SCH58261 (A_{2A} antagonist, 0.5mg/kg) and MRS1754 (A_{2B} antagonist, 9.5μg/kg), respectively, in the last 15 days of the diet. Insulin sensitivity was assessed through an insulin tolerance test. Blood pressure, visceral and total fat were measured. Skeletal muscle, adipose tissue and liver were collected to evaluate, by western blot, alterations in the expression of Glut4, Glut2 and, A_1, A_2A and A_2B adenosine receptors.

Results: In control animals, SCH58261 and MRS1754 decreased insulin sensitivity by 20.40 and 41.45%, respectively. HSu diet decreased insulin sensitivity to 2.41±0.18 %glucose/min from a control value of 4.16±0.28 %glucose/min, an effect that was increased by SCH58261 and MRS1754 administration by 36.92 and 54.32%, respectively. In HSu group, MRS1754 increased total fat and DPCPX reversed visceral fat to control values. HSu animals showed an increase in blood pressure, an effect that was decreased by DPCPX and SCH58261, and completely restored by MRS1754. In skeletal muscle from control animals, DPCPX and SCH58261 administration increased A_1 and A_2A expression by 39.27 and 24.32%, respectively. Moreover, A_1, A_2A and A_2B expression was increased in HSu group, an effect that was restored only by SCH58261 and MRS1754. In liver, A_2A expression is increased in HSu group, an effect that was restored by SCH58261. Moreover, A_2B expression decreased in HSu animals, an effect that was restored by MRS1754. In adipose tissue, SCH58261 increased A_2A expression and DPCPX decreased A_1 expression in HSu animals. Additionally, Glut4 expression in skeletal muscle from control animals decreased with DPCPX and SCH58261 administration.

Conclusion: The effects of chronic caffeine intake on insulin sensitivity are mediated mainly by A_{2A} and A_{2B} adenosine receptors. Additionally, an effect of A_1 adenosine receptors on visceral fat can also account for the effects of the chronic caffeine intake on insulin sensitivity.

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Disclosure: **J.F. Sacramento:** Grants; JFS by PD/BD/105890/2014; MJR by SFR/BD/ 88983/2012.

PS 025 In vivo insulin secretion

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Cystathionine beta synthase deficiency impairs in vivo insulin secretion in mice

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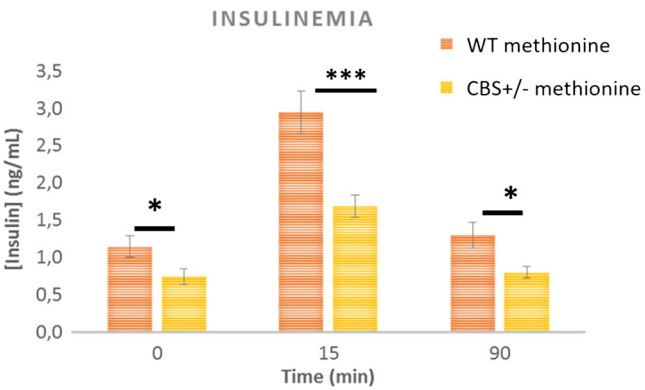
Background and aims: Cystathionine beta synthase (CBS) catalyzes the first step of the transsulfuration pathway form homocysteine to cystathionine, and its deficiency lead to hyperhomocysteinemia (HHcy) in humans and rodents. Although it has been shown that hyperhomocysteinemia promotes insulin resistance by inducing endoplasmic reticulum stress in adipose tissue, and upregulating resistin, scarce information is available about its effect on insulin secretion, and the overall link between CBS activity and the setting of type 2 diabetes is still unknown. We recently established a multiparameter systems-based approach to identified new islet-expressed genes associated with islet functions in mice. Among these genes, we identified CBS whose expression in islet is positively correlated with in vivo insulin secretion. We postulated that a CBS deficiency would be associated with decreased insulin secretion.

Materials and methods: We used a mouse model heterozygous for CBS (CBS+/-) which presents a mild HHcy. Another group of CBS+/- mice, and controls, was supplemented with methionine in drinking water to increase mild to intermediate HHcy, the latter were also submitted to a high-fat diet. We characterized the mice for their food intake, body weight gain, body composition, glucose homeostasis, plasma homocysteine level and CBS activity.

Results: CBS+/- mice without methionine supplementation present a mild glucose intolerance without any change in body weight or insulin sensitivity; they display a significant decrease in basal insulinaemia. Under methionine supplementation, CBS+/- mice have lower body weight and less fat mass than their wild-type controls, under both chow diet and high-fat diet, and these features are not accompanied with changes in food intake. CBS+/- mice show a glucose intolerance due to a defect in insulin secretion in both basal and glucose stimulated conditions (CBS+/- basal insulin secretion in ng/ml : 0,74 ± 0,06 vs. 1,14 ± 0,07 in controls, $p<0.05$; CBS+/- glucose stimulated insulin secretion 1,68 ± 0,07 vs. 2,95 ± 0,12, $p<0.001$). CBS+/- mice with methionine also present a slight improvement in insulin sensitivity after in vivo insulin tolerance test.

Conclusion: We report here that CBS+/- mice with methionine supplementation are leaner under chow diet and protected against high-fat diet induced body weight gain, however they present a glucose intolerance due to insufficient insulin secretion. Whether this defect is due to a direct control of CBS on insulin secretion needs to be further investigated.



Disclosure: **C. Cruciani-Guglielmacci:** None.

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Impaired beta cell function and glucose sensitivity in Chinese individuals with young-onset type 2 diabetes

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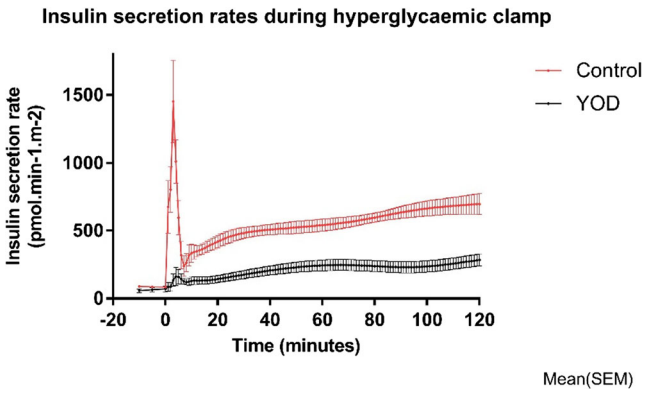
Background and aims: Young-onset type 2 diabetes (YOD) under the age of 40 years occurs in one in five patients with diabetes in Asia. We hypothesise this is driven mainly by beta cell dysfunction, however, detailed physiological studies in Chinese patients are lacking. The aim of this study is to evaluate beta cell function, beta cell glucose sensitivity and insulin sensitivity using the hyperglycaemic clamp in YOD patients with early disease and normal controls.
Materials and methods: We studied 10 YOD patients of Chinese ethnicity (Mean±SD age 36±6 years, BMI 25.7±3.6 kg/m^2, disease duration 2.9±1.5 years) and 10 age- and BMI- matched subjects with normal glucose tolerance and no family history of diabetes . First- and second- phase insulin were evaluated using a two-hour hyperglycaemic (12 mmol/L) clamp. Insulin secretion rates were determined by C-peptide deconvolution. Glucose sensitivity was calculated as the ratio of incremental second-phase insulin secretion to the increment in glycaemia. Insulin sensitivity index (ISI) was calculated as the glucose infusion rate divided by the plasma insulin at steady state.
Results: Basal insulin secretion rate was lower in YOD participants than control subjects (64 [51] *vs* 82 [36] pmol·min^{-1}·m^{-2}, median [IQR], p=0.02). First-phase insulin secretion was reduced by 74% in YOD participants compared to normal subjects (98 [95] *vs* 624 [261] pmol·min^{-1}·m^{-2}, p=0.001) and second-phase insulin secretion was reduced by 64% (227 [158] *vs* 627 [306] pmol·min^{-1}·m^{-2}, p=0.001). Glucose sensitivity was markedly impaired in YOD as compared to controls (24.4 [18.3] *vs* 77.5 [38.0] pmol·min^{-1}·m^{-2}·mM^{-1}, p=0.001) whilst ISI was similar in both groups (22 [39] *vs* 18 [21] mg·kg^{-1}·min^{-1}·pmol^{-1}).
Conclusion: Chinese individuals with YOD display marked deficiency in insulin secretion and beta cell glucose sensitivity even with short duration of disease. While early insulin treatment may slow down the progression of beta cell dysfunction, early detection of these subjects through screening is important to prevent delayed intervention.



Disclosure: **E. Chow:** None.

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55P0251, a novel molecule developed from a herbal backbone structure with potent anti-hyperglycaemic activity in rodents: An imidazoline-like mechanism of action?

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Background and aims: 55P0251 is a novel compound, which we have developed from a molecular backbone structure derived from multiflorine, a compound found in herbal remedies. 55P0251 has distinct anti-hyperglycaemic, but not hypoglycaemic, activity in rodents, which we have attributed to amplification of glucose-stimulated insulin secretion. In preceding experiments, we excluded that 55P0251 acts via mechanisms exploited by established anti-diabetic drugs, including that it did not affect the K_{ATP} channel or the GLP-1 system. Here, we describe further efforts to track down its molecular target and mechanism of action.
Materials and methods: The effects of 55P0251 and its corresponding enantiomer, 55P0250, were examined in a comparative manner in mice. Oral glucose tolerance tests (OGTT), insulin release from perifused pancreatic islets, and oxygen saturation of tail blood were examined. The α_2-adrenoceptor agonist UK14,304 (UK) was used to sort out the potential role of the sympathetic nervous system.
Results: *In vivo*, 55P0251 showed much more pronounced glucose lowering activity than its corresponding enantiomer 55P0250 (AUC in the OGTT, mol/l*min: vehicle, 1.71±0.04; 45mg/kg 55P0251, 1.10±0.03, p<0.0001 vs vehicle; 45mg/kg 55P0250, 1.54±0.06, p=0.03 vs vehicle; enantiomer comparison, p<0.0001). *In vitro*, the two enantiomers augmented glucose-induced insulin release to a similar extent in the absence of adrenergic stimulation (insulin released from perifused islets during 30min of glucose stimulation, pmol/islet: vehicle, 160±30; 500µmol/l 55P0251, 346±69, p=0.02 vs vehicle; 500µmol/l 55P0250, 493±48, p<0.001 vs vehicle; enantiomer comparison, ns), but 55P0251 was clearly superior in counteracting adrenergic inhibition of insulin release by 1µmol/l UK (pmol/islet: UK alone, 52±13; UK+250µmol/l 55P0251, 169±18, p=0.0008 vs UK; 250µmol/l UK+55P0250, 42±14, ns vs UK; enantiomer comparison, p=0.0006). This pattern hinted at sympathetic antagonism of 55P0251, but not 55P0250, which was supported by corresponding interaction with the α_{2A}-adrenoceptor (% inhibition in the binding assay: 10µmol/l 55P0251, -73%; 10µmol/l 55P0250, -1%). α_{2A}-antagonistic activity of 55P0251 *in vivo* was confirmed by increased oxygen saturation of tail blood (indicative of thermoregulatory vasodilation), as it was likewise induced by established α_{2A}-antagonists (vehicle, 64.9±2.0%; 0.5mg/kg yohimbine, 86.5±0.9%; 1mg/kg efaroxan, 85.2 ±3.6%; 90mg/kg 55P0251, 83.4±2.5%; p<0.0001 vs vehicle each). 55P0250 had no such effect (vehicle, 52.4±1.6%, vs 90mg/kg 55P0250, 56.7±2.9%; ns). Accordingly, a low dose of 55P0251 counteracted hyperglycaemia induced by injection of the α_2-agonist UK (AUC of glucose excursion caused by 0.1mg/kg UK: UK alone, 2.22±0.08; vs UK+1.4mg/kg 55P0251, 1.82±0.16; p<0.05).
Conclusion: The novel glucose lowering agent 55P0251 obviously carries α_{2A}-antagonistic properties, which contribute to its effects on insulin release and blood glucose. At high concentrations, 55P0251 appears to amplify glucose-stimulated insulin release via a second mechanism of action that is also addressed by its corresponding enantiomer 55P0250. The observed dual mode of action of 55P0251 is reminiscent of what has been ascribed to imidazolines.
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Disclosure: **C. Fürnsinn:** Grants; Austrian Research Promotion Agency, grant # 820121/18793 SCK/KUG.

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Sphingomyelin synthase 2 deficiency impairs insulin secretion in pancreatic beta cells

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Background and aims: Sphingomyelin synthase 2 (SMS2), which mainly located in the plasma membrane and the Golgi, is known to synthesis

sphingomyelin from substrate ceramide. Previous work demonstrated that plasma membrane sphingomyelin content reduction resulted from knocking out SMS2 improved whole-body insulin sensitivity. Meanwhile, we also noted that serum insulin level in SMS2-KO mice significantly decreased both at fasting and feeding status, but its role in insulin secretion remains unclear.

Materials and methods: We performed experiments on SMS2 whole-body knock out mice. Glucose tolerance test and insulin tolerance test were conducted. Islets number, size and density analysis were based on pancreas HE staining. Islets were isolated for glucose stimulated insulin secretion test, glucose uptake test, RNA extraction, as well as for getting single beta cells for further patch clamp and Immunofluorescence staining. SPSS 19.0 was used for statistics analysis.

Results: First, we validated that lower serum insulin level in SMS2-KO mice upon 2g/kg glucose challenge via intraperitoneal injection. The results were further confirmed by *ex vivo* glucose stimulated insulin secretion test, showing significant insulin secretion capacity in the isolated islets from SMS2 deficient mice when treated with 16.7mmol/L glucose solution. Then, our work indicated the lower insulin concentration was not owing to changes in the number or size of the islets, beta cell proliferation or apoptosis, the ratio between alpha and beta cells or proinsulin synthesis. Thus we came to the hypothesis, SMS2 is fundamental to pancreatic beta cell insulin secretion, which get strongly supported by patch clamp on single beta cells, presenting with almost 2-fold decrease of functional insulin release pool size in SMS2-KO mice. Transmission electron microscopy was applied to observe ultra-structure within beta cells. Insulin granules in SMS2 null group seemed to be smaller in size, lower density and less mature. Meanwhile, glucose response is crucial to beta cell function, and GLUT2 on the plasma membrane is the glucose transporter in beta cell, serving as the first-line glucose sensor. We found that glucose uptake was dramatically decreased, along with less detectable GLUT2 protein in SMS2-KO beta cells. Since SMS2 KO could directly lead to decline in sphingomyelin content on the plasma membrane, anchored membrane protein such as GLUT2 would be influenced by local lipid composition changes.

Conclusion: Herein, we reported insulin secretion got impaired in SMS2 deficient mice, and changes in GLUT2 on the membrane may account for the decrease in glucose stimulated insulin secretion capacity.

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Disclosure: **H. Zhou:** None.

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Metabolic consequences of residual beta cell function during the early years after diagnosis of type 2 diabetes

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Background and aims: Beta cell function in the years following diagnosis of type 2 diabetes is a major therapeutic target, and understanding the metabolic effects of residual function is important. The Direct Remission Clinical Trial (DiRECT) is a controlled trial of weight loss versus conventional therapy for people with early Type 2 Diabetes. We report the characteristics of a subgroup at baseline who had detailed insulin secretion and metabolic assessments.

Materials and methods: Participants were studied during current therapy. 95 participants underwent detailed metabolic testing and complete data was obtained on n=89. Insulin secretion was assessed by the Stepped Insulin Secretion Test with Arginine (SISTA) after an overnight fast and during 2.8 mmol/l increments in plasma glucose. A deconvolution method was used to reconstruct insulin secretion rates from C-peptide concentrations and calculate the 6 minute incremental insulin secretion response. Fasting very low density lipoprotein triglyceride (VLDL-TG) production rate and pool size was measured by a non-isotopic competitive blocking method. 3-point Dixon Magnetic Resonance Imaging was used to estimate the liver and the pancreatic fat. Data are shown as mean ± SD or median with range.

Results: This subgroup of DiRECT was representative of the United Kingdom type 2 diabetes population with early type 2 Diabetes: 57% male; 53.1±7.8 years; 99.5±16.2 kg; BMI 34.4±4.2 kg/m^2, diabetes duration 2.9±1.7 years. Fasting plasma glucose (FPG) was 8.4±2.6 mmol/l with fasting plasma insulin (FPI) 92±57 pmol/l. Liver fat was markedly elevated at 14.7±9.8%. Mean pancreatic fat was also elevated at 8.3 ±2.3%. The production rate of VLDL1-TG was 545±179 mg/kg/day with VLDL1-TG pool size of 2291±1645mg. Fasting NEFA was 0.61±0.20 mmol/l and 0.31±0.13mmol/l at 70min of the SISTA. Liver fat positively correlated with VLDL1-TG production rate (R=0.43; p<0.0001), VLDL1-TG pool size (R=0.31; p=0.003), FPI (R=0.52; p<0.0001), and FPG (R=0.35; p=0.001).The median first phase insulin secretion was 0.138 nmol/min/m^2 (range -0.068 to +0.321).Total cohort first phase insulin response correlated negatively with FPG (R=-0.35; p<0.001). This correlation was much stronger in males (R=-0.54; p<0.0001). FPI correlated negatively with fasting NEFA in the whole cohort (R=-0.23; p=0.015). The first phase insulin response was independent of age, duration of the diabetes, liver fat and VLDL1-TG pool size.

Conclusion: In this cohort of people with short duration type 2 diabetes, median first phase insulin response was low although a wide range of responses were observed. This was associated with fasting plasma glucose control, reflecting the importance of beta cell function for overall control in the early years of type 2 diabetes. There was no correlation between first phase insulin response and duration within the first 6 years of diabetes. Liver fat was nearly 3 times over the upper limit of the population normal range. The data provide new insight into the consequences of beta cell dysfunction in early type 2 diabetes and form a baseline from which the effect of the weight loss intervention of DiRECT can be measured.

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Supported by: DiRECT study is funded as a Strategic Research Initiative by Diabetes UK.

Disclosure: **S.V. Zhyzhneuskaya:** Grants; Diabetes UK.

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Beta cell secretory responses to oral ethanol and isoethanolaemic i.v. ethanol in healthy subjects

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Background and aims: The macronutrients glucose, protein and fat are well known stimulators of the secretion of the incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) from the enteroendocrine K and L cells, respectively, and thus elicit what is denominated the incretin effect; i.e. a greater insulin response after oral vs i.v. administration. Ethanol can also be considered an energy-rich macronutrient, but it is not known whether oral administration of ethanol elicits an incretin effect. The aim of this study was to investigate a possible incretin effect of ethanol.

Materials and methods: In a double-blinded, cross-over design, we subjected 12 fasted healthy men (age 25.3±3.9 (mean±SD) years; BMI 22.6±2.6 kg/m^2) to intragastrically instilled (0.70 g ethanol per kg body weight in a 20% (v/w) solution infused over 5 minutes) and isoethanolaemic i.v. infusion of ethanol (0.70 g ethanol per kg body weight in a 20% (v/w) solution infused over 45 minutes to mimic the plasma ethanol curve from the 'oral' day), respectively, on two separate experimental days. Blood was sampled repeatedly

during both experimental days. The primary endpoint was change in serum insulin levels. Secondary endpoints included plasma/serum responses of glucose and C-peptide during the two ethanol administration forms.

Results: Isoethanoleamia during the two ethanol administration forms was obtained (mean peak plasma ethanol concentrations were reached after 45 minutes during both ethanol administration forms and amounted to 1.8±0.3 (i.v.) and 1.7±0.4 g/l (p=0.22). Plasma glucose levels did not differ between the two days. No increment in serum insulin was observed during the 'oral' ethanol load and a decrease in insulin levels was observed during the isoethanolaemic i.v. infusion (Figure 1). Thus, serum insulin responses (as assessed by incremental $AUC_{0\text{-}180\ min}$) were significantly lower on the i.v. day compared to the day of intragastric administration (-1,478±520 vs -33±652 pmol/l × min (mean±SEM), p=0.05) (Figure 1). No difference between serum C-peptide responses between the two days was observed.

Conclusion: We show that - in contrast to other macronutrients - 'oral' ethanol does not promote an insulin response and that isoethanolaemic i.v. infusion of ethanol decreases serum insulin concentrations. As serum C-peptide concentrations were similar during the two ethanol.

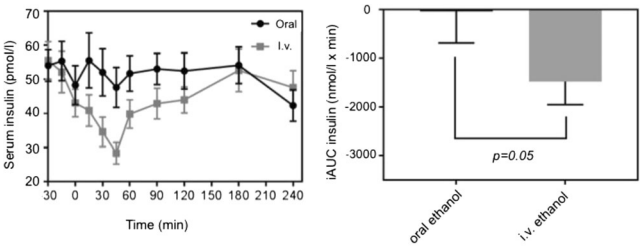

Figure 1. Serum insulin levels after ethanol administered 'orally' (i.e. via an intragastric tube) vs intravenously. iAUC: incremental area under the curve

Clinical Trial Registration Number: H-16026085
Disclosure: **A.R. Lanng:** None.

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ERK7 is a novel regulator of insulin secretion and lipid metabolism in Drosophila melanogaster

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Background and aims: Mitogen Activated Protein Kinases (MAPKs) are Ser/Thr kinases which regulate a wide variety of cellular processes. Although this family is very well studied, some of the members like ERK7 (ERK8/MAPK15) are poorly characterized. ERK7 is an atypical MAP kinase which was shown to be activated by amino acid and serum starvation in *Drosophila* S2 cells. We have developed *in vivo Drosophila* models to further characterize its role in the regulation of insulin sensitivity and lipid metabolism.

Materials and methods: dILP2-Gal4; UAS-GFP driver line was used for Insulin Producing Cells (IPC) specific genetic manipulations. CG-Gal4 was used for fat body (fly counterpart of liver and adipose tissue) specific experiments. RNAi lines and p53 overexpression lines were obtained from VDRC, Austria and Bloomington Stock Center, USA. CRISPR/cas9 technique was employed to generate ERK7 mutants. qRT-PCR assays were used to measure mRNA levels of insulin like peptides and lipogenic genes. Immunostainings using anti-dILP2 antibody were used to assess secretion of insulin like peptides. Gas chromatography was used for quantitative lipidomics.

Results: We hereby show that ERK7 is an important regulator of insulin secretion and lipid metabolism in *Drosophila*. We observed that upon starvation, ERK7 is expressed in median neurosecretory cells (IPCs) which regulate larval growth by secreting insulin-like peptides (dILPs) in diet-dependent manner. We demonstrate that overexpression of ERK7 in IPCs strongly inhibits dILP secretion which consequently impairs larval development. We also identify p53 as an upstream activator of ERK7 in IPCs. Further, we establish that ERK7 function in IPCs is necessary for efficient starvation response. Finally, we also demonstrate that ERK7 has important metabolic functions outside IPCs. We show that ERK7 function in lipogenic tissues of *Drosophila* is important for normal lipid homeostasis. ERK7 expression in fat body (fly counterpart of liver and adipose tissue) disturbs lipid metabolism and leads to insulin resistance. To further explore ERK7 functions, we have generated ERK7 mutants which display obese phenotypes. RNA sequencing of the mutants has revealed a crucial role for ERK7 in lipid metabolism and insulin sensitivity. Hence, we aim to characterize ERK7 as a potential link between diet, diabetes and obesity.

Conclusion: ERK7 regulates insulin secretion in diet dependent manner. In addition, it has a crucial role in modulating insulin sensitivity and lipid metabolism in peripheral adipose tissue.

Disclosure: **K. Hasygar:** None.

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Sex difference in the effect of foetal exposure to maternal diabetes on insulin secretion

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Background and aims: We previously showed that foetal exposure to maternal type 1 diabetes (T1D) is associated with altered glucose-stimulated insulin secretion in adult offspring. Here, we investigated whether this β-cell defect displays a gender dimorphism.

Materials and methods: Twenty nine adult non-diabetic offspring of T1D mothers (ODMs) were compared to 29 non-diabetic offspring of T1D fathers (ODFs). We measured insulin secretion in response to oral glucose and to I.V. glucose ramping followed by I.V. arginine. Insulin sensitivity and body composition were assessed by a euglycemic hyperinsulinemic clamps and dual-energy X-ray absorptiometry respectively.

Results: There was no significant difference between ODMs and ODFs with respect to age (mean (sd): 26.2 (6.1) yrs vs. 25.9 (6.2); p=0.82), female gender (55% vs. 52%), anthropometric variables, routine lab tests and insulin sensitivity (M value: 11.5 (2.9) mg/kg.min vs. 11.7 (2.5); p=0.75). In response to oral glucose, males and females ODMs displayed a reduced early insulin secretion (median (IQR)): 6.0 (3.8 to 9.4) and 9.3 (7.3 to 13.1) μU/mmol vs. 10.4 (5.9 to 14.2) and 13.3 (7.6 to 25.9); p=0.035. In contrast, in response to graded I.V. glucose infusion, a significant interaction (p=0.03) between group and offspring gender was found for the slope of insulin secretion rate against plasma glucose indicating that only females ODMs, but not males, exhibited decreased insulin secretion. There was no defect in response to combined I.V. arginine and glucose suggesting that males and females ODMs exhibit a functional β-cell defect rather than a reduced β-cell mass.

Conclusion: Foetal exposure to maternal type 1 diabetes predisposes to β-cell dysfunction in adult male and female offspring. This β-cell defect is characterized by a sexual dimorphism following I.V. glucose stimulation.

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Supported by: DRCD; ASSERADT; NIH; ADA
Disclosure: **J. Gautier:** Grants; DRCD.

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Impaired beta cell function and reduced incretin effect following surgical acute beta cell loss in humans

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Background and aims: Pancreaticoduodenectomy (PD) causes acute beta-cell loss (~50%) and a compensatory increase in GLP-1 secretion. In this study we explored the effects of PD on quantitative and qualitative parameters of insulin secretion.
Materials and methods: We studied insulin secretion, β cell glucose sensitivity (GS) and incretin effect in 13 non-diabetic patients, (10 F/8 M, 51±15 yrs., BMI 27.9±5.3 kg/m2), before and after PD, using a 2-h hyperglycemic clamp (HC) + arginine and a mixed meal test (MMT). Beta cell-GS was calculated as the ratio of insulin secretion and glucose increments (HC) or modeling (MTT). Incretin effect was estimated as the ratio of GS during MTT and HC and the potentiation factor of several potentiating mechanisms.
Results: As expected, PD caused a significant reduction in total insulin secretion rate, (77.3±6.68 before surgery vs. 37.3±4.90 nmol·m-2 after surgery, nmol·m-2,p<0.001), basal insulin secretion rate, (101±9.82 before surgery vs. 60.2±6.62 pmol·min-1·m-2 after surgery, p<0.01), incremental first phase, (- 58%, p<0.01) second phase, (- 33%, p<0.04) as well as in arginine-stimulated insulin secretion (1919±330.5 before surgery vs. 648.7±244.1pmol/L after surgery, p=0.01). GS was reduced by 38% during HC and by 78% during MMT. Consequently, the incretin effect also decreased significantly following PD (2.87±0.47 before surgery vs. 1.11 ±0.17 after surgery, p<0.01), while no change was observed in the potentiation factor evaluated during MMT (PF before surgery 1.30±0.18 vs. after surgery 1.47±0.22, p=0.58).
Conclusion: These data suggest that acute beta-cell loss determines not only quantitative, but also qualitative alterations of residual beta-cell mass. Further, impaired β-cell GS during MTT following PD may be driven by the decreased incretin effect. The previously observed increased GLP1 secretion following PD does not seem to fully compensate for the reduced incretin effect. Our data are in favor of a pivotal role of the incretin effect (even more than incretin concentration) on the dysregulation of beta-cell responsiveness.
Clinical Trial Registration Number: NCT02175459
Disclosure: **V.A. Sun:** None.

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Insulin sensitivity, beta cell function and hepatic insulin extraction: associations with adiposity and family history of type 2 diabetes in admixed adolescents

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Background and aims: Beta-cell dysfunction and insulin resistance have different manifestations across racial/ethnic groups and there is lack of knowledge regarding the pathophysiology of type 2 diabetes mellitus (T2DM) for ethnic admixed adolescents. The Brazilian people are one of the most admixed populations in the world, which is the result of five centuries of interethnic crosses of peoples from three continents. We aimed to investigate the influence of adiposity and family history of T2DM on aspects of insulin sensitivity, beta-cell function and hepatic extraction of insulin in Brazilian normoglycemic adolescents.
Materials and methods: 82 adolescents (41 girls) underwent the 120-min. hyperglycemic clamp. The insulin sensitivity index was adjusted for free fat mass. Insulin secretion and beta-cell function parameters were obtained from C-peptide deconvolution. The hepatic insulin extraction and the disposition index, which represents the beta-cell function relative to insulin sensitivity, were calculated. The nutritional status was classified based on BMI for age according to the Centers for Disease Control and Prevention growth charts.
Results: After adjusting for pubertal stage, both lean and overweight/obese adolescents had similar glycemic profile (HbA_{1c}: 5.1 ± 0.1 vs. 5.2 ± 0.1%; p = 0.621) and similar disposition index (952 ± 134 vs. 757 ± 90 mg.kg.min*100 per pmol; p = 0.234), respectively. Overweight/obese adolescents had about 1/3 of the insulin sensitivity of lean adolescents (1.1 ± 0.2 *vs.* 3.4 ± 0.3 mg.kg.min.pmol*1000), which was compensated by an increase around 2.5 times in basal (130 ± 7 *vs.* 52 ± 10 pmol.l.min) and total insulin secretion (85839 ± 5076 *vs.* 34407 ± 7273 pmol), and in first (8136 ± 577 *vs*. 3456 ± 827 pmol) and second (69493 ± 4240 *vs*. 27916 ± 6075 pmol) phases of insulin secretion; respectively (p < 0.001 for all). This increase was accompanied by a mean reduction in hepatic insulin extraction of 35% (33 ± 4 *vs.* 51 ± 5 %; p = 0.013), and an increase in beta-cell glucose sensitivity by 2.7 times (86 ± 7 *vs.* 32 ± 10 pmol.min_mmol.l; p = 0.001), respectively. The presence of positive family history of T2DM was not associated with derangements in insulin sensitivity, beta-cells function and hepatic insulin extraction.
Conclusion: In an admixed sample of Brazilian adolescents, the hyperglycemic clamp test demonstrated a strong influence of adiposity and no direct influence of family history of T2DM in different aspects of glucose metabolism.
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Disclosure: **A.C.J. Vasques:** Grants; The National Counsel of Technological and Scientific Development (CNPq), process n. 563664/2010-0, supported the present study.

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Specific GLUT2 invalidation in enteroendocrine cells: impact on glucose homeostasis and enteroendocrine cell plasticity in mice

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Background and aims: The role of glucose transporter GLUT2 in the intestine has been partially uncovered. We have recently published that intestinal GLUT2 invalidation in all epithelial cells leads to a delayed tissue distribution of glucose and reveals unexpected roles in gut homeostasis, including a reduced density of glucagon-like peptide-1 (GLP-1) positive cells. Because GLUT2 is not only located at the membrane of enterocytes but also in enteroendocrine cells, we wanted to understand the specific role of enteroendocrine cells in GLUT2-mediated control of glucose homeostasis and enteroendocrine cell plasticity.
Materials and methods: To address this question, we generated an inducible GLUT2 deficient mouse model specifically in enteroendocrine cells ($GLUT2^{\Delta IEEC}$) by breeding GLUT2 floxed mice with Ngn3-CreERTm mice (gift from G. Gradwohl). GLUT2 invalidation was induced by tamoxifen gavages at 2-months of age (4 days at 1mg/mouse/day). Body weight gain, oral glucose tolerance test (2g/Kg), plasma insulin and GLP-1 measurements were performed 4 weeks after enteroendocrine GLUT2 invalidation. Intestinal GLP-1 content was determined in the jejunum, the ileum and the colon 4 weeks after GLUT2 invalidation.
Results: Body weight gain was similar in $GLUT2^{\Delta IEEC}$ and control mice. Unexpectedly, glucose tolerance was improved in $GLUT2^{\Delta IEEC}$ as compared to control mice (AUC 9241 ± 1191 *vs.* 12472 ± 1006 mg/dL per 120 min., P<0.05). In accordance with this result, we found that basal and glucose-induced plasma insulin was increased in $GLUT2^{\Delta IEEC}$ as compared to control mice (at basal state 0.48 ± 0.07 vs. 0.33 ± 0.03 ng/mL, P<0.05; at 30 min. 0.58 ± 0.04 vs. 0.45 ± 0.04 ng/mL, P<0.05). GLUT2 invalidation in enteroendocrine cells led to a decreased GLP-1 content in all intestinal segments compared to control mice (jejunum 30 ± 2 *vs.* 54 ± 2 pmol/g tissue, P<0.01; ileum 60 ± 3 *vs.* 85 ± 3 pmol/g tissue, P<0.01; colon 106 ± 6 *vs.* 142 ± 11 pmol/g tissue, P<0.05). The impaired intestinal GLP-1 content in $GLUT2^{\Delta IEEC}$ did not alter basal and stimulated plasma GLP-1 (before and 10 min after oral glucose load). We are currently working on the appropriate cell-sorting strategy to demonstrate specific GLUT2 invalidation in enteroendocrine cells.
Conclusion: Our data revealed a new role of GLUT2 on endocrine cell plasticity in the gut but also on glucose homeostasis independently of plasma GLP-1 level.
Supported by: INSERM, UPMC, ICAN Foundation
Disclosure: **A. Grosfeld:** None.

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Effect of high insulin levels and hyperglycaemia on valvular interstitial cells

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Background and aims: Type 2 diabetes (T2D) is a risk factor for the development of calcific aortic valve disease (CAVD). During CAVD, fibrosis, ECM remodeling and finally calcification of the valve cusps take place. Despite the high incidence of T2D and CAVD, knowledge about the effect of a diabetic environment on cellular mechanisms in the valve is limited. In a previous study, we demonstrated that valvular interstitial cells (VIC) are sensitive to insulin and become insulin resistant in response to chronically elevated insulin levels. In this study, diabetic conditions like hyperglycemia and insulin resistance were simulated in vitro to gain better insight in the degeneration of VIC and processes leading to fibrosis and remodeling.
Materials and methods: Primary VIC, isolated from ovine aortic valves, were cultured for 6 d in normal (100 mg/dl; NG) and high glucose (450 mg/dl; HG) medium to simulate hyperglycemic conditions. Additionally, cells were treated with insulin (100 nM) to mimic an environment with increased insulin levels and decreased insulin sensitivity. Cell count was determined every second day and glucose concentration in the supernatant was measured daily in order to describe the metabolic activity of VIC. Degenerative processes and remodeling of the ECM were investigated by gene expression analyses of marker genes, e.g. alpha smooth muscle actin (aSMA) and collagen type I (COL1A1). Calcium accumulation, which occurs during severe degeneration, was quantified with a calcium assay. Data were collected in independent experiments using VIC from six individual sheep and are expressed as mean ± SEM.
Results: At the end of cultivation time, the cell count of insulin-treated VIC under NG conditions was significantly increased compared to NG or HG VIC (NG insulin 1.56 ± 0.16 $x10^6$; NG 1.15 ± 0.09 $x10^6$, p = 0.024; HG 1.08 ± 0.16 $x10^6$, p = 0.005). The glucose concentration in the supernatant decreased under NG conditions significantly at 5 d (NG 51 ± 8 mg/dl, NG insulin 48 ± 7 mg/dl, p < 0.0001) and 6 d (NG 55 ± 10 mg/dl, NG insulin 40 ± 11 mg/dl, p < 0.0001) compared to fresh medium. In contrast, the glucose concentration in HG medium was stable, independent of time point or treatment group. Gene expression analyses showed a significantly decreased aSMA expression after insulin treatment in NG medium (0.53 ± 0.1 fold of NG, p = 0.024) and a similar trend in HG medium (HG 0.97 ± 0.11, HG insulin 0.70 ± 0.13, p = 0.505), whereby HG alone had no effect (p > 0.999). The COL1A1 transcription was increased by trend after insulin treatment (1.83 ± 0.17 fold of NG, p = 0.158) and also under hyperglycemia (1.92 ± 0.43 fold of NG, p = 0.195), whereas the combination of both treatments had an additive effect (2.40 ± 0.66 fold of NG, p = 0.065). However, calcium accumulation was not affected by diabetes-like conditions.
Conclusion: Insulin leads to an increased proliferation rate of VIC and a reduced activation phenotype with decreased aSMA transcription. When further supplemented with hyperglycemia, core markers of ECM remodeling are altered by high insulin levels, representing an in vitro effect mimicking the clinically observed correlation of T2D and CAVD. All in all, the herein presented *in vitro* model may facilitate the investigation of T2D and CAVD in more detail.
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Mid51 regulates not only mitochondrial viability but also glucose-stimulated-insulin secretion in pancreatic beta cells

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Background and aims: The quality of mitochondria is maintained through fusion and fission events that alternate. This dynamic is important for pancreatic beta cells function. Mitochondrial fission is finalized by the dynamin-related protein 1 (Drp1), which is able to constrict mitochondria. The mitochondrial membrane protein Mid51 has been proposed to act as a receptor that can recruit Drp1 from the cytosol to the outer mitochondrial membrane. Several studies suggest that Mid51 promotes mitochondrial fusion by inactivation of Drp1. However, this mechanism remains to be firmly established. Therefore, the aim of this study was to investigate

changes in mitochondrial dynamics, autophagy and cellular function after overexpression of Mid51 in beta cells.

Materials and methods: Overexpression of Mid51 in MIN6 cells was achieved using the pcDNA expression vector. Mid51 gene expression was determined by quantitative RT-PCR. Mid51 protein content and distribution was analysed using western blots and immunofluorescence. Glucose-stimulated insulin secretion was analysed by ELISA. Cellular oxygen consumption was measured with a Clark-type O_2 micro sensor. Confocal microscopy was used to investigate mitochondrial membrane potential, mitochondrial morphology and formation of auotphagosomes after staining with TMRE, MitoTracker Green and a LC3 marker, respectively.

Results: Overexpression of Mid51 resulted in significant enhanced gene and protein expression compared to control transfected MIN6 cells. Control cells showed a homogenous mitochondrial network structure. In contrast, cells with enhanced expression of Mid51 exhibited fragmentation of mitochondria with perinuclear cluster formation. Both, the mitochondrial membrane potential and oxygen consumption were significantly reduced in cells overexpressing Mid51 compared to control transfected cells. Whereas the control transfected cells respond normal after stimulation with 25 mmol/l glucose we found an impairment of glucose-stimulated insulin secretion after overexpression of Mid51. LC3 expression analyses indicated deficits in the regulation of autophagy in cells with enhanced Mid51 level.

Conclusion: Our results suggest that Mid51 is important for regulation of mitochondrial function e.g. membrane potential and oxygen consumption and eventually to maintain glucose-stimulated insulin secretion in MIN6 cells. Because enhanced Mid51 expression disturbed the mitochondrial live cycle and autophagy the protein is likely to be a key player of mitochondrial viability. Future work is important to elucidate if changes in expression and regulation of Mid51 contributes to the development of beta-cell dysfunction in type 2 diabetes.

Disclosure: **J. Schultz:** None.

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The interaction between vitamin D and renin-angiotensin system in the determination of hepatic metabolism in human HepG2 cells under insulin-resistant state

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Background and aims: Vitamin D deficiency or hypovitaminosis D is associated with increased risks of insulin resistance, type 2 diabetes mellitus (T2DM) and its related non-alcoholic fatty liver disease (NAFLD); meanwhile, inappropriate activation of the hepatic renin-angiotensin system (RAS) leads to the liver dysfunction and increased risk of T2DM, such as abnormalities in lipid and glucose metabolism. Our previous findings show that calcitriol (an active metabolite of vitamin D) reduces hepatic triglyceride accumulation and glucose output in diabetic db/db mice and human hepatocellular cell HepG2 under insulin-resistant conditions. Notwithstanding the existence of this finding, the protective action of vitamin D in regulating the activation of hepatic RAS-induced metabolic abnormalities under insulin resistance remains unquestionable. The present study was, therefore, designed to investigate the effects of calcitriol on the expression and function of the RAS components in HepG2 and primary hepatocytes under insulin-resistance state.

Materials and methods: Human hepatocellular cell line HepG2 and isolated c57/BL6J mouse hepatocytes were used as in vitro and ex vivo models, respectively. To mimic T2DM-related insulin-resistance, HepG2 cells and isolated primary hepatocytes were cultured in high-glucose plus high-insulin conditions (30mM glucose; 100nM insulin). Male T2DM db/db mice were also used for in vivo studies. In vitro, ex vivo and in vivo effects of calcitriol on the expression and function of the hepatic RAS components (angiotensin II/ACE/AT1/AT2 receptors and angiotensin 7/ACE2/Mas receptor axes) were then examined.

Results: As expected, major RAS components (e.g. angiotensinogen, AT1/AT2/Mas receptors, and ACE/ACE2) were expressed in the HepG2 and isolated hepatocyte cells. Interestingly, our preliminary data showed that high-glucose plus high-insulin culture conditions significantly upregulated the expression of the key RAS components, notably the AT1 receptor in HepG2 cells. More interestingly, the treatment with different concentrations of calcitriol (0.1-10nM) significantly suppressed the inappropriate activation of the AT1 receptor expression under insulin-resistant conditions in a dose dependent manner (1.66 ± 0.17 vs 0.70 ± 0.09, $p<0.01$), of which these inhibitory effects on AT1 receptor were observed from 4 to 6 hour-calcitriol treatment. In corroboration with the HepG2 cells, similar results on the protective effects of calcitriol were also observed in the isolated hepatocytes.

Conclusion: These data indicate that hormonal vitamin D (calcitriol) has modulatory action on the inappropriate upregulation of the hepatic RAS under insulin-resistant conditions in HepG2 cells, and that potential vitamin D supplementation might provide a cost-effective measure for improving hepatic metabolic dysfunction in T2DM and related NAFLD.

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Novel small molecules targeting lipid phosphatase SHIP2: new treatments for type 2 diabetes

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Background and aims: Lipid phosphatase SH2 domain-containing inositol 5′-phoshatase 2 (SHIP2), a negative regulator of the insulin signaling pathway, is upregulated in muscle, adipose and kidney tissue in experimental models of diabetes, and its inhibition leads to improved insulin sensitivity. Thus, SHIP2 is a potential therapeutic target molecule for the treatment of insulin resistance in type 2 diabetes and diabetic kidney disease. However, to date only a few chemical compounds possessing an inhibitory effect on SHIP2 are known.

Materials and methods: To identify new molecules that inhibit SHIP2 more efficiently than the known inhibitors and possess more favorable properties to be eligible drug candidates, we performed in silico structure-based virtual screening of small molecule chemical libraries. The most potent candidates were validated by biological screening and analyzed using cultured cells, diabetic db/db mice and mice overexpressing SHIP2.

Results: We identified 379 potential SHIP2 inhibitor candidates that had more favorable interaction energies than the previously described SHIP2 inhibitor, AS1949490, and four of them had IC_{50} values lower than 10 μmol. Interestingly, virtual screening revealed anti-diabetic drug, metformin, and compound 118 among these molecules as potential SHIP2 inhibitors. We found that compound 118 (IC_{50} value of 0.75 μmol) and metformin (IC_{50} value of 6.1 μmol) inhibit the catalytic activity of the recombinant SHIP2 phosphatase domain by 100% and 39%, correspondingly. In addition, compound 118 inhibits the catalytic activity of SHIP2 in cultured myotubes and podocytes, and in skeletal muscle and kidney of SHIP2 overexpressing mice more efficiently than metformin. We also observed that, opposite to metformin, compound 118 ameliorates impaired glucose tolerance in SHIP2 overexpressing mice.

Conclusion: Compound 118 inhibits the activity of SHIP2 in vitro and in vivo and ameliorates peripheral insulin resistance. Furthermore, compound 118 has higher SHIP2 inhibition potential than the well-known drug, metformin. This highlights the potential of SHIP2 as a drug target and provides an avenue to identify and design novel molecules, which can be used to develop new insulin sensitizers for future clinical trials.
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Disclosure: **Z. Polianskyte-Prause:** None.

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Identification of basolateral, not luminal, orientation of insulin and IGF1 receptors in gut epithelia

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Background and aims: Due to the robust barrier properties of the gastrointestinal tract, appreciable amounts of insulin are expected to remain unabsorbed following oral administration. Given this, it is critical to understand the orientation of the insulin and insulin-like growth factor 1 receptors (InsR and IGF1R, respectively) expressed on the intestinal epithelia, as these might potentially interact with the insulin present in the intestinal lumen.
Materials and methods: We investigated the expression and distribution of InsR and IGF1R in the gastrointestinal tract of mice and rats by means of Western blotting, quantitative PCR (qPCR) and immunohistochemical (IHC) analyses. Furthermore, we assessed InsR- and IGF1R-mediated signalling by Surefire analysis in in vitro systems of intestinal epithelial or goblet-cell function (i.e. Caco-2, T84 and HT29-MTX-E12 cell monolayers). In addition, insulin-mediated signalling was investigated in rat intestinal tissue mounted in Ussing chambers.
Results: Western blot analysis demonstrated relatively low levels of both receptors in the duodenum, jejunum and ileum of the animals, with receptor expression gradually increasing from the proximal to the more distal regions of the small intestine. Maximal receptor levels were observed in the ascending and transverse sections of the colon which, normalised to tissue weight, expressed approximately 50-75% of the InsR found in liver tissue. qPCR analysis of rat intestinal tissue confirmed the described receptor distribution and supported the observations from the Western blot analyses suggesting similar or higher levels of InsR compared to IGF1R for most of the intestinal regions. Apical stimulation of the cell monolayers with either human insulin (HI) or insulin-like growth factor 1 (IGF1) did not result in significant InsR or IGF1R activation. In contrast, application of the ligands on the basolateral side induced the phosphorylation of their respective receptors in a concentration-dependent manner and with EC50 values in the low nanomolar range. Reflecting the activation of the receptors, basolateral (but not apical) administration of HI or IGF1 resulted in a clear phosphorylation of protein kinase B (Akt). Similarly, basolateral exposure of an insulin analogue (modified to withstand enzymatic degradation) to rat colonic mucosa mounted in Ussing chambers induced a significant increase in Akt phosphorylation compared to that of apically-treated tissue ($p<0.05$). Visualisation of the InsR in murine intestinal samples by means of IHC analyses further confirmed the localisation of the InsR in the basolateral membrane.
Conclusion: Collectively, these results indicate a wide expression of InsR and IGF1R throughout the gastrointestinal tract with a clear basolateral orientation of receptors in the intestinal epithelia which essentially renders these receptors inaccessible to any unabsorbed insulin that may be present in the intestinal lumen following oral dosing of insulin.
Disclosure: **C.E. Stidsen:** Employment/Consultancy; Employee at Novo Nordisk A/S. Stock/Shareholding; Stock/Shareholder of Novo Nordisk A/S.

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Calcineurin is involved in the regulation of human adipocyte glucose uptake

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Background and aims: Calcineurin inhibitors (CNIs), e.g. tacrolimus (Tac), are the cornerstone immunosuppressive therapy used after transplantation to prevent graft rejection. They are associated with metabolic side effects, like dyslipidemia, insulin resistance (IR) and development of diabetes mellitus (DM). CNIs inhibit lipid storage and expression of lipogenic genes in adipose tissue (AT), contributing to the development of dyslipidemia and IR. CNIs also reduce glucose uptake (GU) by reducing the amount of glucose transporter type 4 (GLUT4) on the cell surface via increased internalization in white AT and muscle cells, without altering the total levels of GLUT4 or insulin signaling. Our aim was to investigate possible pathways involved in the decrease of GLUT4 in the plasma membrane and consequent reduction of GU induced by CNIs in human subcutaneous AT (SAT).
Materials and methods: Human SAT biopsies were obtained by needle aspiration from the lower part of the abdomen from nondiabetic subjects (34 females/11 males, HbA1c 34±3.3 mmol/mol, BMI 26.7±4.7 Kg/m^2, mean ±SD). Adipocytes isolated with collagenase were treated for 75 min or 20 h (short- and long-term) with Tac (100 nM), cyclosporine A (CsA, 100 nM), deltamethrin (Delt, 1 μM), okadaic acid (OA, 250 nM), actinomycin D (ActD, 5 μg/ml) or cycloheximide (CH, 25 μM) and the GU was measured in the absence (basal) and in the presence of 25 and 1000 μU/ml insulin for 1h, using D-[U-^{14}C] glucose. The concentration of each drug used did not decrease the cell viability. AT incubated for 20 h with Tac (100 nM) was used to analyse the expression of genes involved in GLUT4 translocation with a PCR array. Top- and down- regulated genes and genes that were statistically different between control and Tac treatment were validated by standard qRT-PCR.
Results: Short- and long-term incubation with Tac, CsA and Delt decreased both basal and insulin-dependent GU in adipocytes to the same extent (about 16% and 34%, respectively, $p<0.05$) and without additive effects when co-incubated. This suggests that the effects of CNIs on GU are due to calcineurin inhibition. OA, used at a concentration (250 nM) that does not inhibit calcineurin but inhibits protein phosphatases 1 and 2A, increased the GU in adipocytes to 154% ($p<0.01$). Thus, the decrease in GU observed with CNIs is specific to calcineurin inhibition and not due to a general phosphatase inhibition. The inhibition of gene transcription by ActD and protein translation by CH also reduced GU by about 45% and 52%, respectively, $p<0.05$, but no Tac effects were seen when gene transcription and protein translation were inhibited. However, the expression of genes involved in GLUT4 translocation was not affected by Tac, suggesting that the GU reduction induced by CNIs may be due to effects on other genes, or protein amount or activation.
Conclusion: The specific inhibition of calcineurin, but not of other protein phosphatases 1 and 2A, decreases GU in subcutaneous adipocytes. This effect requires gene transcription and/or protein synthesis, but not gene expression of well-known genes involved in GLUT4 trafficking and cytoskeleton function. These data suggest that inhibition of calcineurin can contribute to impaired glucose handling in peripheral tissues, as reported with calcineurin therapy in organ-transplanted patients.
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The role of GLUT4 in the kidney proximal tubule in blood glucose control and kidney function

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Background and aims: The contribution of the kidneys to blood glucose control has become increasingly recognized. This includes both endogenous glucose production in fasting states and the reabsorption of filtered glucose; the latter being the target of novel type 2 diabetes drug class, sodium-dependent glucose transporter (SGLT)-2 inhibitors. Our laboratory has identified the presence of the insulin-sensitive glucose transporter, GLUT4, in kidney proximal tubule cells. We aimed to ascertain the role of this transporter in blood glucose control and kidney function.
Materials and methods: Proximal tubule cell-specific GLUT4 knockout mice (GLUT4 KO) were generated by breeding GLUT4 floxed mice with iL1-sglt2-cre mice and compared to wildtype littermates (WT). Twenty-week old male and female mice were studied (n=11-16/group). An oral glucose tolerance was performed and kidney function determined using 24 h urine collections.
Results: Male mice lacking the GLUT4 transporter in renal proximal tubule cells had elevated blood glucose levels in both fed (12.4 ± 0.8 vs 10.5 ± 0.3 mmol.l-1) and fasted (10.7 ± 0.7 vs 9.1 ± 0.3 mmol.l-1) states, and impaired glucose tolerance during the OGTT (AUGC: 2,145 ± 201 vs 1,691 ± 72 mmol.l-1 x 120 min) compared to WT littermates ($p<0.05$). There was a trend ($p=0.06$) for reduced urine flow rate (1.3 ± 0.2 vs 1.9 ± 0.2 ml.24h-1) and smaller kidneys (368 ± 10 vs 411 ± 18 mg) in these male GLUT4 KO mice compared to WT. There were no differences in food or water consumption between groups. Conversely, in female mice, deletion of the GLUT4 transporter from kidney proximal tubule cells did not affect blood glucose levels, kidney weight, or urine production.
Conclusion: These findings highlight sex-specific differences in the role of proximal tubule GLUT4 in whole-body glucose homeostasis and urine formation. This may have implications for therapeutic targeting of renal glucose reabsorption in the treatment of type 2 diabetes. Further assessment of peripheral insulin sensitivity, kidney handling of glucose, and overall kidney function is ongoing.
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Disclosure: **L.A. Gallo:** Grants; National Health and Medical Research Council (Australia), Diabetes Australia, Heart Foundation (Australia).

PS 027 Gastro-entero-pancreatic factors: animals and in vitro

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Estrogens modulate glucose homeostasis by increasing glucagon-like peptide -1 secretion for L and alpha cells

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Background and aims: The implication of estrogens and their associated signalling pathways on metabolism have generated much interest. Clinical and experimental data indicate a beneficial effect on energy and glucose homeostasis associated with improved insulin-sensitivity and positive effects on insulin secretion. The aim of our study is to investigate the impact of estrogen on proglucagon-producing cells, pancreatic alpha- and enteroendocrine L-cells.
Materials and methods: To study the consequences of sexual hormone deprivation, adult female mice from the transgenic GLU-Venus x INS-Cherry mouse strain were ovariectomized (ovx), or sham operated. Conversely, the effects of estrogens were studied in ovx mice after administration of 17β-estradiol (E_2, 80μg/kg), or vehicle for 48 hours. The direct effects of E2 were investigated on primary sorted alpha- and beta-cells from ovx mice or on GLUTag cell line, treated during 48 hours with E_2 (10^{-8}mol/l), or with the estrogen receptor beta agonist Diarylpropionitrile (DPN, 10^{-8}mol/l), for the GLUTag cells only.
Results: Ovx mice exhibited alteration of glucose tolerance during OGTT (29.2 ± 6.0% increase of the area under the curve (AUC)) but not during IPGTT associated to decreased GLP-1 secretion (0.49 ± 0.04 pmol/l and 0.29 ± 0.04 pmol/l for sham and ovx mice respectively, at 5 min after glucose load), an effect that can be reversed by E_2-treatment. E_2 increases insulin (+104.9 ± 32.5%) and decreases glucagon (-63.7 ± 10.5%) pancreatic content and secretion while increasing pancreatic and small intestinal GLP-1 contents. E2 directly acts on alpha-cells to decrease glucagon and increases GLP-1 secretions and contents. Studies on intestinal cells from ovx mice and on the GLUTag cell line showed that E_2 was able to increase intestinal GLP-1 content and secretion. Moreover, by the use of the highly selective estrogen receptor beta agonist, the Diarylpropionitrile (DPN) at the concentration of 10^{-8}mol/l, we could determine that the impact of estradiol on intestinal GLP-1 content and secretion is mainly mediated trough the activation of the estrogen receptor beta.
Conclusion: E_2 has not only beneficial direct actions on insulin and glucagon secretion, but also increases GLP-1 secretion from both intestinal L and pancreatic alpha-cells, highlighting the potential of the estrogenic pathway to reduce type 2 diabetes.
Disclosure: **S. Handgraaf:** None.

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Glucose-dependent insulinotropic polypeptide (GIP) deficiency improves obesity but worsens bone loss in ovariectomised mice

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Background and aims: Ovarian hormone deficiency such as menopause induces osteoporosis and increases the risk of obesity and insulin resistance. Glucose-dependent insulinotropic polypeptide (GIP) is a gut hormone released from enteroendocrine K-cells after food intake and enhances insulin secretion through GIP receptor (GIPR) expressed in pancreatic β-cells. GIPR is also expressed in adipose and bone tissue. GIP deficiency protected the mice from high fat diet-induced obesity and

insulin resistance. Moreover, GIP deficiency showed reduced bone volume. In this study, we assessed the contribution of GIP in ovariectomy (OVX)-induced obesity, insulin resistance, and osteoporosis.

Materials and methods: GIP deficiency in C57BL/6 mice was produced by insertion of a sequence coding green fluorescent protein (GFP) into the preproGIP gene. Homozygous mice ($GIP^{gfp/gfp}$) have a complete absence of circulating GIP while plasma GIP levels are reduced in heterozygous ($GIP^{gfp/+}$) mice. Female wild-type (WT) C57BL/6 mice served as controls. At the age of 8 weeks, ovariectomies (OVX) were performed on WT, $GIP^{gfp/+}$, and $GIP^{gfp/gfp}$ mice, with a subgroup of WT mice given a sham operation. Mice were weighed weekly during experiments. At 20-30 weeks of age, oral glucose tolerance tests and insulin tolerance tests were performed. Fat mass, energy expenditure, and locomotor activity were measured at 25 weeks of age. At 16 weeks of age, bone analysis was performed by micro-computed tomography. Plasma osteocalcin levels were measured by ELISA.

Results: Weight gain following OVX was substantially reduced In in $GIP^{gfp/gfp}$ mice and the associated increase in subcutaneous and visceral fat mass was completely prevented by the elimination of GIP. The OVX-induced increase in food intake and decreases in locomotor activity and energy expenditure were not significantly altered in $GIP^{gfp/+}$ or $GIP^{gfp/gfp}$ mice. OVX lead to glucose intolerance in WT mice but glucose tolerance in $GIP^{gfp/gfp}$-OVX mice was similar to WT controls given sham surgery. Insulin sensitivity remained similar among all groups. Cancellous bone mineral density was reduced in WT mice by OVX, but the reduction was even greater in $GIP^{gfp/gfp}$-OVX mice and GIP depletion also promoted reduced bone cortical thickness, in association with reduced plasma osteocalcin levels.

Conclusion: GIP deficiency ameliorates excess weight gain and adiposity after ovariectomy but enhances ovariectomy-induced osteoporosis, particularly in cancellous bone by suppressing bone formation.

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The nuclear receptor FXR decreases enteroendocrine L cell response to gut microbiota metabolites, the short chain fatty acids

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Background and aims: Diabetes mellitus involves many metabolic disorders including a decrease in incretin effect. One of the incretine hormones, Glucagon-Like Peptide-1 (GLP-1), is produced and secreted by enteroendocrine L cells which represent 1% of the intestinal epithelial cells. We have recently shown that bile acid nuclear receptor Farnesoid X Receptor (FXR) activation in enteroendocrine L cells decreases glucose-induced ChREBP-dependant proglucagon (GLP-1 precursor) gene transcription. By inhibiting glycolysis pathway, FXR also decreases glucose-induced GLP-1 secretion. We hypothesize that FXR could globally decrease L cell response to other GLP-1 secretagogues. The aim of this study is thus to investigate the role of FXR in the L cell response to short chain fatty acids (SCFA) which are metabolites produced by the gut microbiota via fermentation of non digestible polysaccharides. Indeed, in addition to their contribution of 5 to 10% of the daily energetic resources, SCFA are also signalling molecules as they bind to the transmembrane receptor FFAR2, thereby promoting GLP-1 secretion by L cells.

Materials and methods: FXR was activated *in vitro* in the murine cell line GLUTag, in the human cell line NCI-H716 and *in vivo* in C57Bl6/J mice by the synthetic agonist GW4064. GLP-1 secretion tests (ELISA) in response to natural (propionate and butyrate) and synthetic FFAR2 agonists were performed *in vitro* in GLUTag and in NCI-H716 and *ex vivo* on murine colonic explants. FFAR2 mRNA levels were evaluated by qPCR in colon from mice treated with GW4064, from KO FXR mice and from mice treated with colesevelam, a bile acid sequestrant, which display a drastic down regulation of intestinal FXR transcriptional activity.

Results: *In vivo* FXR activation decreases FFAR2 mRNA levels and the subsequent *ex vivo* colonic GLP-1 secretion in response to butyrate. As a mirror effect, FXR KO and colesevelam treated mice exhibit increased colonic FFAR2 mRNA levels. Moreover, *in vitro,* both in GLUTag and NCI-H716 cells, FXR activation decreases GLP-1 secretion in response to butyrate and to synthetic agonists of FFAR2 which is, at least in GLUTag cells, in parallel to a decrease in proglucagon and FFAR2 mRNA levels.

Conclusion: FXR activation decreases L cell response to SCFA in terms of GLP-1 secretion at least in part via the decrease of proglucagon and FFAR2 mRNA expression. Inhibiting FXR in intestine, pharmacologically or by modulating the bile acid pool, leading to an increased L cell response to various nutrients, may be a promising approach in Type 2 Diabetes therapies.

Disclosure: **S. Ducastel:** None.

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Inhibition of hepatic bile acid uptake prolongs bile acid signalling leading to reduced adiposity, increased thermogenesis and enhanced intestinal GLP-1 secretion

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Background and aims: Bile acids, known for their facilitating role in fat absorption, are now also recognized as signalling molecules that regulate glucose metabolism, inflammation, and energy expenditure via activation of their receptors FXR and TGR5. Hepatic uptake of bile acids is mediated by the sodium taurocholate co-transporting polypeptide (NTCP, *SLC10A1*) and different members of the organic anion-transporting polypeptide (OATP) family. Here, we propose that (partially) inhibiting hepatic bile acid transport will delay hepatic clearance of bile acids from portal and peripheral blood, thereby prolonging bile acid signalling and ameliorate metabolic diseases such as diabetes.

Materials and methods: To investigate the effect of prolonged bile acid signalling *in vivo*, NTCP deficient mice (*Slc10a1* knockout) were studied in a diet-induced-obesity (DIO) setting. Also, short and long term effects of pharmacological inhibition of NTCP were studied in OATP1a/1b/– KO mice (that lack all NTCP-independent bile acid uptake). The SGBS pre-adipocyte cell line was used as an *in vitro* model of human adipocytes to study bile acid signalling in fat tissue, while the GLUTag cell line was used to investigate bile acid-induced intestinal L-cell GLP-1 secretion.

Results: NTCP inhibition *in vivo* showed decreased clearance of serum bile acid in both lean and DIO mice. Both male and female NTCP KO mice displayed partial protection against deleterious effects of a high fat diet compared with their wild type counterparts. In this setting, NTCP KO mice had a lower body weight, reduced liver adiposity, and decreased size of white adipose tissue storage depots. Treatment of the OATP1a/1b/– KO mice with a specific NTCP inhibitor resulted in rapid reduction of body weight (~15 % within 3 weeks). They also showed increased serum GLP-1 levels, as well as an increase in body temperature. Importantly, no detrimental effects of NTCP inhibition were seen on plasma ALT levels or liver histology, while plasma bile acid concentrations significantly increased. Stimulation of SGBS cells, a model of human brown adipocytes, with the bile acid TCDCA or a specific TGR5 agonist (TG-1005) for 2 hours, induced a significant upregulation of thermogenesis related genes UCP-1, Dio2, and PGC1α. Injection of the same compounds increased the capacity of mitochondria to use uncoupled respiration within 15 minutes. The same bile acid TCDCA and TGR-5 agonist TG-1005 showed an significant increase in GLP-1 secretion by GLUTag cells, 2 hours after stimulation.

Conclusion: Both long-term and short-term studies in mouse models affected in their BA transport capacity point towards an improved metabolic state upon reduced hepatic bile acid uptake. *In vitro* research confirms that conjugated bile acids execute their beneficial effects in adipocytes and intestinal GLP-1 secretion most likely via TGR5.

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Disclosure: **J.M. Donkers:** None.

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The intestinotrophic effects of glucagon-like peptide 1 and 2 are not important for the metabolic effects of bariatric surgery

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Background and aims: The prevalence of obesity and related comorbidities is reaching pandemic proportions. Today, the most effective obesity treatments are glucagon-like peptide 1 (GLP-1) analogs and bariatric surgery, respectively. Interestingly, the anti-obesity treatment effects of both intervention paradigms have been associated with adaptive growth responses in the gut. The objective of this study was to evaluate the intestinotrophic effects of glucagon-like peptides and the importance of this mechanism in bariatric surgery.

Materials and methods: *In situ* hybridization was used to provide a detailed and comparative anatomical map on the local distribution of GLP-1 receptor (*Glp1r*), GLP-2 receptor (*Glp2r*) and preproglucagon (*Gcg*) mRNA expression throughout the mouse gastrointestinal tract. Gut development in GLP-1R-, GLP-2R- or GCG-deficient mice was compared to their corresponding wild-type (WT) controls, and intestinotrophic effects of GLP-1 and GLP-2 analogs was assessed in WT mice. Lastly, gut volume was determined in a mouse model of vertical sleeve gastrectomy (VSG).

Results: Comparison of *Glp1r*, *Glp2r* and *Gcg* mRNA expression indicated a widespread, but distinct, distribution of these three transcripts throughout all compartments of the mouse gastrointestinal tract. While mice null for *Glp1r* or *Gcg* showed normal intestinal morphology ($Glp1r^{-/-}$ 688±37 mm^3 vs. WT 700±31 mm^3, p=0.81 and $Gcg^{-/-}$ 944 ±43mm^3 vs. WT 938±44mm^3, p=0.92), $Glp2r^{-/-}$ mice exhibited a slight reduction in small intestinal mucosa volume (13±3.7%, 547±10 mm^3 vs 475±20 mm^3, p=0.01). Pharmacological treatment with GLP-1 and GLP-2 analogs markedly increased gut volume (70±6.5% increase, p=0.001 GLP-1+GLP-2 combination vs. vehicle treatment). In contrast, VSG surgery had no effect on intestinal morphology (small intestine volume VSG 885±82 mm^3 vs. sham 879±44 mm^3, p=0.95).

Conclusion: The present study indicates that the endogenous preproglucagon system does not play an essential role in normal gut development in the mouse. Similarly, increased circulating GLP-1 levels in VSG-treated mice could not be coupled to changes in intestinal morphometry. We conclude that pharmacological treatment with long-acting GLP-1 and GLP-2 analogs enhanced gut growth in the mouse, and that intestinotrophic effects of GLP-1 and GLP-2 are revealed upon supraphysiological stimulation of their cognate receptors only.

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Disclosure: **P. Wismann:** Grants; Innovation Fund Denmark.

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Development of an antagonistic GLP1R antibody to block GLP-1 signalling in vivo

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Background and aims: Glucagon like peptide-1 (GLP-1) and GLP-1 mimetics enhance glucose-dependent insulin secretion by binding to GLP-1 receptors (GLP1R) on pancreatic beta cells. Despite the therapeutic success of GLP-1 mimetics for the treatment of type 2 diabetes, several clinical effects of GLP-1 remain unexplained at a mechanistic level, particularly in extra-pancreatic tissues such as the cardiovascular system. The objective of this study was to generate and characterise a monoclonal antagonistic antibody for the GLP1R that can be used to block GLP1R signalling in vivo.

Materials and methods: A naïve phage display selection strategy was used for isolation of single chain variable fragments (ScFv) that bound to GLP1R. The first two rounds were soluble selections on biotinylated human GLP1R extracellular domain, the third round was a cell surface selection using CHO cells overexpressing mouse GLP1R. This led to the isolation of 18 ScFv clones for functional analysis. The clone with the highest affinity, Glp1R0017, was converted into a human IgG1 and characterised further. In vitro antagonistic activity was assessed in a number of assays; a cAMP based HTRF assay in GLP1R overexpressing cell lines, a live cell cAMP imaging assay using INS-1 832/3 cells transfected with the Epac2-camps probe, and an insulin secretion assay in INS-1 832/3 cells. Glp1R0017 was further tested in immunostaining of mouse pancreas, and the ability of Glp1R0017 to block GLP1R in vivo was assessed by intraperitoneal glucose tolerance tests (ipGTT) in C57/Bl6 mice (n=8 per group).

Results: Naïve phage display led to the selection of Glp1R0017. Glp1R0017 was found to be antagonistic against mouse, human, rat, cyno and dog GLP1R with IC_{50} values of 5.2 nM, 43.3 nM, 5.3 nM, 9.0 nM and 11.7 nM, respectively. This antagonistic activity was specific to GLP1R; no antagonistic activity was found in cells overexpressing the glucose dependent insulinotropic peptide receptor (GIPR), GLP-2 receptor or glucagon receptor. INS-1 832/3 imaging experiments, measuring cAMP changes in real time, showed that GLP-1 stimulated cAMP was reduced 2.8 fold after 15 minute pre-incubation of cells with Glp1R0017 ($p<0.001$). Also in INS-1 832/3 cells, Glp1R0017 reduced insulin secretion stimulated by GLP-1 from 1.4 fold to 0.8 fold ($p<0.001$). Immunostaining of mouse pancreas tissue with Glp1R0017 showed specific staining in the islets of Langerhans, not observed in GLP1R KO tissue. In vivo, Glp1R0017 reversed the glucose-lowering effect of liraglutide during ipGTT in mice.

Conclusion: Glp1R0017 is a monoclonal antagonistic antibody to the GLP1R that blocks GLP-1 actions on pancreatic beta cells, including elevation of cAMP and insulin secretion. In vivo inhibition of GLP1R has also been demonstrated by ipGTT. This antibody holds the potential to investigate the physiological importance of GLP1R signalling in extra-pancreatic tissues where less is known about the cellular targets and signalling pathways activated by GLP-1.

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Disclosure: **E.K. Biggs:** None.

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Exploring the autophagy mechanism in pancreatic alpha cell

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Background and aims: It has been reported that autophagy plays a pivotal role in intracellular quality control through degradation of subcellular damaged organelles and components. While autophagy has been demonstrated to play an essential role in β-cell homeostasis, it remains to be elucidated how the cellular autophagy affects the homeostasis and function of glucagon-secreting α cells, another type of pancreatic endocrine cells.
Materials and methods: In order to generate a mutant mouse model lacking Atg7, a key molecule for autophagosome formation, specifically in α cells (αAtg7KO), we crossed the *Atg7*$^{flox/flox}$ mice with the *Gcg-CreERTM* mice that induce Cre-mediated recombination under the control of the glucagon (*Gcg*) locus.
Results: When the αAtg7KO mouse was administered tamoxifen at the age of four weeks, accumulation of p62 was observed specifically in α cells, suggesting that α-cell autophagy was specifically disrupted as designed. Histological analysis revealed that the number of α cells was increased in αAtg7KO mice compared to control littermates, and multilayered glucagon-positive cells were observed in the islets of αAtg7KO mice. On the other hand, metabolic parameters such as body weight, blood glucose levels and glucose tolerance were comparable between αAtg7KO mice and control littermates. In addition, there was almost no difference in serum glucagon levels between the groups.
Conclusion: These findings suggest that α-cell autophagy plays a role in regulating α-cell mass and islet formation. Further investigation would be needed to investigate underlying mechanisms of this phenotype.
Disclosure: **M. Himuro:** None.

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Islet amyloid induces beta cell dysfunction in mice independent of toll-like receptor 2 signalling

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Background and aims: Islet amyloid, formed by the aggregation of beta cell-derived islet amyloid polypeptide (IAPP), is thought to contribute to beta cell dysfunction in type 2 diabetes, however the mechanism is unclear. IAPP aggregates recruit macrophages to pancreatic islets and induce secretion of pro-inflammatory cytokines, contributing to islet inflammation and impaired beta cell function in mice. *In vitro,* the induction of pro-inflammatory cytokine expression by IAPP aggregates is mediated by toll-like receptor 2 (TLR2) and its downstream adaptor molecule Myeloid Differentiation Primary Response Gene 88 (MyD88). We hypothesized that IAPP aggregates induce beta cell dysfunction and subsequent diabetes development through TLR2-dependent signalling.
Materials and methods: To test this, we examined the impact of TLR2 or MyD88 deletion on islet amyloid induced islet inflammation and beta cell dysfunction in mice. As rodent IAPP does not aggregate to form islet amyloid, we utilised mice expressing the human form of IAPP in beta cells (hIAPP Tg/0 mice), which develop islet amyloid, islet inflammation, and diabetes. We first crossed hIAPP Tg/0 mice with *Tlr2*$^{-/-}$ mice to generate *Tlr2*$^{-/-}$ hIAPP Tg/0 and *Trl2*$^{+/+}$ hIAPP Tg/0 mice, as well as hIAPP 0/0 littermate controls. As IAPP aggregates could induce islet inflammation through redundant TLR pathways, many of which signal through MyD88, we next generated hIAPP Tg/0 mice with myeloid-specific deletion of MyD88 by crossing hIAPP Tg/0 mice with *Myd88*$^{flox/flox}$ *Lysm*$^{cre/+}$ mice.
Results: *Tlr2*$^{+/+}$ hIAPP Tg/0 mice were significantly hyperglycemic and glucose intolerant relative to hIAPP 0/0 littermate controls on high fat diet. Surprisingly, *Tlr2*$^{-/-}$ hIAPP Tg/0 mice did not have attenuated hyperglycemia relative to *Trl2*$^{+/+}$ hIAPP Tg/0 mice (18.8 ± 2.0 vs 16.0 ± 1.4 mmol/L at 18 weeks, n=6-11, p=0.4) nor improved glucose tolerance. Beta cell function was impaired in both *Tlr2*$^{-/-}$ hIAPP Tg/0 and *Trl2*$^{+/+}$ hIAPP Tg/0 mice, evidenced by ~5 fold reductions of plasma insulin levels and ~2 fold increases in proinsulin: insulin ratios compared to their respective hIAPP 0/0 controls. Furthermore, inflammatory markers were elevated in islets of both *Tlr2*$^{-/-}$ hIAPP Tg/0 and *Trl2*$^{+/+}$ hIAPP Tg/0 relative to hIAPP 0/0 controls, revealing that deletion of TLR2 did not resolve islet amyloid induced islet inflammation. Deletion of MyD88 in myeloid cells yielded similar results. *Myd88*$^{flox/flox}$ *Lysm*$^{cre/+}$ hIAPP Tg/0 and *Myd88*$^{flox/flox}$ *Lysm*$^{+/+}$ hIAPP Tg/0 mice displayed similar levels of hyperglycemia (20.5 ± 2.3 vs 19.6 ± 1.9 mmol/l at 22 weeks, n=10) compared to their respective hIAPP 0/0 controls (11.4 ± 1.4 and 11.5 ± 0.9 mmol/l at 22 weeks, n=5-8) on high fat diet. Relative to hIAPP 0/0 controls, hIAPP Tg/0 mice also had impaired glucose tolerance and reduced plasma insulin regardless of myeloid MyD88 deletion.
Conclusion: While TLR2 and MyD88 are necessary for the proinflammatory response to IAPP aggregates *in vitro*, these data demonstrate that deletion of TLR2 signalling pathway components does not resolve islet amyloid induced inflammation or beta cell dysfunction in mice, indicating that alternate innate signalling pathways likely contribute to the inflammatory and deleterious effect of islet amyloid *in vivo*.
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Disclosure: **H.C. Denroche:** Grants; CIHR. Other; JDRF postdoctoral fellowship, CDA postdoctoral fellowship.

PS 028 Gastro-entero-pancreatic factors in humans

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Influence of meal olfactory and visual stimuli on GLP-1 plasma concentration in healthy volunteers

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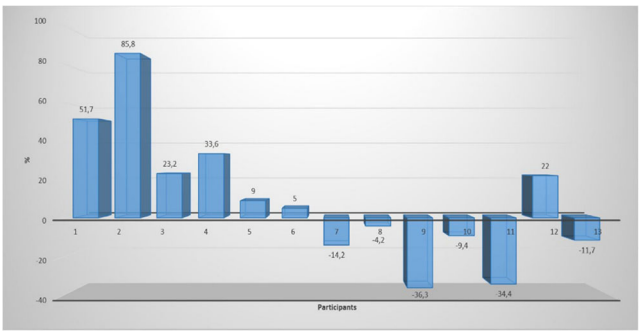
Background and aims: Food ingestion is well known to induce a rapid increase in plasma glucagon-like peptide-1 (GLP-1) level much faster than food reaches the distal intestine where GLP-1 is produced. The aim of the study is to investigate whether the olfactory and visual alimentary stimuli can provoke the plasma GLP-1 rise before meal.
Materials and methods: 13 healthy volunteers (5 men and 8 women) 21-22 years old with a mean BMI 21,70 kg/m2 (18,74 - 25,45 kg/m2) without DM and metabolic disorders had highly caloric delicious breakfast. Venous plasma glucose, insulin and GLP-1 were examined twice at fasting state (-5', 0' min), then twice (10'and 20' min) when smelling and looking at food (not eating), and then twice after the start of the meal (30' and 120' min). Data analyzed by descriptive statistics.
Results: Baseline characteristics: HbA1c 4,97% ±0,26% mean waist-hip ratio 0,77 ± 0,05, mean HOMA-IR 1,46 ± 0,69. All participants had normal glucose tolerance. Mean fasting glucose was 4,62 mmol/l (min 3,90; max 5,22 mmol/l), glucose at 120' 4,84 mmol/l (min 3,90; max 6,28 mmol/l). No increase in insulin level in response to olfactory and visual stimuli was observed. Seven participants (54%) had GLP-1 plasma level increased from baseline in average by 33,2% in 10 minutes after they started smelling and watching the food. The increase in plasma GLP-1 was associated with higher BMI (Figure) and waist-hip ratio. The meal-stimulated GLP-1 peak was observed at 30 min of the study as expected. The other 6 volunteers showed no increase or slightly decrease in GLP-1 plasma level at 10' min of smelling and watching the meal, while the meal-stimulated GLP-1 peak was also observed at 30 min.
Conclusion: We found that increase in GLP-1 concentration in half of the healthy volunteers is starting before food ingestion at the stage of smelling and watching the food, mostly in participants with higher BMI. These visual and olfactory stimuli might activate central neurotransmitters that provoke the GLP-1 secretion in distal intestine. Heterogeneity of the GLP-1 response to such stimuli needs further investigation. Figure. Increase in plasma GLP-1 (%) from baseline to min 10 of visual and olfactory alimentary stimuli in healthy volunteers. The results of participants are ranged from higher (1)(25,4 kg/m2) to lower (13) (18,7 kg/m2) BMI



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Disclosure: **E.A. Shestakova:** None.

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Plasma proneurotensin and diabetes risk in Iraqi immigrants and native Swedes. The MEDIM population based study

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Background and aims: Proneurotensin has recently been shown to predict type 2 diabetes and cardiovascular morbidity and mortality. Type 2 diabetes is highly prevalent in Middle Eastern populations that represent the largest non-European immigrant group in Sweden today. The aim of this study was to investigate differences across ethnicities in proneurotensin and its associations with type 2 diabetes risk.
Materials and methods: The MEDIM study is a population-based, cross-sectional study conducted 2010-2012 in the general community, City of Malmö, Sweden. Residents 30 to 75 years of age born in either Iraq or Sweden were invited from the census register. Anthropometrics and fasting plasma samples were collected and oral glucose tolerance tests performed.
Results: Iraqi born individuals had significantly higher fasting proneurotensin concentration than Swedish born (137.5 vs 119.8 pmol/L, $p<0.001$), irrespective of age, sex or body mass index (BMI). Insulin sensitivity index and insulin secretion respectively, displayed stronger associations with proneurotensin within the Iraqi than within the Swedish born population as reflected by significant interactions between country of birth and proneurotensin ($P_{interaction\ ISI}=0.050$; $P_{interaction\ DI}=0.007$). Iraqi born participants within the highest tertile of proneurotensin had almost five times the odds of having type 2 diabetes as compared to Swedes within the lowest tertile of proneurotensin.
Conclusion: This study reports that the Iraqi immigrant population compared to the native Swedish population have higher levels of proneurotensin irrespective of age, sex or body mass index. In this study the effect of proneurotensin on insulin secretion and action is modified by ethnic background in that the associations between proneurotensin and insulin action and secretion are stronger in Iraqi immigrants than in native Swedes. Our data indicate that part of the excess diabetes risk in the Middle Eastern immigrant population as compared to the native Caucasian population, can be explained by higher levels of proneurotensin.
Supported by: ALF; Regino Skåne; Swedish Society of Medicine; Swedish research council
Disclosure: **L. Bennet:** Other; JS is employed by sphingotec GmbH, a company having patent rights in the proneurotensin assay and commercializing it. AB is CEO of sphingotec GmbH and holds shares in this company.

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Differences in glucose and insulin metabolism after Roux-en-Y gastric bypass and sleeve gastrectomy

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Background and aims: The two surgical procedures Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (SG) induce major and sustainable weight losses and result in improved glycaemic control in patients with pre-operative type 2 diabetes. The mechanism behind these beneficial effects has been suggested to be related for the two procedures, but many details remain to be elucidated. We therefore compared postprandial glucose metabolism after similar weight losses in SG and RYGB operated subjects as well as in obese controls (CON).

Materials and methods: In a cross sectional design 12 SG operated subjects (1.8±0.4 [mean±SD] years post SG), 12 RYGB operated subjects (2.2±0.4 years post RYGB) and 12 obese CON subjects were included and underwent a 4-hour liquid mixed meal test (400 kcal, 50 E% carbohydrate, 35 E% fat, 15 E% protein). All subjects had normal glucose tolerance (HbA1c<48 mmol/mol and fasting plasma glucose (PG) <6.1 mmol/L) and the groups were matched on BMI (SG: 33.4±8 kg/m2, RYGB: 33.5±7, CON: 33.4±6, ANOVA p=0.99), age (SG: 42.8±11 years, RYGB: 43.0±7, CON: 44.9±12, p=0.86), and sex (3/9 (men/women) in all groups). Surgical groups were also matched on post-operative weight loss (Pre-operative BMI: SG: 44.0±7 vs. RYGB 44.1±8, p=0.96).
Results: Fasting PG was similar in the two surgical groups (SG: 5.1 mmol/L vs. RYGB: 4.9, p=0.23), but was higher in CON subjects (5.3, p<0.01 vs. RYGB). Postprandial glucose excursions differed between all 3 groups and were largest after RYGB with numerically higher peak (p=0.08 vs SG, p<0.01 vs. CON) and lower nadir (p=0.02 vs. SG and <0.01 vs CON) (Δmax-min plasma glucose: SG: 5.6±1.5 mmol/L, RYGB: 7.2 ± 1.6, CON: 4.3±1.5, nadir PG: SG: 4.3±0.5, RYGB: 3.8 ±0.6, CON: 4.4±0.4), whereas iAUC of PG was comparable between groups (ANOVA p=0.77). Peak of insulin was also greatly exaggerated after RYGB (p<0.01 vs. SG and CON), but was comparable after SG and CON (p=0.42). In contrast, iAUC of insulin did not differ between the groups (ANOVA p=0.41). Postprandial lactate followed the PG excursions (Peak lactate: SG: 1.8±0.2, RYGB 2.1±0.2, CON: 1.4 ±0.3, ANOVA p<0.01). Insulin clearance was larger in both surgical groups (C-peptide/Insulin: SG: 25 (20;27) [median (IQR)], RYGB: 21 (15;23), CON: 13 (11;19)). Insulin secretion related to insulin resistance (Disposition Index (Insulinogenic index * 1/HOMA-IR): SG: 162 (119;202), RYGB: 199 (127;323), CON 89 (57;118)) was comparable between RYGB and SG (p=0.26), but higher when compared with CON subjects (p<0.05 vs. both SG and RYGB).
Conclusion: Postprandial glucose metabolism after RYGB and SG differs with respect to glucose excursions, nadir of glucose and peak of insulin despite similar iAUC of both glucose and insulin. The metabolic fate of ingested glucose also differs significantly after RYGB and SG with larger postprandial lactate formation after RYGB, suggesting that differential mechanisms underlie the improved glycaemic control in the two surgical situations. In contrast, beta cell function in relation to the ambient insulin resistance was similarly improved after SG and RYGB.
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Supported by: Grant from Capital Region Research Foundation
Disclosure: **M.S. Svane:** Grants; Capital Region Research Foundation.

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Weight loss by two different diets increases the postprandial response of GLP-1 but only the Paleolithic diet increases the postprandial response of GIP

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Background and aims: Weight loss by diet intervention has shown conflicting results on postprandial levels of glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). We therefore investigated a Paleolithic diet (PD) and a prudent control diet according to the Nordic nutrition recommendations (CD) and aimed to compare the effect of the two diets on postprandial levels of GLP-1 and GIP.
Materials and methods: Seventy healthy, obese, postmenopausal women were randomized to either the PD or the CD. In the PD group participants were advised to eat vegetables, fruit, lean meat, fish, nuts and eggs. Cereals, dairy products, added sugar and salt were excluded. With the CD participants were advised to increase their intake of whole grain, fruit, vegetables and fish. Dairy products and meat were supposed to be low fat. Both diets were without calorie restriction. Plasma levels of GLP-1 and GIP were measured after ingestion of 75 g glucose at baseline and after 6 and 24 months of diet intervention. The incremental area under the curve (iAUC) of GLP-1 and GIP was calculated during 120 min after glucose intake.
Results: The PD group showed a more pronounced weight reduction after 6 months (9.2 ± 4.2 kg (mean±SD)) and 24 months (8.1 ± 5.6 kg) compared to the CD group (4.7 ± 4.2 kg at 6 months and 4.9 ± 4.8 kg at 24 months; P<0.001 and P<0.05 for the difference between groups at 6 months and 24 months). For the PD group the iAUC of GLP-1 increased by 34 % after 6 months and by 45 % after 24 months compared to baseline. For the CD group the iAUC of GLP-1 increased by 11 % after 6 months and by 59 % after 24 months. For the PD group the iAUC of GIP increased by 23 % after 6 months compared to baseline but decreased by 3 % in the CD group (P<0.05 for the difference between groups).
Conclusion: Postprandial levels of GLP-1 increased through diet-induced weight loss by the Paleolithic diet and the control diet. The postprandial GIP response increased through weight loss by the Paleolithic diet but not by the control diet.
Clinical Trial Registration Number: Dnr 05-098M
Disclosure: **J. Otten:** None.

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Acute lowering of circulating fatty acids does not improve the incretin effect in patients with type 2 diabetes

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Background and aims: Plasma glucose is the main stimulus for insulin secretion (IS), but non-esterified fatty acids (NEFA) and the incretin hormones (mainly, GLP-1 and GIP) also are important modulators of IS. The incretin effect (IE) accounts for the ~40-80% higher IS after oral ingestion compared to intravenous glucose. Palmitate impairs IE by downregulating GLP-1 receptor signaling in beta-cell lines and isolated mouse islets. Insulin resistance and defective IE are common in T2DM. Thus, increased NEFA levels could impact beta-cell function through an impairment of IE. Our aim was to test whether an acute NEFA reduction induced by acipimox (ACP, a potent inhibitor of lipolysis) improves IE in T2DM.
Materials and methods: Thirteen patients (10F/3M; 54.8±7.6 years, mean±SD; BMI = 32.8±5.6 kg/m^2; HbA_{1c} = 7.24±0.48 %) received a 3-hour OGTT (75 g) and a 3-hour isoglycaemic glucose infusion (IV) on separate days. Both tests were repeated after ACP ingestion (200 mg two hours before and 1hour after starting glucose administration). C-peptide deconvolution was used to calculate IS rates, and mathematical modelling to quantitate beta-cell function and IE. The main parameters are insulin secretion rate (ISR); glucose sensitivity (ßGS), *i.e.,* the slope of the IS/glucose dose-response curve; glucose-induced potentiation (P_{GLU}), a time-dependent modulation of the dose-response; and incretin-induced potentiation (P_{INCR}) calculated as the fold IS increment during OGTT compared to IV glucose.
Results: On the OGTT, ACP decreased NEFA OGTT-area-under-curve (AUC) by 55±14% (64±28 *vs* 27±9 mol·L^{-1}·h^{-1}, p<0.01). Fasting glycaemia, OGTT and IV glucose AUCs were similar before and after ACP, while ACP decreased incremental OGTT-glucose AUC (744±163 *vs* 902± 262 mol·L^{-1}·3h^{-1}, p<0.05). ISR was lower during IV than OGTT

both in the control (58±19 *vs* 70±23 nmol·m^{-2}; p<0.01) and ACP studies (53±15 *vs* 64±20 nmol·m^{-2}, p<0.01). ACP reduced ISR (64±20 *vs* 70±23 nmol·m^{-2}, p<0.05) and did not change ßGS (32±11 *vs* 26±9 pmol·min^{-1}·m^{-2}·mM^{-1}), P_{INCR} (1.17±0.14 *vs* 1.12±0.19 fold), P_{GLU} or plasma glucagon and GIP (all p=ns). In contrast, ACP improved insulin sensitivity, estimated as the oral glucose sensitivity index, OGIS (326±44 *vs* 291±60 ml·min^{-1}·m^{-2}, p<0.05). Changes in ISR were directly related to changes in NEFA (rho=0.62, p=0.03) and inversely related to OGIS (rho= -0.73, p=0.01).

Conclusion: In patients with type 2 diabetes, acute pharmacological NEFA reduction lowers glycaemia and enhances insulin sensitivity but does not improve the incretin effect.

Supported by: EFSD/Sanofi

Disclosure: **V.B. Chueire:** None.

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Glucagon response in patients with type 1 diabetes during meal-related hypoglycaemia and hyperinsulinaemic, hypoglycaemic clamp conditions

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Background and aims: It has been suggested that in long-standing, C-peptide negative type 1 diabetes not only insulin secretion is disturbed but also counter-regulatory response to hypoglycaemia and in particular adequate glucagon secretion is blunted. Glucagon response to hypoglycaemia was tested in a hypoglycaemic clamp setting (clamp) vs. mimicking real-life hypoglycaemia conditions by using a n increased insulin to carbohydrate ratio and exercise challenge (real-life) in C-peptide positive (CP+) vs. C-peptide negative (CP-) subjects with T1D.

Materials and methods: Data from one clamp study and three real-life studies were pooled. In the clamp study 21 (10 CP+, 11 CP-) subjects underwent a hyperinsulinemic, hypoglycaemic clamp (target 3.5mmol/L) after an overnight stabilisation phase at 5.5mmol/L ± 10%. In the real-life studies 27 subjects (9CP+, 18CP-) were investigated at the research centre and received standardised meals with 180% of their regular short-acting insulin dose. Glucagon response during hypoglycaemia was defined as the area under the glucagon curve during the first hypoglycaemic episode (≤3.5 mmol/L). In both experiments, glucose was measured every 5min (Super GL glucose analyser); glucagon samples were collected using P800 (BD) blood tubes and analysis was performed via specific sandwich ELISA (Mercodia). The area under the curve (AUC) for glucagon was calculated using the trapezoidal method. CP+ was defined as C-peptide concentrations ≥0.05 nmol/L.

Results: Baseline characteristics are given in Table 1. During real-life both CP+ and CP- patients had significantly higher AUC-glucagon levels as compared to clamp (CP+: p=0.0146 and CP-: p=0.0022, respectively). During clamp, CP+ patients showed a higher glucagon response (AUC-glucagon) compared to CP- patients (p=0.0074). This finding was not observed in the real-life setting.

Conclusion: Patients during the real-life experiment had overall higher glucagon response than patients undergoing the clamp experiment. CP+ showed a higher glucagon response compared to CP- during hypoglycaemic clamp conditions, but not during real-life conditions. Whether this is due to differences in the patient population investigated (e.g. difference in diabetes duration between clamp and real-life population) or due to the differences in hypoglycaemia induced glucagon stimulation remains to be elucidated.

	Clamp		Real-life	
	CP+ (n=10)	CP- (n=11)	CP+ (n=9)	CP- (n=18)
Age (a)	39.6 ± 12.8	38.3 ± 12.4	32.5 ± 13.7	31.3 ± 9.5
BMI (kg/m²)	23.6 ± 1.8	25.0 ± 2.3	21.8 ± 1.9	25.1 ± 3.4
HbA1c (mmol/mol)	56 ± 10	58 ± 9	49 ± 8	59 ± 11
Diabetes duration (a)	2.5 ± 2.2	23.9 ± 10	7.5 ± 11	16.8 ± 9.3
C-peptide (nmol/L)	0.2 ± 0.1	0.0 ± 0.0	0.2 ± 0.1	0.0 ± 0.0
Fasting BG (mmol/L)	5.5	5.5	7.2 ± 1.8	7.6 ± 2.8
Fasting glucagon (pM)	3.3 ± 2.1	0.8 ± 0.9	4.6 ± 3.3	4.5 ± 2.8

Table 1 - Baseline characteristics

Clinical Trial Registration Number: NCT02028078, DRKS00009604, NCT02614768, DRKS00011488

Disclosure: **J. Muenzker:** None.

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Insulin resistance and beta cell function in pregnancies after gastric bypass surgery

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Background and aims: Obesity has become a major health care concern, related to impaired quality of life and increased risk for metabolic disorders. Surgical interventions are provided already in younger populations at reproductive age if conservative strategies fail to derive satisfactory weight reduction. Previous studies have shown altered glucose kinetics in pregnancies following gastric bypass surgery, however, the pathophysiological alterations beyond these observations are not fully discovered.

Materials and methods: A total of 64 females (26 with history of gastric bypass (RYGB), 19 obese and 19 normal weight controls) were consecutively recruited among women visiting our pregnancy outpatient department between April 2014 to February 2016. A detailed metabolic characterization of the study population was performed between 24 and 28 weeks of gestation including an extended 3h-75g-OGTT as well as a short frequently sampled intravenous glucose tolerance test (IVGTT) to provide estimates of insulin sensitivity and insulin secretion.

Results: We found major alterations in glucose kinetics during the OGTT including an early rise of plasma glucose followed by hypoglycemia in 86% of RYGB women. This was accompanied by increased insulin, C-peptide and glucagon concentrations after oral glucose load. IVGTT data suggested improved insulin sensitivity (CSI in RYGB vs. obese vs. normal weight: 2.31 vs. 0.81 vs. 3.72 (10^{-4} min^{-1} [µU/ml]$^{-1}$, p<0.001) and reduced acute insulin response to intravenous glucose (ΔAIRG: 81.9 vs. 149.7 vs. 89.2 µU/ml). Hence, the disposition index (CSI×ΔAIRG) was improved in pregnancies after RYGB as compared to obese women but subtle alterations in β-cell function were observed in RYGB vs. normal weight controls. We further evaluated data on glucose disposal in n=15 RYGB and n=15 controls, which were matched for their actual BMI and found improved insulin sensitivity in the RYGB subgroup.

Conclusion: Pregnancies after gastric bypass surgery are affected by altered postprandial glucose insulin and C-peptide dynamics, associated with higher glycemic variability. Insulin sensitivity is improved, however, subtle alterations in β-cell function were still observed in pregnancies following bariatric surgery. Longitudinal studies are at need to assess potential consequences for fetal development and pregnancy outcome.

Disclosure: **C.S. Göbl:** None.

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Glucagon-like peptide-1 (GLP-1) has no effect on peripheral vasodilation but augments coronary microcirculatory flow in humans

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Background and aims: We have previously demonstrated that GLP-1 (7-36) improves post-ischemic left ventricular contractility (stunning) induced by coronary balloon occlusion in humans. The mechanism for this cardioprotection is elusive; GLP-1 receptors are not expressed on human myocardium. We hypothesised that GLP-1 (7-36) mediated vasodilatation may be responsible either by dilating coronary arteries, or dilating the peripheral circulation, or both.
Materials and methods: 10 patients requiring left anterior descending coronary artery stenting were recruited to undergo a forearm bloodflow study, and a coronary microcirculation study. On the first visit we gave a local intra-arterial infusion of 5 incremental doses of GLP-1 (7-36) (0.0375, 0.075, 0.15, 0.3, and 0.6pmol/kg/min) for 6 minutes each and assessed forearm bloodflow using standard plethysmography technique. On the second visit, we recorded paired coronary haemodynamic measures (Pd, Pa, transit time (T_{mn}) at rest and during maximal adenosine hyperaemia) at baseline (10 minutes after stent implantation) and after a 20-minute continuous infusion of GLP-1 (7-36) at 1.2pmol/kg/min. We calculated Fractional Flow Reserve (FFR), Basal Microcirculatory Resistance, Index of Microcirculatory Resistance (IMR), Coronary Flow Reserve (CFR), and Coronary Collateral Flow Index (CFI_p).
Results: The results are shown in Table 1 and illustrate that there was no significant effect of GLP-1 on absolute change in forearm bloodflow (1.838 to 1.909, p=0.78), forearm bloodflow ratio (1.152 to 0.974, p=0.19), or percentage change in forearm bloodflow ratio (-14.372%, p=0.2) from baseline to the top dose of 0.6pmol/kg/min. There were no significant effects of GLP-1 on FFR (0.87 vs. 0.89, p=0.68), IMR (17.8 vs. 21.2, p=0.61), CFR (4.4 vs. 3.2, p=0.40) or CFI_p(0.11 vs. 0.14 p=0.32). Resting T_{mn} was faster following GLP-1 (0.97 vs. 0.68, p=0.02) and this was responsible for lowering basal microcirculatory resistance (BMR) (82.4 vs. 55.5, p=0.02).
Conclusion: We did not observe any dilation in peripheral vessels with GLP-1. Therefore it is unlikely that increasing left ventricular ejection fraction by reducing afterload contributes to the mechanism of cardioprotection in humans. GLP-1 appears to act as a coronary microcirculatory vasodilator in humans. Augmenting microcirculatory flow opens stretch-activated calcium channels in adjacent cardiac myocytes, increasing intracellular calcium, which in turn enhances contractility, a mechanism known as the Gregg effect.

Coronary study				Forearm bloodflow study				
	Baseline	GLP-1 (1.2pmol/kg/min)	P value	GLP-1 dose (pmol/kg/min)	Mean control flow	Mean infused flow	Forearm bloodflow ratio	% change forearm bloodflow ratio
FFR	0.87	0.89	0.68	Baseline	1.796	1.838	1.152	0
IMR	17.8	21.2	0.61	0.0375	2.191	1.986	0.957	-16.551
CFR	4.4	3.2	0.40	0.075	2.126	2.165	1.073	-7.566
CFI_p	0.11	0.14	0.32	0.15	2.120	1.807	0.960	-13.859
T_{mn}	0.97	0.68	0.02	0.3	2.002	1.858	1.024	-8.967
BMR	82.4	55.5	0.02	0.6	2.136	1.909	0.974	-14.372
				P value (trend)	0.17	0.78	0.19	0.2

Table 1: Haemodynamic effects of GLP-1 in coronary arteries and radial arteries

Disclosure: **S.J. Clarke:** Employment/Consultancy; MSD.

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The glucagon-like peptide-1 receptor agonist lixisenatide reduces postprandial glucose excursions in totally pancreatectomised patients

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Background and aims: The extrapancreatic effects of glucagon-like peptide-1 (GLP-1) have been difficult to disentangle. Treatment of diabetes secondary to total pancreatectomy remains a challenge. We investigated the effects of the GLP-1 receptor agonist, lixisenatide, on postprandial glucose metabolism in totally pancreatectomised patients.
Materials and methods: In a double-blinded, randomised, cross-over study, we recruited 12 totally pancreatectomised patients (3 women; age: 65.0±9.5 [mean±SD] years; BMI: 22.9±3.9 kg/m^2) and 12 control subjects (4 women; age 64.4±7.6 years; BMI: 24.0±2.9 kg/m^2). Both groups were examined during two 3-hour liquid mixed meal tests (with 1.5 g paracetamol for evaluation of gastric emptying) after single-dose injection of 20 μg of lixisenatide or placebo, respectively. Patients received their regular dose of basal insulin the night before each experimental day; no insulin was given during the meal tests. Blood was sampled for measurements of plasma/serum concentrations of glucose, glucagon, C-peptide and paracetamol.
Results: Compared to placebo**,** lixisenatide significantly reduced postprandial plasma glucose excursions in totally pancreatectomised patients (AUC±SEM: 2,715±179 vs 3,473±177 mmol/l × min, p=0.006) and controls (AUC: 814±14 vs 1,152±57 mmol/l × min, p<0.0001) (figure 1). In totally pancreatectomised patients, C-peptide was undetectable in plasma and lixisenatide significantly reduced gastric emptying as well as postprandial glucagon responses (AUC: 620±162 vs 1,479±271 pmol/l × min, p=0.003). In the control subjects, lixisenatide reduced postprandial plasma C-peptide responses (AUC: 105±19 vs. 261±34 nmol/l × min, p=<0.001) and decelerated gastric emptying significantly whereas postprandial glucagon responses were unaffected (AUC: 1,283±159 vs 1,253 ±225 pmol/l × min, p=0.879).
Conclusion: The GLP-1 receptor agonist lixisenatide reduces postprandial plasma glucose excursions in totally pancreatectomised patients. The mode of action seems to involve deceleration of gastric emptying and reduced postprandial responses of gut-derived glucagon.

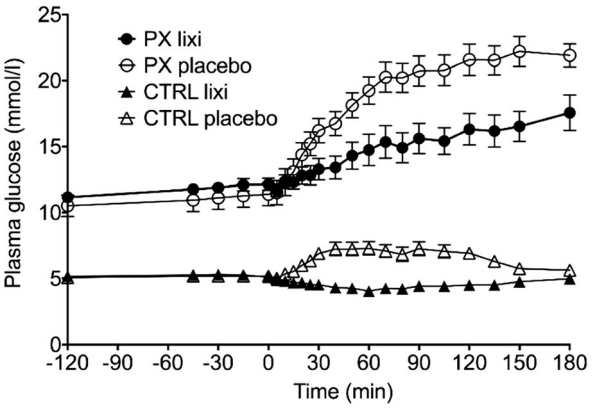

Figure 1. Plasma glucose (mean±SEM) during a liquid mixed meal test with and without prior administration of 20 μg lixisenatide or placebo in 12 totally pancreatectomised patients (PX) and 12 matched control subjects (CTRL)

Clinical Trial Registration Number: NCT02640118
Supported by: Sanofi Aventis
Disclosure: **C.T.B. Juel:** None.

PS 029 Exercise physiology

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Early exercise performance predicts late-onset development of type 2 diabetes in New Zealand obese mice

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Background and aims: Regular exercise training improves insulin sensitivity and glycaemia. However, individuals with type-2 diabetes (T2D) may largely differ in their response to exercise and subsequent improvements in glycaemic control, implicating that the genetic background affects the ability to respond to exercise. Our aim was to investigate on the molecular level exercise-induced alterations in glucose homeostasis in an established polygenic mouse model for T2D, the inbred New Zealand Obese (NZO) mouse strain.
Materials and methods: NZO mice were fed a high-fat diet with 30 % fat from calories after weaning. At week 6 of life, mice were subjected to a 6-weeks chronic interval training on treadmills, or kept as sedentary controls. Endurance capacity was assessed by acute running tests before and after the training intervention. Fasting (6h) plasma glucose levels (FPG) and glucose tolerance tests (GTT) were determined to evaluate glycaemic control. Liver and skeletal muscle triglycerides were determined using a colorimetric assay. Statistical analysis was performed using student's t-test and two-way ANOVA.
Results: At 6 weeks of age, all NZO mice were normoglycaemic (90-100 mg/dl FPG). At 12 weeks, approx. 50% of the mice had developed diabetes (FPG ≥300 mg/dl) in both the trained (11/19) and sedentary (13/26) group, respectively. However, after six weeks of chronic interval training, NZO mice showed improved physical condition, measured by increased time to exhaustion during an acute running test (trained vs sedentary; 13.7 ± 0.5 min vs 11.5 ± 0.4 min, n = 5-19, $p<0.05$). Post-hoc analysis of the acute running tests revealed that diabetic (T2D) mice showed significantly reduced endurance capacity compared to non-diabetic (ND) mice, even before the training (14.1 ± 0.4 min vs 16.7 ± 0.6 min, n = 8-11, $p<0.001$). Furthermore, only ND mice significantly improved their glucose tolerance (trained vs sedentary; AUC 46790 ± 2982 vs 71370 ± 4907, n = 8-12, $p<0.01$), whereas T2D mice remained glucose intolerant (trained vs sedentary; AUC 129300 ± 2633 vs 127500 ± 2465, n = 7-9) in an i.p.GTT, despite the training. Liver triglycerides were increased after chronic interval training, both in ND (trained vs sedentary; 33.5 ± 1.7 μg/mg liver vs 26.7 ± 1.7 μg/mg liver, n = 6, $p<0.05$) and T2D (trained vs sedentary; 37.5 ± 2.3 μg/mg liver vs 31.5 ± 1.4 μg/mg liver, n = 6-7, $p=0.06$) mice. In contrast, skeletal muscle triglycerides only tended to be reduced after chronic exercise in ND and T2D mice.
Conclusion: NZO mice, a polygenic model for early onset obesity and type 2 diabetes, show a heterogeneous response to chronic exercise training with respect to glycaemia. While our results demonstrate that regular training is able to improve exercise performance in NZO mice, an improvement of glycaemic control is observed only in a subgroup of the animals. In fact, we identified reduced endurance capacity as an efficient predictor for the development of T2D, indicating that metabolic programming of non-responder NZO mice has already occurred at a very early age and may not be reversed by acute exercise training. Furthermore, elevated liver fat content indicates abnormal lipid storage and distribution in the NZO model that is not improved by exercise training. Future studies will focus on the identification of epigenetic and metabolic factors that determine the response to exercise.
Disclosure: **C.A. Springer:** None.

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High physical activity levels are associated with better glycaemic control in patients with type 1 diabetes

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Background and aims: The beneficial effects of exercise on metabolic control of patients with type 1 Diabetes (T1D) are still controversial. The aim of this work was to study PA levels and the relationship with glycaemic control and health-related parameters, as body composition and nutritional habits in adults with T1D.
Materials and methods: We included 182 patients with T1D and 185 controls without diabetes between 18 and 50 years old. We assessed PA levels using IPAQ questionnaire, classifying individuals as low (inactive), moderate or high PA levels. Also, we analysed cardiorespiratory fitness by gas analysis exchange (VO2/VCO2) during a maximal incremental exercise test on a cycle-ergometer, obtaining the maximum oxygen consumption (VO_{2max}).We collected medical history that included clinical records and nutrition analyses using a 4days dietary register. Body composition was calculated using dual energy X-ray absorptiometry (DXA). Glycaemic control was established as the last value of HbA1c. We studied values of HbA1c, VO_{2max}, body composition and nutritional habits in relation of PA levels in T1D and control groups.
Results: Patients with T1D and control subjects presented no differences on age, BMI or total PA levels. Patients with T1D present lower levels of cardiorespiratory fitness (VO_{2max}: 30.1±11.1 vs 33.5±11.8 mlO_2/kg/min; $p<0.01$) compared with control group as well as lower amounts of carbohydrates intake (185.9±62.7g vs 212.1±70.1g;$p<0.05$). Regarding T1D individuals, inactive patients had similar values of HbA_{1c} than those with high PA levels (7.8±1.2 vs 7.6±1.4,$p=0.5$). However, analysing just a group of the more trained ones (more than 5 training sessions a week), they showed lower levels of HbA1c than those classified as inactive (7.2±1 vs 8.0±1.3,$p<0.05$).
Conclusion: Subjects with T1D show significant differences in cardiorespiratory fitness and nutrition habits compared to controls. Also, we found a favourable relationship between physical activity levels and glycaemic control, but only in those individuals with higher levels of physical activity.
Supported by: 2014_SGR_520 and CIBERDEM
Disclosure: **S. Murillo:** None.

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Associations of physical activity intensity levels and cardiorespiratory fitness with glucose-induced GLP-1 responses: the ADDITTION-PRO study

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Background and aims: Physical activity and cardiorespiratory fitness may affect the secretion of the gut hormone GLP-1 whose involvement in glucose- and appetite regulation is well-established. However, little is known about the role of physical activity levels during daily living on GLP-1 secretion. Therefore, we examined the association of physical activity intensity levels, including sleep and sedentary time, and fitness with GLP-1 responses to an oral glucose tolerance test.
Materials and methods: We analysed cross-sectional data from 1,326 individuals from the Danish ADDITION-PRO study without known diabetes (53% men, mean age 66.0 years). Circulating GLP-1 levels were

measured at 0, 30, and 120 minutes during a 75-g oral glucose tolerance test. Physical activity was measured using a combined heart rate and movement sensor during 7 days. The intensity of physical activity was classified using metabolic equivalents (METs). The cardiorespiratory fitness level was estimated by an 8-min step test in combination with heart rate measurement. Associations between physical activity measures as explanatory variables and GLP-1 response as outcome were examined by linear regression analysis adjusted for age and sex (model 1), and further adjusted for insulin sensitivity and BMI (model 2). Moderate-to-vigorous physical activity (MVPA) (hours/day) and sedentary time (hours/day) were further adjusted for overall daily physical activity energy expenditure (PAEE) in both models. This was in order for an increase in MVPA to be at the expense of a decrease in a less intensive activity.

Results: The total area under the curve (AUC) for GLP-1 was 10.3-12.7% lower for every hour increase in physical activity spent in moderate-to-vigorous intensity (MVPA) compared to light intensity activity, independent of obesity and insulin sensitivity (See Table 1.). Furthermore, a lower total GLP-1 response with increasing fitness levels was observed, independent of obesity and insulin sensitivity (See Table 1.). PAEE, sedentary and sleep time did not influence the total GLP-1 response to glucose (See Table 1.).

Conclusion: The results suggest that individuals who spend physically active hours at moderate-to-vigorous intensity have lower total GLP-1 response compared to individuals who spend physically active hours at light intensity independent of obesity status and insulin sensitivity. Perspectives: A better understanding of how physical activity is associated with GLP-1 response to oral glucose will be a powerful tool in developing more successful strategies for prevention and management of type 2 diabetes and obesity.

Table 1: Estimated percentage change (95% CI) in GLP-1 response by a unit increase in physical activity measure

	Model	PAEE (kJ/kg/day)	p	MPVA (hours/day)	p	Fitness (ml O_2/kg/min)	p	Sedentary (hours/day)	p	Sleep (hours/day)	p
GLP-1											
$tAUC_{0\text{-}30}$ (pmol/l x min)	1	-0.2 (-0.4;0.0)	0.08	**-12.5 (-21.9;-1.9)**	**0.022**	**-1.0 (-1.8;-0.2)**	**0.020**	-0.6 (-2.7;1.7)	0.6	-0.2 (-4.2;4)	0.9
	2	-0.2 (-0.4;0.0)	0.08	**-12.7 (-22.1;-2.2)**	**0.019**	**-1.3 (-2.2;-0.4)**	**0.004**	-0.7 (-2.9;1.5)	0.5	0.1 (-3.9;4.4)	0.9
$tAUC_{0\text{-}120}$ (pmol/l x min)	1	-0.1 (-0.3;0.1)	0.2	-9.9 (-18.8;0.1)	0.052	-0.7 (-1.4;0.1)	0.085	-0.3 (-2.3;1.7)	0.7	0.5 (-3.2;4.4)	0.7
	2	-0.2 (-0.4;0.0)	0.08	**-10.3 (-19.2;-0.4)**	**0.042**	**-1.2 (-2.0;-0.4)**	**0.005**	-0.3 (-2.3;1.7)	0.7	0.5 (-3.2;4.3)	0.8

Data are percentage change with 95% CI.
P: p-value for test of significance of the association
Model 1: adjusted for age and sex
Model 2: further adjusted for BMI and $ISI_{0\text{-}120}$
Abbreviations: BMI= Body mass index. $ISI_{0\text{-}120}$= Insulin sensitivity index. PAEE = Physical activity energy expenditure (kJ/day/kg). MVPA = Moderate-to-vigorous physical activity (hours/day). MVPA and sedentary time (hours/day) were further adjusted for overall daily physical activity energy expenditure (PAEE). $tAUC_{0\text{-}30}$= total area under the curve for GLP-1 response to glucose 0-30 min.; $tAUC_{0\text{-}120}$ = total area under the curve for GLP-1 response to glucose 0-120 min.

Disclosure: **S.S. Torekov:** None.

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Ghrelin is related to the maintenance of the exercise pattern synchronised with circadian clock under the condition of constant darkness

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Background and aims: We previously reported a bimodal diurnal rhythm of ghrelin with its peaks at the beginning and at the end of the dark period concomitant with a simultaneous increases of voluntary exercise in the wild type (WT) mice. Those accelerations of exercise were attenuated in the ghrelin knockout (GKO) mice, suggesting the relevance of ghrelin surge to the motivation for voluntary exercise (52nd EASD annual meeting). However, the pattern of voluntary exercise was quite similar between WT and GKO mice. It has not been elucidated whether the exercise pattern is synchronized with circadian clock and whether it is entrained by light on-off or ghrelin surge which has been considered to be necessary for the entrainment of circadian rhythm to 24 h daily rhythm. In the present study, we investigated the exercise pattern in WT and GKO mice under constant darkness to evaluate the relevance of circadian clock and ghrelin independently.

Materials and methods: Eight-week-old male WT and GKO mice were individually housed in cages equipped with running wheels under a 12 h light: 12 h dark cycle (L/D, light on 7:00-19:00) at a controlled ambient temperature with ad libitum access to food and water. The number of revolutions was acquired every 15 minutes. At 15 weeks of age, mice were subjected to wheel running under constant dark conditions (D/D) and kept for the next 60 days to measure the circadian rhythmicity of voluntary exercise. At 23 weeks old, light was turned on at 7:00 and the L/D condition was resumed for the next 10 days in order to ascertain whether re-entrainment appeared or not in either group of mice.

Results: A marked increase of wheel-running activity was observed both at the beginning and at the end of dark period in either WT or GKO mice under L/D condition. Under D/D condition, shorter circadian periods than 24 h were observed in both WT and GKO mice with no difference between both groups. In WT mice the wheel-running activity as voluntary exercise was synchronized with circadian rhythmicity with the repetition of clear peaks of exercise and no exercise. In contrast, the exercise pattern was quite different and acrophase of voluntary exercise was unclear and dispersed in GKO mice. Both WT and GKO mice were re-entrained after the resumption of L/D condition (Figure).

Conclusion: It was demonstrated that ghrelin in itself is not relevant to the maintenance of the exercise pattern under L/D condition because of the immediate re-entrainment in either WT or GKO mice. In contrast, it is plausible that ghrelin is related to the maintenance of the exercise pattern synchronized with circadian clock under D/D condition. Thus it is conceivable that the role of ghrelin in voluntary exercise is different between under L/D and D/D condition, that is the relevance to the motivation for exercise under L/D and to the maintenance of exercise pattern under D/D.

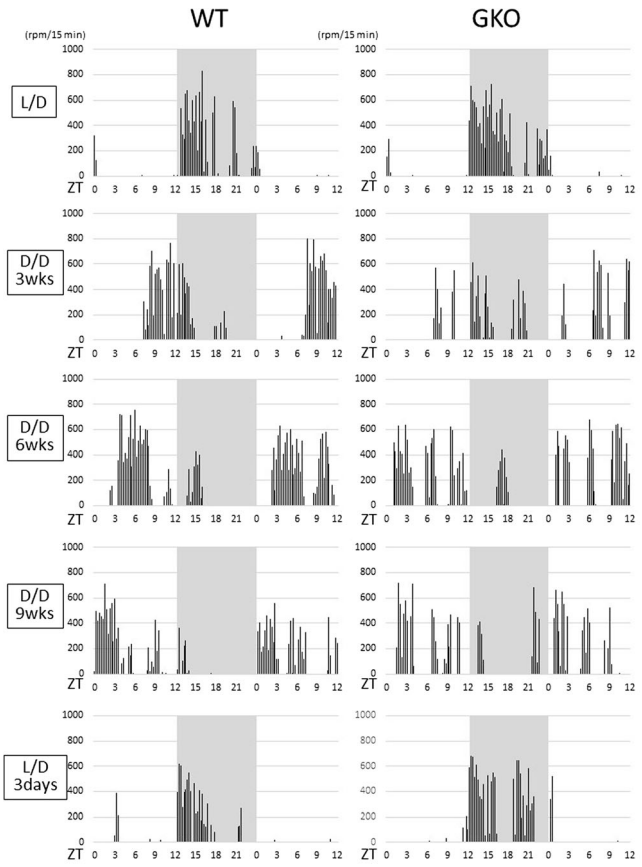

Figure. Wheel-running activity under either L/D or D/D condition in WT or GKO mice

Disclosure: **H. Mifune:** None.

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Hypothalamic regulation is involved in the AMPK activation in skeletal muscles during physical exercise

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Background and aims: Physical exercise is a potent stimulator of AMPK activation in skeletal muscles. AMPK has been believed to play pivotal roles in metabolic changes in contracting skeletal muscles, as we demonstrated that pharmacological activation of AMPK causes alterations in metabolome quite similar to those in contracting skeletal muscles. The AMPK activation has been considered to be due to local consumption of ATP by muscle contraction. On the other hand, we have demonstrated that leptin activates AMPK in liver as well as skeletal muscles through central pathways. Thus, we investigated possible involvement of the central regulation in the AMPK activation by physical exercise.
Materials and methods: Male ddY mice were forced to swim in the current water pool for 30 or 60 min following to treatment with various blockers. Neonatally monosodium glutamate-treated mice (MSG-mice), whose hypothalamic neurons were damaged, were also evaluated. Gastrocnemius and biceps muscles were isolated just after the exercise and isoform-specific AMPK activities were determined by immunoprecipitation and canonical kinase assay.
Results: The swimming exercise evoked alpha2 isoform-specific AMPK activation in the skeletal muscles with increase in phosphorylation level of Thr172 residue of alpha subunit. In contrast, we did not find AMPK alpha2 activation after swimming exercise in the MSG-mice. Chemical sympathectomy by guanethidine treatment almost completely inhibited the swimming-induced AMPK activation. While pretreatment of propranolol, a blocker against beta-adrenoreceptors, did not affect it, the AMPK activation was completely inhibited by prazosin, an alpha1-adrenoreceptor antagonist. Administration of propranolol or prazosin slightly decreased basal plasma lactate levels, but we confirmed that these treatment did not affect change in the plasma lactate levels during the swimming exercise.
Conclusion: Our observations suggest that physical exercise causes isoform-specific activation of AMPK alpha2 in skeletal muscles primarily via hypothalamus-sympathetic nervous systems with alpha1-adrenoreceptors. Suppression of the AMPK activation in MSG-mice suggested involvement of hypothalamic neurons, however, the MSG-mice exhibited obesity. Thus, intensity of exercise might be different between control and MSG-mice. Therefore, we determined the effects of sympathetic blockers. Guanethidine-induced chemical sympathectomy as well as prazosin administration completely blocked the AMPK activation after swimming, suggesting that alpha1 receptor-dependent sympathetic control should be more important for AMPK activation we observed than local ATP consumption by muscle contraction itself. These treatments did not affect rise in plasma lactate levels by swimming, which suggests no difference in the exercise intensity. In conclusion, AMPK activation in skeletal muscles during physical exercise should not be simply due to local changes in energy status by ATP consumption. Systemic regulations including hypothalamic pathways should play significant roles in the AMPK activation and its downstream metabolic alterations during physical exercise.
Disclosure: **L. Miyamoto:** None.

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Poor glycaemic control is associated with altered physiological responses during cardio-pulmonary exercise testing in type 1 diabetes

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Background and aims: There is equivocal research detailing functional capacity impairment in people with type 1 diabetes compared to those without the condition. It is unclear where the mechanistic basis for impairment may lie but it has been shown in some studies to involve cardiovascular, pulmonary and/or musculoskeletal systems, with glycaemic control suggested to play a role. Cardio-pulmonary exercise (CPX) testing provides assessment of the integrative responses of cardiovascular, pulmonary and musculoskeletal systems during sub-maximal and maximal exercise intensities. It is non-invasive and highly sensitive and can be used to determine the location of fatigue of cardiovascular or respiration origin. Little work has assessed the impact of glycaemic control on physiological markers during acute CPX testing in type 1 diabetes. This study sought to measure changes in sub-maximal and peak cardio-pulmonary physiological markers in response to cardio-pulmonary exercise testing and determine the impact of glycaemic control on exercise responses.
Materials and methods: Following ethical approval and after informed consent sixty-four patients with type 1 diabetes (age: 34.7±7.7 years; 13 females; length of diabetes: 17.1±9 years; HbA_{1c}: 7.81±0.95 % (61.9±13.1 mmol/mol) performed a cardio-pulmonary cycle ergometer exercise test until maximum volitional exhaustion (female/male; start: 30/40 watts, increase: 30/40 watts every 3 min). Data were averaged every 10 s and expressed as absolute and relative physiological and power values. Metabolic oxidative data were determined using principles of stoichiometry. Pearson's product moment correlations (r) were used to explore relationships between glycaemic control and physiological and performance markers, with $P \leq 0.05$.
Results: Peak physiological parameters were heart rate (HR_{peak}) 185±11 bpm, maximum oxygen uptake (VO_{2max}) 36.7±5.2 $ml.kg^{-1}.min^{-1}$ and power output (P_{peak}) 230±46 watts. Total exercise time was found at 21.2±2.9 min. HbA_{1c} was associated with several markers at the sub-maximal threshold of the heart rate turn point (HRTP); namely, exercise time (r=-0.23, P=0.07), P_{HRTP} (watts) adjusted for body mass (kg) (r=-0.24, P=0.06), O_2 uptake at P_{HRTP} (r=-0.31, P=0.01). Furthermore, HbA_{1c} was correlated to the carbohydrate oxidation rate at HRTP when expressed relative to percentage of VO_{2max} (r=-0.27, P=0.02). HbA_{1c} was also related to the time to exhaustion (r=-0.30, P=0.01), P_{peak} (watts) adjusted for body mass (kg) (r=-0.25, P=0.04), O_2 uptake at P_{peak} (r=-0.24, P=0.06) and HR_{peak} (r=0.29, P=0.02).
Conclusion: Our data demonstrate an efficacious role for cardio-pulmonary exercise testing in a large group of type 1 diabetes patients in determining performance capacity. This study found an important influence of glycaemic control on acute exercise tolerance during cardio-pulmonary exercise testing in type 1 diabetes. Higher HbA_{1c} values were related to lower economy of O_2 use at sub-maximal work rates that translated to shorter exercise test duration and higher peak heart rates. The influence of glycaemic control on cardio-respiratory responses and exercise tolerance in type 1 diabetes warrants further investigation.
Clinical Trial Registration Number: NCT01704417
Supported by: Novo Nordisk A/S.
Disclosure: **O. Moser:** Grants; Sêr Cymru II COFUND fellowship/ European Union.

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Evaluation of invasive versus non-invasive thresholds during cardio-pulmonary exercise testing in patients with type 1 diabetes

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Background and aims: Cardio-pulmonary exercise (CPX) testing protocols with small stage-to-stage increments can be used to determine two thresholds defining three phases of metabolism. The second threshold such as the second ventilatory threshold (VT2) or the second lactate turn

point (LTP_2) serve as upper limits for endurance training. Both methods are expensive, complex, time consuming or invasive. As a non-invasive technique, the dynamics of the heart rate to performance curve (HRPC) can be used to identify a heart rate turn point (HRTP) because the HRPC deviates near to maximum effort, which allows detecting the HRTP. This point was shown to be related to LTP_2 in healthy individuals. As it is currently not known if the HRTP is in congruence with LTP_2 in patients with type 1 diabetes (T1D) this method was investigated as it might offer a useful and easy to apply non-invasive detection method for the upper limit of sustainable endurance exercise.

Materials and methods: 14 male subjects were recruited for this study (7 T1D patients/7 healthy individuals). T1D patients and healthy individuals' characteristics were: BMI 23.9 ± 2.5 vs. 23.4 ± 1.8 kg/m^2, age 25.3 ± 5.3 vs. 23.4 ± 4.1 years and HbA_{1c} 7.4 (57) ± 0.6 % (6.3 mmol.mol^{-1}) and duration of diabetes 16.9 ± 8.1 years. All subjects performed CPX test on a cycle ergometer (start 40 watts, increase 20 W.min^{-1}). Both lactate turn points (LTP_1/LTP_2) were identified by a computer-aided linear regression break point analysis from the power output (P) and lactate concentration relationship, and HRTP was defined as the point of intersection of two regression lines in the HRPC between LTP_1 and P_{max} with minimal standard deviation of the two straight lines. Pulmonary gas-exchange variables were measured continuously. Lactate concentration (LA), heart rate (HR), oxygen uptake (VO_2), carbohydrate oxidation rate (CHOoxi) and P were compared between HRTP and LTP_2 via ANOVA, post-hoc testing and Pearson's product moment correlations (r), with P≤0.05.

Results: Starting blood glucose prior the CPX testing was 10.58 ± 3.42 mmol.l^{-1} in T1D. HRTP and LTP_2 were significantly associated for physiological variables and P for both T1D patients (r=0.94) and healthy individuals (r=0.91) (p<0.01). Mean differences and confidence intervals for HRTP and LTP_2 were found for LA at -0.30 mmol.l^{-1} (-0.35 to 0.05, p=0.08), HR -1.71 b.min^{-1} (-4.86 to 1.43, p=0.23), VO_2 -1.61 ml.kg^{-1}.min^{-1} (-4.64 to 1.40, p=0.23), CHOoxi 0.23 g.min^{-1} (-0.59 to 1.05, p=0.51) and P -6.57 W (-14.67-1.52, p=0.09) for T1D patients. Similar results were found for healthy controls for LA at 0.21 mmol.l^{-1} (-0.41 to 0.84, p=0.43), HR -0.14 b.min^{-1} (-2.72 to 2.44, p=0.89), VO_2 -0.34 ml.kg^{-1}.min^{-1} (-1.64 to 0.95, p=0.53), CHOoxi 0.36 g.min^{-1} (-0.27 to 1.01, p= 0.21) and P 0.57 W (-6.09 to 7.23, p=0.84).

Conclusion: Comparable to healthy individuals the HRTP is an easy to apply non-invasive method to determine the upper limit for endurance exercise in T1D patients which is equivalent to lactate derived thresholds. Furthermore, no expensive and sophisticated equipment is needed and anxious patients with fear of breathlessness do not need to wear a constricting facemask.

Clinical Trial Registration Number: NCT02075567

Disclosure: **M.L. Eckstein:** Grants; KESS II.

PS 030 Non-insulin hormones

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The role of glucagon in gastrointestinal-mediated glucose-disposal and incretin effect in patients with type 2 diabetes and normal glucose tolerant individuals

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Background and aims: Gastrointestinal-mediated glucose-disposal (GIGD) after OGTT reflects the percentage of glucose disposal caused by the oral route. GIGD is reduced in patients with type 2 diabetes (T2D) because of a reduced incretin effect, but possibly also due to OGTT-induced hyperglucagonaemia. Utilising the glucagon receptor antagonist (GRA) LY2409021, we investigated the effect of glucagon on GIGD, incretin effect and plasma glucose.

Materials and methods: In a double-blinded, randomised, cross-over study, 10 patients with T2D (male/female: 5/5; age: 57.1±4.1 [mean ±SEM] years; BMI: 33.0±1.7 kg/m^2; fasting plasma glucose: 8.1 ±0.3 mmol/l; HbA1c: 46.2±1.9 mmol/mol) and 10 gender, age and BMI-matched controls (male/female: 5/5; age: 57.4±4.0 years; BMI: 31.8±1.3 kg/m^2; fasting plasma glucose: 5.6±0.1 mmol/l; HbA1c: 33.9±0.9 mmol/mol) underwent two 50 g-OGTTs with preceding single-dose administration of 100 mg GRA and placebo, respectively, and two corresponding isoglycaemic i.v. glucose infusions (IIGIs).

Results: Compared to placebo, GRA reduced fasting plasma glucose by 2.4±0.4 mmol/l (p=0.0001) in T2D patients and by 0.6±0.04 mmol/l (p<0.0001) in controls. Plasma glucose excursions during OGTT assessed by baseline-subtracted AUC were greater with GRA compared to placebo in both groups (T2D: 998±64 vs. 694±66 pmol/l × min, p=0.0004; controls: 307±40 vs. 209±44 pmol/l × min, p=0.005). No differences were observed between GRA and placebo on GIGD or the incretin effect.

Conclusion: Fasting plasma glucose was lowered by LY2409021 in patients with T2D and controls, but baseline-subtracted plasma glucose excursions increased with GRA in both groups. GIGD and incretin effect are unaffected by glucagon receptor antagonism.

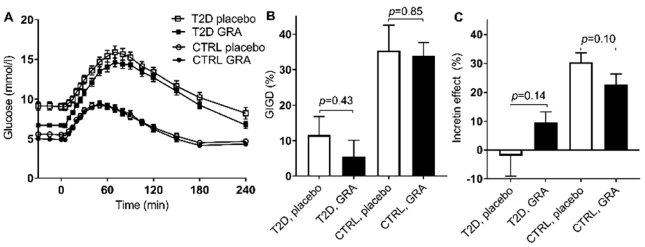

Figure 1. Plasma glucose excursions (A) during 50 g-OGTT, gastrointestinal-mediated glucose disposal (GIGD) (B) and incretin effect (C) after single-dose administration of glucagon receptor antagonist (GRA) and placebo in patients with type 2 diabetes (T2D) and controls (CTRL). Data are means±SEM

Clinical Trial Registration Number: NCT02669524

Supported by: The Danish Diabetes Academy supported by the Novo Nordisk Foundation

Disclosure: **E. Nielsen-Hannerup:** Grants; The Danish Diabetes Academy supported by the Novo Nordisk Foundation, Eli Lilly and Company, The A.P. Møller Foundation for the Advancement of Medical Sciences.

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Novel approach to estimate glucagon turnover in humans: use of ^{13}C glucagon

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Background and aims: Unlike the C-peptide model used to estimate insulin secretion, there are currently no available methods to measure glucagon secretion and kinetics in humans. A better understanding of these parameters could lead to valuable insights into future research into dysregulation of pancreatic alpha cell function in diabetes. Translationally, this knowledge could further inform the development of multi-hormone algorithms for closed loop control in T1D.
Materials and methods: In order to do so, we applied the isotope dilution approach by synthesizing a non-radioactive, ^{13}C labeled glucagon isotope (FF-glucagon; Phe 6 $^{13}C_9$, ^{15}N; Phe 22 $^{13}C_9$,^{15}N) that was reproducibly detectable in plasma (LOD: 5 pg/ml) by nanoLCMS/MS. To estimate post-absorptive glucagon turnover, we conducted a pilot study in 8 non-diabetic (ND) (age 31.1±10.8 yrs., BMI 22.9±4.5kg/m^2; HbA1c 5.0±0.1%) and 2 type 1 diabetes (T1D) (age 32.8±13.2 yrs., BMI 28.8 ±5.5kg/m^2; HbA1c 6.6±0.1%) subjects with normal liver and kidney functions. Subjects were admitted at 6 PM to the Clinical Research Trials Unit, consumed a standard meal at 7 PM and remained NPO except water for the rest of the study. Insulin was infused in T1D subjects to maintain euglycemia. FF-glucagon was infused intravenously for 2 hours starting at 7 AM at a constant rate to achieve steady plasma enrichment. The heated hand vein method was used to collect blood periodically to measure glucose, insulin, glucagon concentrations and FF-glucagon enrichment. Glucagon concentration (native + FF-glucagon) was measured by a radioimmunoassay (Millipore) and FF-glucagon enrichment by nanoLCMS/MS.
Results: Plasma glucose (4.8±0.3 mM), insulin (24.4±12.3 pM) and glucagon (20.9±4.4 pM) concentrations did not change during and after cessation of FF-glucagon infusion. A stable tracer-tracee ratio (TTR) enabled measurement of Glucagon kinetics with the steady-state equation: $Ra_{glucagon} = ([F_{FF\text{-}glucagon}/TTR] - F_{FF\text{-}glucagon})$, where $Ra_{glucagon}$ = rate of systemic post-hepatic glucagon appearance; $F_{FF\text{-}glucagon}$ = rate of infusion of FF-glucagon and TTR = Ratio of FF-glucagon to total glucagon. $Ra_{glucagon}$ was 0.18±.02 pM/kg/min in ND and 0.32±.04pM/kg/min in T1D; glucagon clearance was 11.5±3.3 ml/kg in ND and 11.1±4.5 ml/kg in T1D.
Conclusion: The results provide proof of concept that the method works and hints that the inappropriately elevated plasma glucagon concentrations observed frequently in T1D could be due to increased systemic glucagon appearance rather than due to altered glucagon clearance. We believe this represents the first report of a novel method to measure in vivo glucagon kinetics in humans.
Supported by: NIDDK
Disclosure: **A. Basu:** None.

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Glucagon elimination is increased in patients with type 2 diabetes

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Background and aims: Type 2 diabetes (T2D) is characterised by hyperglucagonaemia, which contributes significantly to the hyperglycaemic state of the disease. The aetiology behind the hyperglucagonaemia is complex and thought to involve hypersecretion of glucagon from the pancreas. However, no study has so far investigated whether a decreased metabolic clearance rate of glucagon could contribute to hyperglucagonaemia in patients with T2D
Materials and methods: Glucagon was infused intravenously (4 ng/kg/min) for 1 hour in 16 patients with T2D (age [mean±SD]: 59±8 years, BMI: 31±6 kg/m^2, HbA1c: 52±16 mmol/mol (6.9±1.4%), estimated glomerular filtration rate (eGFR): 89±12 ml/min/1.73 m^2) and 16 age, gender and BMI-matched non-diabetic controls (age: 59±9 years, BMI: 31±6 kg/m^2, HbA_{1c}: 34±4 mmol/mol (5.3±0.4%), eGFR: 83±13 ml/min/1.73 m^2). Plasma glucagon was measured frequently before, during and after the 1-hour infusion (Figure 1).
Results: Compared to controls, metabolic clearance rate of glucagon was higher (40.6±2.7 [mean±SEM] vs. 29.7±1.2 ml/kg/min, p=0.002) and elimination $t_{½}$ of glucagon was lower in the T2D patients (4.4±0.3 vs. 5.5±0.4 minutes, p=0.02).
Conclusion: From these accurate estimates of the metabolic clearance rate and $t_{½}$ of glucagon in patients with T2D and matched non-diabetic controls, we conclude that glucagon elimination is increased in our cohort of patients with T2D, suggesting that increased secretion rather than diminished metabolic clearance rate of glucagon contributes to the hyperglucagonaemic state of T2D.

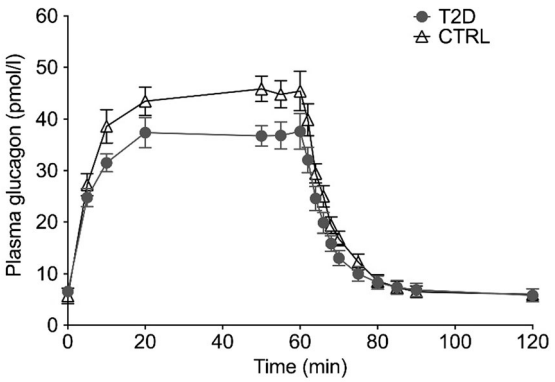

Figure 1. Plasma glucagon (mean±SEM) following infusion of glucagon (4 ng/kg/min) from 0-60 minutes in 16 patients with type 2 diabetes (T2D) and 16 matched non-diabetic control subjects (CTRL)

Clinical Trial Registration Number: NCT02475421
Disclosure: **A.B. Lund:** Lecture/other fees; Novo Nordisk.

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Baicalin attenuates glucose intolerance by modulating pancreatic hormones in high-fat induced obese diabetic mice

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Background and aims: Baicalin, a naturally occurring flavonoid found in in the genus *Scutellaria*, was originally known as an allosteric modulator of the benzodiazepine sites of the γ-aminobutyric acid A receptor ($GABA_AR$) producing anxiolytic effects in mice without sedative or myorelaxant effects. Baicalin has been recently shown to exert metabolic effects by attenuating hyperglycemia-induced mitochondrial damage in 훽;-cells in diabetic rats, high-fat diet- (HFD-) induced body weight gain, and lipid deposits in the liver and systemic inflammation in mice. This study investigated the effects of baicalin on islet functions and diabetic status in HFD-induced obese diabetic mice.
Materials and methods: C57BL/6 mice were grouped into normal control, HF control, HF sham, and 4 different baicalin dose-administered groups (25, 50, 100, and 150 mg/kg). HFD containing 60% fat and water were fed ad libitum for 24 weeks. Designated doses of baicalin or 0.9%

saline were administered intraperitoneally 5d/wk. Body weight (BW) and conventional glucose homeostasis parameters (FPG, FPI, AUC-glucose, AUC-insulin, and etc.) were monitored; in addition, ex vivo glucose-stimulated insulin secretion (GSIS) and glucagon secretion (GSGS) with isolated islets were performed.
Results: Baicalin attenuated HFD-induced hyperglycemia in dose-dependent manner without affecting BW. Insulin secretion in response to high glucose stimulation (16.7mM) was significantly higher in islets isolated from the groups administered 50, 100, 150 mg/kg baicalin compared to HF control; glucagon secretion was significantly suppressed by high glucose stimulation in islets from the same groups.
Conclusion: This study showed that baicalin had positive effects on glycemic control by regulating secretion of insulin and glucagon, possibly through $GABA_AR$ mediation.
Disclosure: **J. You:** None.

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NADPH as a metabolic coupling factor: A player in glucagon secretion?

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Background and aims: NADPH is an important coupling factor in the mechanisms controlling insulin secretion by pancreatic β-cells where stimulus-secretion coupling largely depends on mitochondrial activity. It has been reported that in β-cells, NADPH plays an important role in the amplifying pathway of insulin secretion in response to elevated $[Ca^{2+}]_i$. In α-cells, both intrinsic and paracrine mechanisms have been postulated. The causes of glucagon hypersecretion in type-2 diabetes (T2D) are only beginning to be elucidated. The aim of this study is to access the role of NADPH in the metabolism and pathophysiology of α-cells using both αTC1 clone 6 cell line and intact mouse islets.
Materials and methods: Expression was down regulated by small interfering RNA of four NADPH generating enzymes in αTC1 clone 6 cells: isocitrate dehydrogenase-1(IDH-1), the mitochondrial form IDH-2, Malic enzyme 1 and the mitochondrial form Malic enzyme 3 (ME3). C75 was used to inhibit Fatty acid synthase (FAS) in attempt to increase the levels of NADPH. Glucagon secretion was measured in isolated mouse islets from chow- and high fat diet (HFD) -fed C57/bl6-J mice for 8 weeks. Islets were pretreated with C75 (150 μM) in DMSO 2 hours prior to secretion experiment.
Results: siRNA downregulation of ME3 by 50% resulted in decreased levels of NADPH by 50% and reduced glucagon gene expression by 67%. After knockdown of ME3, glucagon secretion was increased in 30% and C75 treatment more than doubled glucagon secretion at 1 mM glucose (to 158 ±40 pmol/l from 65±19 pmol/l; n=6, p<0.05) in HFD mouse islets but was ineffective in islets from mice fed the control diet. Glucagon secretion at 11 mM glucose was also increased 2.5-fold (n=6, p<0.05).
Conclusion: These results suggest that (1) both ME3 and FAS are involved in α-cell regulation and (2) that C75 may have deleterious effects on islet function under diabetogenic conditions, exacerbating hyperglucagonemia.
Supported by: VR, Wilhems and Martina Lundgren R, Sigurd and Elsa Goljes MF, Adlerbert RF
Disclosure: **C. Miranda:** None.

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Endogenous progestogen steroid hormone modifies the positive association between total estradiol and type 2 diabetes risk in postmenopausal women: a prospective study

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Background and aims: Recent evidence indicates that higher levels of endogenous estradiol might be associated with increased risk of type 2 diabetes (T2D) in postmenopausal women. It is not known whether progesterone attenuates the deleterious effects of estrogen on T2D. We analyzed whether endogenous estradiol levels were associated with T2D risk in postmenopausal women and whether this association was modified by the levels of the endogenous progestogen steroid hormone.
Materials and methods: This study was embedded in the Rotterdam Study, a prospective follow-up study among subjects aged ≥45 years (n= 3117). At baseline, total estradiol and 17-hydroxyprogesterone levels were measured. T2D events were diagnosed on the basis of medical records and glucose measurements from Rotterdam Study visits. Multivariable adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated using Cox regression models.
Results: During a median follow-up of 11.1 years, we identified 384 incident cases of T2D. Total estradiol was associated with increased risk of T2D (3rd tertile vs. 1st tertile: RR: 1.42; 95%CIs=1.02 -2.00, P-trend=0.04). A significant interaction between total estradiol and 17-hydroxyprogesterone was found (*P-interaction* =0.02). After stratification by median 17-hydroxyprogesterone, an increased risk of T2D was observed for total estradiol only in subjects with levels of 17-hydroxyprogesterone lower than median (3rd tertile vs.1st tertile: HR = 1.99, 95% CI=1.22-3.24, *P*-trend=0.007). No association was observed in subjects with levels of 17-hydroxyprogesterone higher than median (3rd tertile vs.1st tertile: HR = 1.05, 95% CI=0.67-1.36, *P*-trend=0.88).
Conclusion: These findings suggest that high levels of endogenous estradiol levels increase T2D risk in postmenopausal women, but this risk may be reduced by high levels of 17-hydroxyprogesterone.
Disclosure: **M. Taulant:** None.

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Dysregulated iron homeostasis in type 2 diabetes

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Background and aims: Iron and diabetes are strictly related. This is clinically evident in patients affected by hereditary hemochromatosis, in which the prevalence of diabetes is about 20%. On the other side, recent studies demonstrate that in diabetic patients plasma iron and/or ferritin levels could be prognostic factors for the development of the disease. The aim of this study is to understand the molecular mechanisms that cause diabetic iron overload and to elucidate the role of increased iron levels in the pathogenesis of diabetes and in the generation of the diabetic late complications.
Materials and methods: Patients with diabetes mellitus type 2 (T2DM) from the HEIDIS cohort were analysed for systemic iron parameters and hepcidin expression. Lepr(db/db) mice were used as a model of T2DM to identify the mechanisms by which diabetes affects iron homeostasis. Tissue iron content and distribution have been assessed in histology while alterations in the expression of iron-related genes have been analysed in qRT-PCR and western blot. Systemic iron content and hepcidin levels have been measured using the BDA method and with a specific ELISA. To assess how increased iron levels affect the pathogenesis of diabetes, Lepr(db/db) mice have been mated with the FpnC326S mouse model of hereditary hemochromatosis type 4 and diabetic and iron parameters have been measured.
Results: 115 T2DM patients and 26 healthy controls were analyzed for systemic iron parameters. CRP>2 was used as exclusion criteria to avoid inflammatory interferences. Diabetic patients show a significant increase in serum iron content (p=0,035), transferrin saturation (p=0,025) and

ferritin levels (p=0.01). Serum hepcidin levels were inappropriately low (p<0,0001) explaining the elevated systemic iron levels. To study how diabetes affects iron homeostasis, we analyzed the Lepr(db/db) mouse model of T2DM. 10-week old mice show the hallmarks of the early T2DM: obesity, hyperglycemia, hyperinsulinemia and increased HbA1c. Consistent with the patients, these mice have elevated serum iron, transferrin saturation and ferritin levels while circulating hepcidin is significantly reduced. Analysis of the liver revealed an overall iron deficiency despite the elevated systemic iron content and the increased TfR1 mRNA expression. Interestingly, the hepatic levels of the iron storage protein ferritin are unchanged. Molecular analysis revealed the typical BMP/SMAD activation pattern that mediates hepcidin upregulation upon systemic iron overload. FpnC326S/Lepr(db/db) hemochromatotic diabetic mice show a similar phenotype when compared to hemochromatotic non-diabetic controls, having a 1,5-fold increase in systemic iron levels and a decrease in non-heme liver iron content.

Conclusion: This study revealed that hepcidin levels are inappropriately decreased both in patients and in Lepr(db/db) mouse model of T2DM, causing elevated systemic iron levels. T2DM causes a lack of systemic iron uptake by the liver, generating hepatic iron deficiency. This phenotype persists in hemochromatotic/diabetic mice, which show decreased hepatic iron content and increased systemic iron levels compared to hemochromatotic non-diabetic mice. Experiments are ongoing to identify the consequences of increased systemic iron content in organs affected by the diabetic late complications.

Supported by: SFB1118

Disclosure: **S. Altamura:** None.

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Analysis of heterogeneity of individual donor and islet glucagon responses to glucose stimulated hormone secretion and drug therapy

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Background and aims: Human islets have become a critical resource for researchers studying islet physiology and hormone secretion. Notably, donor islet heterogeneity influences the development of diabetes and its response to treatment, and often poses a high risk of data reproducibility and misinterpretation compared to rodent islets. Dapagliflozin (an SGLT2 inhibitor) has been shown to elevate plasma glucagon levels independent of the action of insulin. The aim of this study was to determine the reproducibility of human islet cultures in response to glucose and drug treatment across a large number of donors.

Materials and methods: Human islets were isolated from deceased non-diabetic donors. Islet preparations were treated in quadruplicate with 1 mM or 6 mM glucose, with or without dapagliflozin (12 μM) for 1h. Healthy male C57BL/6J mice were treated with one-shot dapagliflozin (10 mg/kg of body weight *n= 30*) or vehicle (*n=30*) after an overnight fast. Glucagon secretion was measured by ELISA.

Results: Islets from 26 donors (BMI range, age range, HbA1c range) were studied. Mean glucagon secretion was 6,06±5,70% of content (range 16,17% to 0,27%) at 1mM and decreased to 3,68±3,56% of content (range 12,37% to 0,23%) at 6mM. However, donor effect was a major determinant of glucagon secretion (p<0.0001). When analyzed with two-way ANOVA, dapagliflozin significantly induced glucagon secretion at 6mM to 6,16±5,58% of content (range 22,99% to 0,42%), (p<0.0001, dapagliflozin / donor interaction). Sample size analysis predicted that for 80% power with 5% significance as a measure of sensitivity, the minimal sample size required to draw firm conclusion is n=10 human islet donors. *In vivo*, *dapagliflozin consistently and significantly induced glucagon secretion in C57BL/6J* mice (vehicle vs. dapagliflozin; p<0,0001).

Conclusion: The use of human islet cultures for drug testing is recommended in conjunction with adequately powered group sizes for dependable preclinical testing of new therapeutic drugs.

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PS 031 Substrate metabolism

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What happens to glucose metabolism if we combine fructose and prednisolone intake? It is never too late to repair bad habits

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Background and aims: It is well known that regular ingestion of fructose and continuous treatment with glucocorticoid (GC), separately, causes diabetogenic effects. Thus, we sought to evaluate the impact of a combination of fructose and prednisolone ingestion on glucose and lipid homeostasis as well as the impact of the interruption of this combined treatment.
Materials and methods: Male Wistar rats were divided into five groups as follows: control (C) group, fructose (F) group (20% fructose in the drinking water), prednisolone (P) group (12.5 μg/mL prednisolone in the drinking water), fructose plus prednisolone (FP) group (a combination of fructose and prednisolone in the drinking water as for F and P groups) for 90 consecutive days. Half of the rats (group R) from the FP group were maintained for 90 more days with a discontinuation of fructose and prednisolone to monitor the parameters.
Results: The FP and P groups exhibited reduced body weight gain even though they had comparable caloric intake with the C and F groups (n=10, p<0.05). The combination of fructose and GC intake rendered rats hypertriacylglyceridemic from the fourth week of treatment until the end of treatment (n=10, p<0.05). The FP group also developed increased abdominal adiposity, reduced insulin sensitivity, hyperinsulinemia, increased circulating uric acid, and impaired hepatic redox balance with increased hepatic triacylglycerol content (n=10, p<0.05). No major impact on fasting glycemia or glucose tolerance was observed in the FP group. This metabolic phenotype observed in the FP group was accompanied by an increase in the fat accumulation in the liver and by augmented beta cell mass per body mass (n=8-10, p<0.05). In addition, the combination of fructose and GC treatment led to a decrease in the total protein kinase B (PKB) content in the liver and to a reduction in the phosphorylated 5' AMP-activated protein kinase (AMPK) content in the adipose tissue (n=6, p<0.05). Quantification of several proteins involved with the proinflammatory pathway showed no alteration caused by the combination of fructose and GC intake. Interruption of fructose and prednisolone ingestion was satisfactory for the improvement of body weight gain and the normalization of circulating triacylglycerol and uric acid in the R group. Cessation of treatments also led to normalization of the peripheral insulin sensitivity, fat accumulation and redox state in the liver (n=7-14, p<0.05). Finally, protein content of PKB in the liver and phosphorylated AMPK in the adipose tissue were all improved in the R group (n=6, p<0.05).
Conclusion: We conclude that combination of fructose and prednisolone intake impairs the body growth and induces a metabolic syndrome-like phenotype. We also conclude that the removal of fructose and GC from water ameliorates practically all metabolic adverse effects. These findings point to the risk of associating GC-based therapies with a regular intake of sweetened beverages and draws attention to the plasticity of the organism and the benefits of removing the causal factors, whenever possible.
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Pyruvate dehydrogenase kinase as a regulator of hepatic gluconeogenesis

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Background and aims: Hepatic gluconeogenesis is delicately controlled by fasting and feeding. In fasting or diabetes, for example, gluconeogenic genes are transcriptionally activated by upregulation of cAMP-PKA-CREB signaling pathway. Pyruvate dehydrogenase (PDC), a gatekeeper determining the fate of pyruvate by catalyzing it into acetyl CoA, becomes inactivate when phosphorylated by pyruvate dehydrogenase kinase (PDK). It was previously known that inhibition of PDK4 in the muscle increases pyruvate oxidation thereby limit availability of substrate utilized for gluconeogenesis in the liver. However, the role of PDK4 in the liver is less studied.
Materials and methods: Mice were treated with either adenovirus carrying PDK4 or GFP intravenously, and their pyruvate tolerance and mRNA expression of gluconeogenic genes were examined. PDK inhibitor dichloroacetate was treated to evaluate the pharmacologic effect on hepatic gluconeogenesis. After hepatic PDK4 was knocked down by injecting adenovirus carrying shPDK4, hepatic glucose production was examined by pyruvate tolerance test and gluconeogenic gene expression was evaluated by qPCR.
Results: PDK4 overexpression in the liver was sufficient to upregulate PEPCK, G6Pase mRNA expression and increase blood glucose levels as well as hepatic glucose production in normal mice via augmenting PKA-CREB signaling pathway, whereas knockdown of hepatic PDK4 by adenoviral shPDK4 infection in diet-induced obesity mice decreased blood glucose level and hepatic glucose production. Mice treated with PDK inhibitor dichloroacetate in the long-term also exhibited attenuated gluconeogenic gene expression and gluconeogenesis, thus their blood glucose levels became normalized.
Conclusion: Collectively, we propose the novel and critical role of hepatic PDK4 which inhibits gluconeogenesis by counteracting glucagon signals. This finding strengthens the possibility of PDK4 as an ideal target against diabetes where glucagon signaling is pathologically stimulated.
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The impact of a meal with high fructose content on glycaemic control and hepatic gluconeogenesis

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Background and aims: Over the past 3 decades there has been a massive increase in the intake of high-fructose corn syrup (HCFS) in Western countries. HFCS, which consists of 55% fructose and 45% glucose, is implicated in the surge of glucose intolerance, insulin resistance, Type 2 Diabetes and fatty liver disease over the same period. Paradoxically, fructose per se is traditionally regarded as being beneficial for diabetic subjects, since it is described as having a low glycemic index. Our objective was to characterize the kinetics metabolic profile of glycemic control in healthy subjects following ingestion of a meal containing 50 grams of fructose and glucose in proportion to that of HFCS (55% fructose/45% glucose- HF) and compare to ingestion of a load with catalytic levels of fructose (5% fructose/95% glucose- LF) - which has previously been shown to have beneficial effects on glycemic control and glucose disposal in diabetic subjects.
Materials and methods: Seven overnight fasted subjects were given a meal consisting of 50 g 55% fructose/45% glucose and powdered whey protein dissolved in 330 ml water having ingested ^2H_2O to 0.3% body water 2 hr before the meal. 0.5g Paracetamol was ingested 1 hr before and

1 hr after the meal. Plasma glucose was sampled periodically with a glucometer and a 30 ml sample was collected at 180 min post-load for analysis of ^{2}H enrichment by ^{2}H NMR. Urine was also sampled from 120-240 min for paracetamol glucuronide (PG) and body water enrichments. Four of the subjects repeated the study with 50 g 5% fructose/95% glucose. The contribution of glucose absorption and gluconeogenesis to plasma glucose levels was estimated from the enrichment of glucose hydrogen 5 relative to body water (H5/BW). The contributions of direct and indirect pathways of hepatic glycogen synthesis were determined from PG H5/BW.

Results: Glycemic excursions were identical for HF and LF loads. However, H5/BW was significantly higher for HF compared to LF (0.51±0.03 vs. 0.29±0.01) translating to a significantly higher fractional contribution of gluconeogenesis to plasma glucose levels (51±3% vs. 29 ±3%). In addition, HF had a tendency for higher indirect pathway contributions to glycogen synthesis compared to LF (56±3% vs. 44±3%).

Conclusion: High-fructose sugar formulations presents the liver with both fructose and glucose with the conversion of fructose to gluconeogenic precursors being relatively uncontrolled. Our data indicate that plasma glucose excursions have a higher gluconeogenic component following ingestion of a carbohydrate load with fructose/glucose proportions resembling HFCS. Nevertheless, glycemic control is maintained by healthy subjects presumably through hepatic autoregulation.

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Glucose tolerance in adolescents with obesity

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Background and aims: Prevalence of obesity in children and adolescents is growing and currently affects about 20% of the world population. Childhood obesity is associated with glucose intolerance. dyslipidemia and hypertension. The aim of the study was to assess the glucose metabolism in the adolescents with obesity.

Materials and methods: 151 patients with obesity aged 9-18 years. BMI>95pc. without any additional diseases and drugs affecting metabolic parameters were included in the study. Height. body weight. waist circumference. BMI were measured; pubertal maturity to Tanner stage criteria were assessed. HbA1c were measured and 2-hours OGTT (glucose and insulin) was performed in all children. Insulin resistance was determined by HOMA-IR. In the second stage of the study. in 46 adolescents 4-hours OGTT and Mixed Meal Tolerance Test (glucose and insulin) were performed and continuous glucose monitoring system CGM (iPro-Medtronic) were submitted to 5-6 days.

Results: Among 151 patients (66 girls. 85 boys. at the age of 13.5 ±1; 2.5 years) BMI 30 ±4.5kg/m^{2}. BMI z-score 2.1 ± 0.3. waist circumference 96.4 ±10.6cm. 13.2% (20 children) was in the prepubertal period. Based on OGTT impaired fasting glucose was found in 4 patients (2.6%), glucose intolerance in 25 patients (16.5%) and diabetes in 2 patients (1.3%). HbA1c was 5.3 ±0.3%. HOMA-IR was 3.8 ± 2.1. In 53 children (35.1%) insulin resistance was found. A positive correlation (R = 0.25; p = 0.0025) between HOMA-IR and BMI z-score was observed. Hyperinsulinemia was present in 103 patients (68.2%). Comparing 4-hours OGTT and MMTT lower glycemic values were observed at all time points after consuming a standardized meal than in the OGTT. However the mean value of insulin was statistically higher in 15 and 30 minute of the test in MMTT than in OGTT. In CGM the mean glycemic value was 101.3 mg/dl. 98.64% of glucose readings were in the range of 70-140 mg/dl. Only 0.91% of the readings were above 140 mg/dl and 0.45% of readings were below 70mg/dl. The mean glycaemia value at night was 95.9 mg/dl. A positive correlation (R = 0.36; p = 0.013) between HOMA-IR and mean of nighttime glycaemia was observed.

Conclusion: With the rise in obesity insulin resistance increases, which leads to higher glycaemia at night. Mixed meal stimulates higher insulin secretion than glucose in the first 30 minutes after ingestion.

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Impact of dietary carbohydrate composition on metabolic benefits of low protein-high carbohydrate diets

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Background and aims: Nutrition is the major modifiable factor that influences health and lifespan, but the relationship is complex and poorly understood. Recent research using Geometric Framework (GF), a state-space nutritional modelling method, showed that 'low protein-high carbohydrate' (LPHC) diets generate the best cardio-metabolic health and lifespan outcomes (Solon-Biet 2014). This seems at odds with advice to reduce intake of simple carbohydrates because there is evidence that they promote metabolic disease. However, the discrepancy may relate to the fact that the LPHC diets used starch derived *complex* carbohydrates. Therefore, we aimed to evaluate which *types* of carbohydrate contribute to the benefits of LPHC diets.

Materials and methods: Male C57BL/6 mice (n=300; 8 wk old) were fed ad libitum one of 15 isocaloric LPHC diets composed of differing percentages of low protein (5, 10 and 15%) and high carbohydrate (75, 70 and 65%), while the fat was fixed at 20%. Diets were further factored by systematically changing the sucrose-starch ratios (20/80, 35/65, 50/50, 65/35, 80/20) in their carbohydrate component. This feeding array allows examination of the impact of each individual food component (protein, sucrose and starch) and their interactions using GF methodology. Food intake and body weights were monitored fortnightly, parameters of metabolic health were assessed after 5-6 and 13-14 wks, and tissues were harvested after 18 wks on diets. For data analysis by GF, means/mouse/cage or data/mouse were used (as appropriate), using thin-plate spline procedures in R, constructed upon nutrient axes for sucrose, starch and protein across diet composition and nutrient intake spaces.

Results: GF analysis showed that mouse weights were markedly affected by protein intake and peaked on a combination of increased protein-high starch consumption. In contrast, energy intake increased on lower protein-high starch diets and declined with an increase in dietary protein and sucrose content. Water intake was negatively regulated by dietary protein and increased with sucrose ingestion. Glucose tolerance and insulin concentrations were adversely affected by protein intake, but surprisingly, moderate sucrose intake did not impair glucose homeostasis, while extreme consumption paradoxically improved insulin sensitivity due at least in part to decreased food intake on very high sucrose diets. Consistently, adiposity increased with protein and starch intake, but high sucrose consumption reduced body fat and consequently led to increased % lean mass. On the contrary, liver fat was maximum on low protein diets with no effect of sucrose intake. Circulating concentrations of triglyceride and urea increased with protein consumption but increased sucrose intake did not induce hypertriglyceridemia or hyperuremia. Similarly, weights of liver, kidney and pancreas and glucose stimulated insulin secretion from the islets increased with protein intake, but the source of dietary carbohydrate did not have remarkable effects on these measurements.

Conclusion: Our work suggests that in the context of LPHC diets, protein intake is the major determinant of the metabolic phenotype, while compared to starch, very high sucrose intake paradoxically improves parameters of metabolic health in this setting.

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Modelling postprandial absorption of complex carbohydrates

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Background and aims: We have previously developed a novel isotope dilution method to estimate complex carbohydrate metabolism in humans that exploits the natural abundance of ^{13}C contained within the ingested polysaccharide (PS). With this technique, it is possible to estimate the systemic appearance of meal carbohydrates (Ra_{meal}), a surrogate for rate of carbohydrate absorption. The aim of this analysis was: 1) to assess the reproducibility of the method, 2) to model the dynamics of complex carbohydrates vs. simple carbohydrates absorption.
Materials and methods: Sixteen healthy subjects (9M, age, 33±2 y, BMI 23±1 kg/m^2; mean±SE) were randomized into two matched groups. Each subject was studied at a Clinical Research Trials Unit on three occasions in random order: once with simple carbohydrates (glucose; G) as Jell-O (labeled with [^{13}C]glucose), and twice (to assess reproducibility) with [^{13}C]PS containing mixed meals, with either sorghum (S) or rice (R) as the complex carbohydrate. All meals had ~50g carbohydrates and similar macronutrient composition amounting to a 600 calorie mixed meal. In addition, [6-^3H]glucose was infused for 360 min to enable model independent estimation of Ra_{meal}. The model of glucose absorption assumes that the stomach is described by two compartments, representing the solid and the liquid phase, while the gut is described by one compartment. In addition the grinding rate of the stomach and the absorption rate from the gut are assumed to be constant, while gastric emptying depends on the total amount of carbohydrates in the stomach. The model estimates the amount of carbohydrates in the stomach and thus the gastric retention (GR) as percentage of the ingested carbohydrates. The time when GR reaches 50% ($T_{50\%}$) and the GR at t=60 min (GR_{60min}) were calculated. Reproducibility of the results was assessed using intra-class correlation (ICC) analyses. ICC>0.9 was considered to be a robust representation of reproducibility. Differences among model parameters were assessed with the Wilcoxon signed rank test ($p<0.05$ was considered statistically significant).
Results: Data suggests that rice is more reproducible than sorghum for iAUC of Ra_{meal}: ICC^R=0.93 (95% CI: 0.66-0.99) vs ICC^S=0.66 (95% CI: 0.66-0.87). Average GR profiles are reported in Figure 1. $T_{50\%}$ and GR_{60min} were both significantly higher in R and S than in G: $T_{50\%}{}^R$=122±13 min (visit 2: 108±7 min) vs. $T_{50\%}{}^G$=42±10 min (p=0.0156); $T_{50\%}{}^S$=118±7 min (visit 2: 110±7 min) vs. $T_{50\%}{}^G$=43±7 min (p=0.0156); $GR_{60min}{}^R$=72±4% (visit 2: 71±3%) vs. $GR_{60min}{}^G$=39±7% (p=0.0156); $GR_{60min}{}^S$=76±2% (visit 2: 70±3%) vs. $GR_{60min}{}^G$=41±4% (p=0.0156). Finally, the model does not highlight any difference in gut absorption.
Conclusion: Rice provided more reproducible results for Ra_{meal} than sorghum and is thus more suitable for in future clinical research studies on PS metabolism. The higher GR estimated with complex vs. simple carbohydrates is likely responsible for the delayed Ra_{meal}.

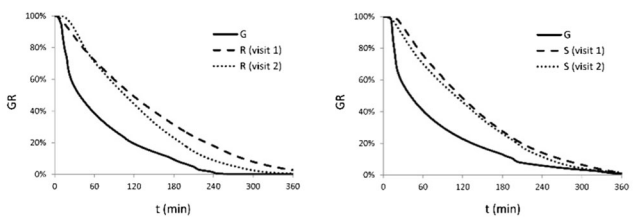

Figure 1: Left panel: Gastric Retention (GR) as percentage of the ingested dose for G (solid) and R (visit 1, dashed) and R (visit 2, dotted line). Right panel: Gastric Retention (GR) as percentage of the ingested dose for G (solid) and S (visit 1, dashed) and S (visit 2, dotted line).

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Toxicological impact of high fructose intake on gut microbiota and liver/intestine integrity: Any differences between solid and liquid formulations?

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Background and aims: We previously demonstrated the deleterious effects of fructose feeding on liver through production of advanced glycation end products (AGEs) and the induction of hepatic steatosis and inflammation. Moreover, it has been reported that high fructose intake alters microbiota composition, resulting in reduced bacterial diversity and altered expression of genes involved in specific metabolic pathways. A recent paper demonstrated that liquid high-sugar compared to solid high-sugar diets differentially modulate intestinal sugar transporters and hormone expression. To date, however, the peculiar effects of fructose intake in different forms, liquid or solid, on intestinal integrity and microbiota, and hepatic outcomes, have never been investigated.
Materials and methods: For this aim, C57 mice were fed a standard diet (SD) plus water to drink, a standard diet plus 60% fructose syrup (L-Fr), or a 60% fructose solid diet plus water (S-Fr), for 12 weeks. At the end of protocol, analysis on liver lipogenesis, fibrosis, and inflammation and on intestinal absorption, AGEs accumulation, and integrity were performed by western blotting, biochemical and histological analysis. Gut microbiota population has been characterized by metagenomic sequencing.
Results: L-Fr intake induced higher levels of hepatosteatosis (liver TG: +80% vs. SD, +33% vs. S-Fr, $p<0.05$), with greater activation of the lipogenic SCAP/SREBP signaling, and of markers of fibrosis, than the S-Fr. In contrast, S-Fr evoked a stronger local AGEs accumulation, RAGE expression, and barrier injury in the ileum intestinal mucosa, leading to higher concentration of LPS in the portal plasma (+300% vs. SD, +210% vs. L-Fr, $p<0.05$). This effect was associated to a stronger activation of the LPS-dependent pro-inflammatory pathway NLRP3 inflammasome in the liver of S-Fr mice than of L-Fr mice. Interestingly, the local accumulation of fructose in the intestine led to alterations of the gut microbiota composition depending on the fructose formulation, with increase in the saccharides metabolizing *Lactobacillus* genus in the L-Fr, and increased colonization by populations related to intestinal inflammation and barrier disruption, such as *Clostridium*, in the S-Fr group.
Conclusion: These results suggest that consumption of fructose under different forms, liquid or solid, has a different impact on intestinal mucosa, thus differently affecting liver homeostasis. We hypothesize that the liquid fructose is more rapidly absorbed by intestine and metabolized by the liver to produce considerable amounts of lipids. In contrast, the solid form might be slowly absorbed by enterocytes producing glycated proteins and affecting barrier integrity, with developing of systemic inflammation. Such alterations of intestinal integrity and microbial population, also found in diabetic and obese individuals, might predispose to the development of chronic metabolic and inflammatory diseases.
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Disclosure: **R. Mastrocola:** None.

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Metabolic profile of the women after gestational diabetes

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Background and aims: The data from the many studies indicate that GDM is associated with an increased risk of metabolic disorders in later life. The aim of our study was to evaluate the incidence of metabolic disturbances after GDM and to analyze their determinants.
Materials and methods: 144 women with GDM treated in Outpatient Department of Diabetes in Lodz between 2013 and 2016 were initially included in the study. Patients were examined toward metabolic disturbances at 3 and 18 months after delivery. Anthropometric data including BMI, waist and hips circumference, suprailiac, subscapula and biceps skinfolds were analyzed. Body composition was examined with the use of *Tanita* analyzer. Glucose and insulin concentrations in oral glucose tolerance test (OGTT) were evaluated, and the indices of insulin resistance and beta cell function were calculated based on HOMA2 IR, HOMA2 %S, HOMA2 B and QUICKI methods. Haemoglobin A_{1c}, C-reactive protein (CRP), total, LDL and HDL cholesterol and triglycerides concentrations were also measured.
Results: 95 and 68 women attended follow-up visit at 3 and 18 months after delivery, respectively. Normal OGTT result was shown in 82 and 46 women, impaired fasting glucose (IFG) was detected in 6 and 15 patients, impaired glucose tolerance (IGT) in 9 and 5 patients, and diabetes mellitus (DM) was diagnosed in 1 and 1 subjects after 3 and 18 months postpartum, respectively. 3 months after delivery, in a group of healthy women, compared to a group with any glucose disturbances (IFG and/or IGT or DM), significantly lower waist and hip circumference, thinner biceps, suprailiac and subscapula skinfold, as well as lower body weight (all $p < 0.001$), BMI ($p < 0.01$), fat percent ($p < 0.05$), visceral fat ($p < 0.01$) and index of obesity ($p< 0.001$) were noted. Lower CRP ($p < 0.05$) and triglycerides concentration ($p < 0.01$), as well as lower HOMA2 IR ($p < 0.01$), higher HOMA2% S ($p < 0.01$) and higher QUICKI ($p < 0.05$) were also observed in this group. Other parameters did not differ significantly between the groups ($p > 0.05$). 18 months after delivery, in a group of healthy subjects, compared to a group with abnormal OGTT results, significantly lower waist circumference ($p < 0.05$) and fat percent ($p < 0.005$) were shown. Other parameters did not differ significantly between the groups. A significant correlation between the presence of any disturbances in OGTT and waist circumference ($r = 0.266$, $p < 0.05$) and fat percent ($r = 0.293$, $p < 0.05$) was noted.
Conclusion: In the women after GDM less favorable metabolic profile and higher CRP concentration are connected with persistent carbohydrate disturbances shortly after delivery. 18 months postpartum however, most of the differences in the metabolic profile between the studied groups disappeared, and the metabolic disturbances that persisted postpartum in a longer perspective correlated with waist circumferenceand the fat percent only.
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Leptin therapy alters the hepatic transcriptome, the proteome, and the metabolome to suppress amino acid utilisation in diabetic rodents
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Background and aims: Leptin lowers blood glucose levels in diabetic rodents and the typically observed hypoglycemia with fasting suggests suppressed glucose production may be the mechanism. Since the liver is a major site for glucose production, we used an un-biased multi-omics approach to characterize leptin-induced changes in liver.
Materials and methods: We administered leptin or vehicle by pumps to streptozotocin (STZ)-diabetic mice and collected the liver on day 4 for transcriptomics analysis by RNA-sequencing, and proteomics and metabolomics analysis by mass spectrometry. Enrichment analysis independently pointed towards suppressed utilization of amino acids. Thus, we examined amino acid utilization in leptin-treated STZ-mice and non-diabetic controls by performing alanine tolerance tests and pyruvate tolerance tests as a comparison. To further assess the role of suppressed alanine metabolism, we overexpressed the gene responsible for alanine catabolism, glutamic pyruvate transaminase (*Gpt*), in the liver of STZ-diabetic mice by hydrodynamic gene delivery then tested the efficacy of leptin therapy on day 4 post plasmid injection.
Results: Leptin treatment downregulated 993 genes in the liver, including genes involved in amino acid catabolism. Proteomics similarly revealed that proteins involved in amino acid catabolism were downregulated. Metabolites involved in protein biosynthesis and amino acid metabolism were upregulated, suggesting a global build up of amino acids in the liver of leptin-treated STZ-mice. To assess amino acid utilization, STZ-mice were treated with leptin, which normalized blood glucose levels (22.1 ±0.5 vs 6.7±0.7 mM day -1 and day 3), then STZ-leptin and non-diabetic groups were fasted to the point of mild hypoglycemia and injected with alanine or pyruvate on day 4. Alanine did not increase blood glucose levels in leptin-treated STZ-mice (6.0±0.5 vs 6.4±0.4 mM at 0 and 30 minutes), and hypoglycemia worsened (3.9±0.6 mM at 90 minutes). In contrast, pyruvate increased blood glucose levels in leptin-treated STZ-mice (6.5±0.5 vs 9.9±1.8 mM at 0 and 30 minutes). In non-diabetic controls with mild hypoglycemia, blood glucose levels were increased with both alanine (4.7±0.1 vs 6.9±0.4 mM at 0 and 30 minutes) and pyruvate (4.5±0.2 vs 11.1±0.3 mM at 0 and 30 minutes). *Gpt* transcript levels were downregulated by ~2 fold in leptin-treated STZ-mice compared to diabetic controls on day 4 ($p<0.0001$). Administration of plasmid encoding *Gpt* to STZ-mice for overexpression of GPT in the liver did not alter blood glucose (24.1±0.7 vs 24.3±0.7 mM on day 3). Leptin similarly lowered blood glucose levels in mice with hepatic *Gpt* overexpression (10.6±2.5 mM) and controls (9.0±1.1 mM) by day 5 post leptin therapy.
Conclusion: Leptin alters the hepatic transcriptome, the proteome, and the metabolome, to suppress amino acid catabolism. Leptin-treated STZ-mice cannot utilize alanine to produce glucose but their ability to utilize pyruvate, a product of alanine breakdown, remains intact. Overexpression of *Gpt* is insufficient to block leptin action, suggesting that leptin-induced downregulation of *Gpt* alone does not drive the anti-diabetic actions of leptin.
Supported by: CIHR
Disclosure: **M.M. Kwon:** None.

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ERO1Lβ is a key regulator of the endoplasmic reticulum stress resolution in pancreatic beta cells
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Background and aims: Type 2 diabetes (T2D) is characterized by pancreatic beta-cell failure that lead to uncontrolled glucose homeostasis. A relevant contribution to beta-cell loss in type 2 diabetes is endoplasmic reticulum (ER) stress induced by accumulation of misfolded proteins. The unfolded protein response (UPR) is an adaptive response inducing attenuation of protein translation and degradation of misfolded protein in order to alleviate ER stress and prevent beta-cell death. ERO1LB, a disulfide oxidoreductase whose expression is highly enriched in the ER of pancreatic beta-cells, contributes to correct disulfide bonds formation and proper insulin folding. Here we investigated the capacity of ERO1LB to protect against palmitate-induced ER stress and its ability to rescue proper beta-cell function. Furthermore, we aimed to characterize the role of ERO1LB in the UPR signaling pathway response to understand the potential as a therapeutic target for the treatment of T2D.

Materials and methods: To simulate the acute and chronic effect of ER-stress in beta-cells, we examined short (2 days) and longterm (7 days) effects of palmitate-induced ER stress on ERO1LB expression and compared it to classical inducers of ER stress. We further characterized the beta-cell response to a glucose challenge (GSIS) after short and long palmitate exposure, and correlated these results with the activation of UPR, monitored by RT-qPCR, Western Blot and ImmunoFluorescence analysis. Folding capacity assays and measurements of ER Ca^{2+} levels were used to gain insight into the functional and mechanicistic role of ERO1LB protein in a mouse beta-cell line (MIN6). By using overexpression approaches we investigate the ability of ERO1LB to alleviate beta-cells from ER stress induced by glucolipotoxicity condition.
Results: We show that beta cells exposed to chronic lipotoxicity, a condition often present in obese T2D patients with chronically elevated circulating fatty acid levels, induces ER-stress and activates strong UPR response. In our ER stress model we observed a significant increased proinsulin/insulin ratio only after longterm palmitate treatment, as well as activation of the three different arms of the UPR response in a time-dependent manner (PERK, then ATF6 and IRE1α). Decreased ER Ca^{2+} levels and protein folding capacity were also observed in presence of palmitate. ERO1LB is upregulated by short palmitate exposure whereas long palmitate treatment induces a decrease in ERO1LB levels, similar to what has been observed in islets from T2D. Overexpression of ERO1LB in MIN6 cells alleviated the induction of ER stress, and restored appropriate proinsulin folding. This suggests ERO1LB is important for maintaining a sustained insulin folding capacity.
Conclusion: Our data showes that ERO1LB is able to prevent lipotoxicity induced ER-stress by interfering with UPR activation, and suggest that ERO1LB has the ability to rescue beta-cell from ER stress induced loss of function. These findings confirm an important role for ERO1LB in maintaining proper insulin folding and beta-cell function. Thus, ERO1LB is a potential candidate target for novel treatments for T2D.
Disclosure: **D. Amadio:** None.

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Critical assessment of postchallenge hyperglycaemia in subjects with low body weight

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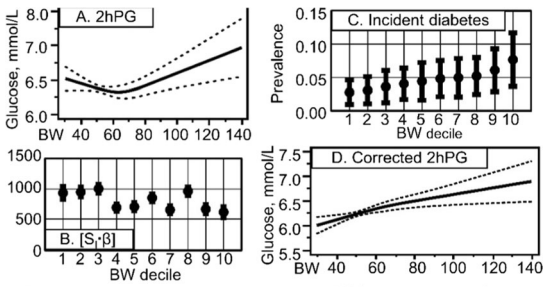
Background and aims: Post-challenge hyperglycaemia (PCH) in the 75-g OGTT due to low glucose volume (G_V) is theoretically possible in subjects with low body weight (LBW) because G_V is a function of BW. The present study aimed to critically evaluate this issue.
Materials and methods: Data from 11,411 non-diabetic subjects (Matsumoto cohort; male-to-female ratio, 7,077:4,334; mean age, 52.6 years; BW, 63.3 kg) were analysed. Among these subjects, 5,282 were followed up for a mean of 5.3 years. All participants underwent a standard 75-g OGTT and HbA1c evaluation, and glucose tolerance was determined using the ADA definition. In an independent group of non-diabetic subjects (Iida cohort; n = 1,537; male-to-female ratio, 950:587; mean age, 52.8 years; BW, 62.7 kg), insulin sensitivity (S_I, 1/HOMA-IR), β (Stumvoll 1st phase), and the index of whole body insulin action (a product of the two variables) were determined. Considering BW or BW decile as an explanatory variable, multiple correlation analysis, analysis of covariance, or logistic regression analysis was performed with adjustment for age, sex, and percentage body fat. The possible non-linear effect of BW was evaluated using cubic spline fitting. In subjects with BW ≤ 59 kg, correction of 2-h plasma glucose (2hPG) in proportion to reduced estimated extracellular water (ECW, a surrogate of G_V) was attempted, i.e., $2hPG_{corr}$ = fasting plasma glucose (FPG) + (ECF/16.1 [males] or 15.1 [females]) · δPG_{2h}. ECF denotes the estimated ECW in a given individual, and 16.1 and 15.1 were ECF values of males and females, respectively, with BW of 59 kg.
Results: BW, across the entire range, was positively correlated with FPG ($p < 0.01$). In contrast, BW was correlated in a skewed J-shape fashion with all post-challenge glucose values, such as 1hPG, 2hPG (Panel A), δPG_{1h}, and δPG_{2h}, with inflections at around 60 kg (p for non-linearity <0.01, for each). The prevalence of impaired glucose tolerance (IGT) as a function of BW was also J-shaped. S_I significantly increased as BW decreased (p for trend <0.01), and there was no significant BW-related trend for β. As a result, insulin action ($S_I \cdot \beta$) tended to be high with LBW (Panel B, p for trend = 0.09). On follow-up, diabetes developed less frequently in subjects with LBW (Panel C). Analysis in IGT subgroup showed essentially the same results. Correcting 2hPG mitigated the J-shape correlation between BW and 2hPG (Panel D).
Conclusion: PCH against BW showed a J-shape distribution. However, insulin action was not impaired in subjects with increased prevalence of PCH at the lower end of BW. Of note, despite a high prevalence of PCH, the future development of diabetes was less frequent in LBW subjects than in their heavier counterparts. Correcting 2hPG on the basis of the estimated ECW mitigated the J-shape for 2hPG. PCH, especially IGT, in subjects with BW ≤ 59 kg may be, in part, due to reduced G_V rather than impaired glucose metabolism.



Panels A, B and D, estimated means and 95%CIs at the mean of covariates;Panel C, mean and SD of p obtained by logistic regression.

Disclosure: **T. Sakuma:** None.

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The lipid peroxidation by-product 4-hydroxy-2-hexenal impairs insulin sensitivity in skeletal muscle

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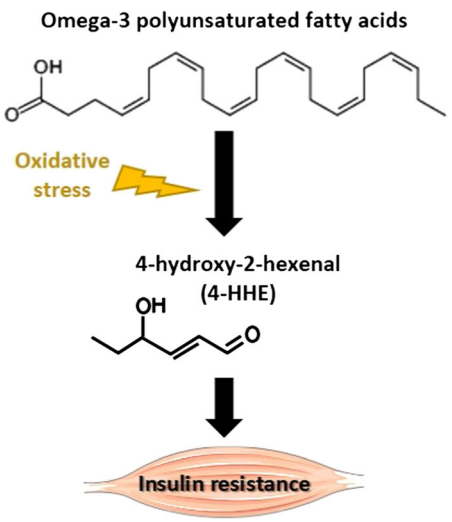
Background and aims: Oxidative stress is involved in the pathophysiology of many chronic diseases and in particular contributes to the development of insulin resistance and its progression towards type 2 diabetes. The peroxidation of cell membrane phospholipids associated with oxidative stress produces deleterious reactive species, including hydroxy-alkenals. Peroxidation of omega-6 polyunsaturated fatty acids leads to the production of 4-hydroxy-2-nonenal (4-HNE), while 4-hydroxy-2-hexenal (4-HHE) is generated during the oxidation of omega-3 polyunsaturated fatty acids. These lipid aldehydes exhibit potent electrophilic properties making them able to make covalent adducts with amino phospholipids such as phosphatidylethanolamine, proteins and nucleotides. Because of their relative stability and high reactivity, these aldehydes are thought to interfere with crucial physiological processes such as cell cycle, apoptosis or metabolic pathways. Only a handful of studies have shown activation of stress signalling pathways by 4-HHE, but the data about its pathophysiological effects remains scarce. Especially, the putative role of 4-HHE in the development of insulin resistance has not been investigated. The aim of the present study was to investigate the effect of 4-HHE in the development of insulin resistance.
Materials and methods: 4-HHE concentration was measured in plasma from humans and rats by GC-MS. Insulin resistance was estimated in healthy rats using hyperinsulinemic euglycemic clamps. In L6 muscle cells and 3T3 adipocytes, glucose uptake was measured using 2-deoxy-D-glucose and signalling pathways by western blotting. Intracellular glutathione was measured using fluorimetric assay kit and boosted using 3H-1,2-dithiole-3-thione (D3T).
Results: In T2D subjects, plasma 4-HHE was twice the level of healthy volunteers. Circulating levels of 4-HHE were elevated in Zucker Fatty diabetic rats. During hyperinsulinemic euglycemic clamps in rats, acute intravenous injection of 4-HHE significantly altered whole body insulin sensitivity and decreased glucose infusion rate. In vitro, 4-HHE impaired insulin-stimulated glucose uptake and signaling (PKB/Akt and IRS1) in L6 muscle cells. 4-HHE induced carbonylation of cell proteins and reduced glutathione concentration. Increasing intracellular glutathione pools using D3T prevented 4-HHE-induced carbonyl stress and insulin resistance.
Conclusion: These results demonstrate that 4-HHE is produced under diabetic conditions and blunts insulin action. 4-HHE may therefore play a causal role in the pathophysiology of T2D and lipid peroxidation might constitute a potential therapeutic target to taper oxidative stress-induced insulin resistance.



Supported by: Johanssons foundation, Sweden
Disclosure: **L. Sardón Puig:** None.

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Association of circulating betatrophin levels with insulin resistance in polycystic ovary syndrome: cross-sectional and interventional studies

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Background and aims: Betatrophinis a secreted protein of 198 amino acids that is highly conserved in all mammalian species. Polycystic ovary syndrome (PCOS) is characterized by heterogeneity in phenotypic manifestations mainly related to reproductive and hormone aberrations and metabolic disturbances. IR is the most significant and highly prevalent parameter among PCOS women, while IR is associated with T2DM and obesity. Recent studies have demonstrated the relationship between betatrophin and insulin resistance (IR) inT2DM, but the reports were inconsistent. Furthermore, the physiological role of betatrophin remains poorly understood. The aim of this study was to investigate the association of betatrophin with IR in PCOS women and explore the physiological role of betatrophin*in vivo* and *in vitro*.
Materials and methods: Plasma betatrophin levels were measured with ELISA. Insulin sensitivity was assessed with EHC. Gene expressions at mRNA and protein levels were determined with qRT-PCR and Western blotting. Influences of insulin, metformin, rosiglitazone and over- or knockdown-expression of betatrophin were analyzed ex vivo.
Results: PCOS women had higher betatrophin levels compared with the controls ($P<0.01$). Circulating betatrophin was positively correlated with BMI, WHR, TG, TC, LDL-C, AUC glucose, AUC insulin, LH, FAI and HOMA-IR but negatively with M-value. Metformin treatment in newly diagnosed PCOS women led to a reduction of betatrophin levels. Insulin stimulation in hepatocytes increased betatrophin expression, but not promoted its secretion. Metformin or rosiglitazone led to a reduction of betatrophin expression in insulin-stimulated hepatocytes. In hepatocytes/macrophages co-culture systems, betatrophin expressions were significantly increased, whereas this increase was eliminated by rosiglitazone. In hepatocytes, overexpression and knockdown of betatrophin decreased and increased insulin-stimulated InsR, AKT and IRS-1 phosphorylation respectively. Serum from metformin-treated PCOS women decreased betatrophin expression and reinforced insulin signals.
Conclusion: The present study provide the first evidence indicating a significant increase of circulating betatrophin in untreated PCOS patients and different effects of elevated insulin on betatrophin*in vivo* or *in vitro*. We also present novel data suggesting that metformin or rosiglitazone treatment, possibly *via* a direct effect and/or an indirect effect of improved insulin sensitivity, decreases circulating levels and expression of betatrophin *in vivo* or *in vitro*. In addition, over-expression and knockdown of betatrophin impair and improve insulin signaling in hepatocytes, respectively. Finally, serum from metformin-treated PCOS women decreases betatrophin expression and reinforces insulin signal transduction in hepatocytes. Therefore, betatrophin is a useful marker of IR in PCOS.
Clinical Trial Registration Number: ChiCTR-OOC-14005314
Supported by: NSFC(81570752, 8167075)
Disclosure: **M. Yang:** None.

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Insulin resistance in hepatitis C virus infection: relative contribution from liver vs extrahepatic sites

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Background and aims: Chronic hepatitis C (CHC) has been associated with the development of metabolic disorders, such as insulin resistance (IR). Albeit hepatitis C virus (HCV) infects only hepatocytes, peripheral IR is also observed in HCV infected patients. In the present study, we are interested to (1) identify the putative liver-derived inducers of IR in extrahepatic tissues (i.e. muscle and adipose tissue), and (2) elucidate the underlying molecular mechanisms.
Materials and methods: CHC genotype 3a patients were treated with an interferon-free regimen (ledipasvir/sofosbuvir combined with ribavirin). These patients underwent a 2-step euglycemic hyperinsulinemic clamp with tracers, together with indirect calorimetry measurement, to measure IR at start and after 6 weeks of antiviral therapy. Blood and adipose and muscle tissue biopsy samples were collected at the same time points. *In vitro*, human primary adipocytes were treated with the collected sera. Insulin signaling and lipid/glucose metabolism were investigated by immunoblot and RT-PCR. Insulin-stimulated glucose uptake was assessed by incubating cells with [1,2-3H(N)]-2-deoxy-D-glucose.
Results: As of today, 13 patients have been enrolled, and complete viral suppression was achieved in 10/10 who received 6 weeks of therapy. Clamp analyses have been completed in 6 patients: all exhibited a significant improvement of the peripheral insulin sensitivity (p=0.0004), whereas no difference was observed in insulin-mediated lipolysis suppression, as measured by non-esterified fatty acid levels. Treatment of adipocytes with sera from virally-suppressed patients increased the insulin ability to promote glucose uptake and to induce Akt phosphorylation compared to exposure to sera collected before treatment. No difference in the expression of lipid metabolism genes was observed.
Conclusion: Taken together, these data indicate that HCV clearance might result into an improved peripheral insulin sensitivity. Clarification of the role of skeletal muscle in the systemic IR, and identification of liver-derived circulating factors potentially involved in the regulation of peripheral insulin sensitivity (metabolome analysis) are underway.
DG and GG have equally contributed to the work
Clinical Trial Registration Number: 15-063
Supported by: FNS, GILEAD, Fondation pour la Recherche sur le Diabète
Disclosure: **D. Gomes:** Grants; Gilead Sciences.

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Type 2 diabetes prevention through hormone sensitive lipase inhibition: elongase ELOVL6 at the center of attention

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Background and aims: Hormone sensitive lipase (HSL) is an enzyme expressed in adipose tissue where it hydrolyzes triglycerides into fatty acids. We previously demonstrated the protective effect of HSL inhibition on the development of insulin resistance in obese HSL haploinsufficient mice. However, the mechanisms involved in the improvement of insulin sensitivity were still unclear. In this study, we demonstrate the prominent role of adipocyte de novo lipogenesis (DNL i.e glucose-derived fatty acid synthesis) and of the Elongation of Very-Long-Chain Fatty Acids protein 6 (ELOVL6) in the beneficial effect induced by HSL inhibition.
Materials and methods: Human hMADS adipocytes (human multipotent adipose-derived stem cells) were used for in vitro studies. Murine models of HSL, elongase ELOVL6 and Carbohydrate Responsive Element Binding Protein (ChREBP) transcription factor deficiency were employed for in vivo studies. Human data were obtained from patients with differing obese and metabolic status and from morbidly obese subjects undergoing a bariatric surgery.
Results: Robust induction of DNL was observed in parallel with better activation of insulin signaling in human adipocytes with siRNA-induced HSL knockdown. A strong elevation of the expression and activity of elongase ELOVL6, one of the enzymes of DNL, was measured in vitro and in vivo during genetic and pharmacologic HSL inhibition. Increased ELOVL6 activity was responsible for a shift in fatty acid composition leading to an accumulation of the C18-fatty acid oleate in triglycerides and phospholipids. Concomitant knockdown of HSL and ELOVL6 reversed oleate accumulation and the improvement of insulin signaling observed in HSL-inhibited adipocytes. The beneficial effect of ELOVL6 was triggered by an increased oleate content in phospholipids and a higher plasma membrane fluidity. In human subcutaneous adipose tissue, ELOVL6 gene expression was reduced during insulin resistance and restored after a bariatric surgery-induced weight loss, showing a close association between ELOVL6 and insulin sensitivity. In vitro and in vivo, the DNL-transcriptional factor ChREBP exerts a strong and specific control on ELOVL6 expression. Dual HSL-ChREBP inhibition mirrored ELOVL6 deletion on fatty acid profile and insulin signaling.
Conclusion: In this study, we demontrate that HSL inhibition protects from insulin resistance. HSL inhibition promotes ChREBP activity and induces ELOVL6 which modulation of plasma membrane fatty acid composition and fluidity improves insulin signaling.
Disclosure: **P. Morigny:** None.

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Effect of insulin on renal maximum glucose transport capacity in healthy volunteers and patients with type 2 diabetes

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Background and aims: Most studies, though not all, indicate that the maximum glucose transport capacity of the proximal tubule (Tm_G) is increased in patients with type 2 diabetes (T2D). Furthermore, one old study from 1951 suggested that insulinization may reduce Tm_G. In contrast, in human embryonic kidney 293T cells insulin increases the activity of transfected human SGLT2. To assess these issues *de novo*, we examined the effect of physiological hyperinsulinaemia on Tm_G and urinary glucose excretion (UGE) in healthy subjects (NGT) and T2D patients under steady-state conditions of plasma glucose and insulin concentrations.
Materials and methods: Nine T2D (5F/4M; 55±5 years, mean±SD; BMI=28.1±5.3 kg/m^2; HbA_{1c}=7.6±0.6 %) and 7 NGT participants (1F/6M; 36±8 years; BMI=24.9±4.7 kg/m^2) received a 7-hour constant somatostatin infusion (400 μg/h) and a variable glucose infusion designed to achieve steady-state glycaemias of ~22 mmol/L in 1 hour and to maintain this level for the next 3 hours (CT period). Then, a constant insulin infusion (1 mU·min^{-1}·kg^{-1}) was started and maintained for an additional 3 hours (INS period) while clamping glycaemia at CT levels with the use of an *ad hoc* algorithm. Urines were collected separately for the two study periods for the measurement of glucose and creatinine.

Results: Plasma glucose plateaus were similar across group and study period (CT=21.7±0.2 *vs* INS=21.5±5.2 mmol/L in T2D; 22.8±2.1 *vs* 23.0 ±3.6 mmol/L in NGT, all *p*=ns). Exogenous glucose infusion rate (GIR) was similar in T2D and NGT during the CT period (30±7 *vs* 30±15 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; *p*=ns); during the INS period, GIR rose much higher in NGT than T2D (128±20 *vs* 69±29 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, *p*<0.0001). Creatinine clearance was similar between groups and study periods (144±25 and 140±31 $ml \cdot min^{-1}$, CT *vs* INS in T2D; 158±39 and 169 ±28, CT *vs* INS in NGT, all *p*=ns). During the CT period, UGE was significantly lower in T2D than in NGT (486 [309] *vs* 706 [347] $\mu mol \cdot min^{-1}$, median and [IQR], *p*=0.03) whereas fractional glucose excretion (FE_G) was similar (17±5% and 22±7%, respectively; *p*=ns). During the INS period, both UGE and FE_G rose above CT levels in NGT (to 1,155 [547] $\mu mol \cdot min^{-1}$ and 32±12%, respectively, *p*<0.03 for both *vs* CT), whereas they were unchanged in T2D (at 531 [319] $\mu mol \cdot min^{-1}$ and 18±6%, respectively, *p*=ns). In the pooled data of T2D and NGT participants, FE_G was positively associated with GIR during the INS period (*r*=0.63, *p*=0.02).
Conclusion: Acute physiological hyperinsulinaemia during steady-state hyperglycaemia increases urinary glucose excretion in NGT subjects. This insulin effect is impaired in patients with type 2 diabetes, and may be related to insulin resistance.
Disclosure: **E. Muscelli:** None.

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Three days low-carbohydrate/high-fat diet increases insulin clearance in non-obese healthy men

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Background and aims: It has been reported that 1-wk overfeeding impairs insulin sensitivity and elevates insulin level, and the latter is partly induced by impaired metabolic clearance rate of insulin (MCRI). Thus, MCRI is considered as a regulatory factor to maintain euglycemia during short term dietary change. However, the effect of low-carbohydrate/high-fat (LCHF) diet on MCRI has not been elucidated.
Materials and methods: To clarify the effect of 3-day LCHF diet, we studied 48 non-obese healthy men (BMI; 22.6±2.5 kg/m^2). Each subject consumed a 3-day control diet, which was followed by a 3-day eucaloric LCHF diet (20% carbohydrate, 20% protein, 60% fat). After the completion of both diet protocols, we performed hyperinsulinemic euglycemic clamp (insulin infusion rate (IIR) = 100 mU/m^2 per min) to evaluate glucose infusion rate (GIR) and MCRI, calculated as a ratio of IIR to steady state serum insulin (SS_{SI}).
Results: The 3-day LCHF diet increased MCRI (497.4±71.4 ml/min per m^2 to 540.1±85.0 ml/min per m^2, P < 0.05); however, the individual changes were highly variable. To further investigate the role of MCRI, we divided the subject into high-responder (HR) and low-responder (LR) based on the median %change of MCRI by LCHF diet. At baseline, HR group showed higher GIR level compared with LR group (13.0±1.9 vs. 11.8±2.0 mg/kg FFM per min, P<0.05), while MCRI and SS_{SI} levels were comparable. After LCHF diet, MCRI was increased (481.9±58.8 to 582.5±90.8 ml/min per m^2, P<0.001) and SS_{SI} was decreased (210.2 ±23.3 μU/mL to 175.1±23.5 μU/mL, P<0.001) in HR group, but those were not altered in LR group. In parallel with decreased SS_{SI}, GIR was decreased in HR group (from 13.0±1.9 to 11.7±2.4 mg/kg FFM per min, P=0.003) after LCHF diet, but not in LR group.
Conclusion: In conclusion, MCRI was increased after 3-day LCHF diet; however, the effect was highly variable. The decreased GIR after LCHF diet in HR group may be partly due to decreased SS_{SI} and contribute to maintain euglycemia during low-carbohydrate availability in insulin sensitive subjects.
Disclosure: **R. Suzuki:** None.

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Effect of liver transplantation on beta cell function and insulin sensitivity and clearance in cirrhotic patients. A 2 years-FU study

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Background and aims: Abnormalities of glucose (G) homeostasis are common in cirrhotic patients and may improve after liver transplantation (LT). Aim of the study was to assess the impact of LT on beta cell function (BCF) and insulin (I) sensitivity/clearance.
Materials and methods: 89 cirrhotic patients were studied before and 3, 6, 12, 24 months after LT by a frequently sampled OGTT, which was analyzed by well established mathematical models and provided 4 key outputs: 1. derivative control (DC: I secreted in response to the rate of plasma G increase; units: $[pmol \cdot m^{-2}$ BSA$]/[mM \cdot min^{-1}]$) and 2. proportional control (PC: the stimulus-response curve linking G per se to I secretion rate) of BCF from the G/C-peptide curves; 3. OGIS (units: $ml \cdot min^{-1} \cdot m^{-2}$ BSA), a robust index of I sensitivity, from the G/I curves; 4. I Clearance (units: $L \cdot min^{-1} \cdot m^{-2}$ BSA) from the combined analysis of G/C-peptide/I curves
Results: After LT, 26 patients recovered from DM (regressors, R), 15 did not (non-regressors, NR), 4 developed DM, and 44 stayed non-diabetic. Before LT, R group was similar to NR group, but for higher I sensitivity (OGIS 373.65±91.85 VS 316.51±82.82, p=0.048). In all groups I clearance increased (p<0.03) from 1.3±0.2 (before LT) to 1.9±0.1, 1.8±0.1, 1.7 ±0.2 and 1.6±0.1 before and 3, 6, 12, 24 months after LT, respectively. In the R group, the trajectories over time of I sensitivity (from 367±92 to 435 ±62 to 432±73 to 423±54 to 449±62) and DC of BCF (from 522±142 to 965±282 to 1207±238 to 1572±360 to 1309±287) showed significant improvements in the R group only (p=0.0001 and p=0.001, respectively). A declining trajectory over time of PC of BCF was detected in the NR group (p<0.05-0.01).
Conclusion: LT brings about a generalized increase in I clearance and is associated to regression of DM in ~30% of patients. Our data ascribe this beneficial change in G homeostasis to improvements in both I sensitivity and specific facets of BCF
Clinical Trial Registration Number: NCT02038517
Disclosure: **V. Grancini:** None.

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Plasma soluble leptin receptor predicts insulin sensitivity in humans

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Background and aims: Soluble leptin receptor (sOb-R) concentration is inversely associated with risk of type 2 diabetes (T2D) and gestational diabetes (GDM) in humans, but little is known about the underlying mechanisms. Both T2D and GDM are characterized by insulin resistance in combination with beta-cell dysfunction. Insulin resistance and leptin resistance usually appear simultaneously, and insulin and leptin signaling share many of the same signaling pathways. sOb-R is formed by ectodomain shedding of membrane-bound leptin receptors, and the sOb-R concentration may reflect leptin sensitivity. The aim of this study was to explore the association between sOb-R and insulin sensitivity.
Materials and methods: MyoGlu was a clinical trial of 26 sedentary men (13 with impaired glucose metabolism [IGM] and 13 controls) aged

40-65 years, showing that a 12-week exercise intervention increased insulin sensitivity by 30%. Insulin sensitivity was measured as the total glucose infusion rate (GIR) during a hyperinsulinemic euglycemic clamp. Plasma sOb-R and leptin were measured with ELISA kits. Global gene expression was quantified using high throughput mRNA sequencing from muscle and adipose tissue biopsies. Pathway analyses were performed based on correlations between sOb-R and gene expression in muscle and adipose tissue.

Results: At baseline, IGM men had higher total body fat (mean ± SD; 14.2 ± 0.9 vs. 7.8 ± 0.6 L, p < 0.001), lower GIR (4.2 ± 0.5 vs. 7.6±0.4 mg/kg/min, p < 0.001) and lower sOb-R (4.4 ± 0.2 vs. 5.6 ± 0.4 ng/mL, p = 0.016) than controls. Baseline sOb-R was strongly correlated with baseline GIR (linear regression coefficient β [95 % CI]: 1.19 [0.57-1.82] mg/kg/min per increment increase in sOb-R, p= 0.001). This association was weakened but persisted after adjustment for age, IGM status, total body fat and plasma leptin concentration (0.67 [0.09-1.24], p = 0.026).In contrast to insulin sensitivity, sOb-R did not change following the intervention (5.0 ± 1.3 vs 5.1 ± 1.2, p = 0.20). However, baseline sOb-R was positively associated with change in GIR (Figure 1). The association was strengthened after adjustment for IGM status, age, baseline GIR, total body fat and plasma leptin concentration (0.75 [0.17-1.33] mg/kg/min, p = 0.014). In pathway analyses of both adipose tissue and skeletal muscle, sOb-R was positively correlated with increased metabolism (e.g. oxidative phosphorylation, branched chain amino acid degradation and PPAR signaling) and negatively correlated with inflammatory pathways (e.g. chemokine, B and T cell receptor signaling pathways).

Conclusion: Plasma sOb-R was positively correlated with insulin sensitivity, and predicted change in GIR following a 12-week exercise intervention, independently of covariates. High plasma sOb-R was correlated with upregulation of genes related to increased metabolism. These findings may partly explain the observed inverse association between sOb-R and risk of GDM and T2D.

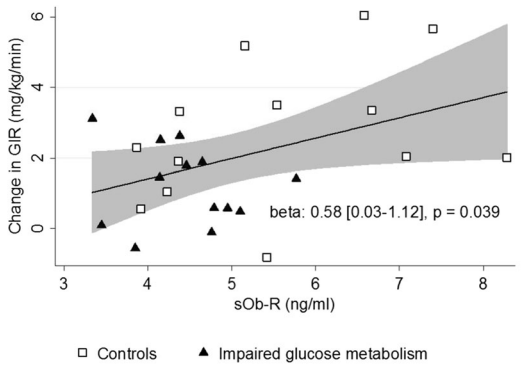

Figure 1. Crude relationship between baseline sOb-R and change in glucose infusion rate (GIR) following a 12-week exercise intervention in men with impaired glucose metabolism and controls. Linear prediction (full line) with 95 % CI (grey area).

Clinical Trial Registration Number: NCT01803568
Disclosure: **C. Sommer:** None.

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Higher insulin sensitivity in recent-onset latent autoimmune diabetes of adults compared with recent-onset type 2 diabetes

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Background and aims: Latent autoimmune diabetes of adults (LADA) exhibits features of both type 1 diabetes (T1D: islet-cell antibodies), and type 2 diabetes (T2D: late onset and initial insulin-independence). However, less is known about the key abnormalities of T1D and T2D, insulin sensitivity and beta-cell function, in humans with LADA. This study aimed to compare the metabolic phenotype of persons with recent-onset (disease duration <1 year) LADA, T1D, T2D and nondiabetic humans (CON).

Materials and methods: We compared sex-, age- and BMI-matched participants of the German Diabetes Study: LADA: n[m/f]=42[26/16], age 50.5±9.5 years, BMI 27.9±5.3 kg/m^2 ; T1D: n[m/f]=42[26/16], 47.9±7.8 years, 26.3±4.6 kg/m^2 ; T2D: n[m/f]=42[26/16], 50.6±9.9 years, 28.4±4.9 kg/m^2 and CON: n[m/f]=42[26/16], 50.3±10.6 years, 27.6±4.6 kg/m^2. LADA was diagnosed by age >30 years at manifestation, positivity for glutamic acid decarboxylase (GAD) autoantibody and/or islet-cell antibodies (ICA) and >6 months of an initial insulin-free period. All participants underwent glucagon tests and IVGTT to assess insulin secretion and hyperinsulinemic-euglycemic clamp tests, to assess insulin sensitivity (M). Data were analyzed using generalized ANOVA, taking the matched design into consideration and adjusted for age, sex and BMI.

Results: Glycemic control in LADA was better than in T1D (HbA1c: 6.6 ±1.1 % [49±12 mmol/mol] vs. 7.1±1.6 % [54±18 mmol/mol], p<0.05), but similar to T2D (6.6±1.1 % [49±12 mmol/mol] vs. 6.5±0.8 % [48±8 mmol/mol]) and lower than in CON (6.6±1.1 % [49±12 mmol/mol] vs. 5.3±0.3 % [34±4 mmol/mol], p<0.05). Beta-cell function, as assessed from both C-peptide and insulin rise following glucagon stimulation, was 66% higher in LADA than in T1D, but 33% lower than in T2D and 95% lower than in CON (all p<0.05). Total C-peptide secretion during IVGTT was 3.1 times higher in LADA compared to T1D (84.0±67.2 vs. 27.0±26.6 ng/ml), 17% lower than in T2D (98.5±59.1 ng/ml) and 2.4 times lower than in CON (200.7±68.4 ng/ml) (all p<0.05). Insulin sensitivity in LADA was comparable to T1D (8.2±2.9 and 8.3±3.2 mg/kg*min), 15% higher than in T2D (7.0±2.1 mg/kg*min, p<0.05), but 26% lower than in CON (10.3±3.1 mg/kg*min, p<0.05).

Conclusion: Persons with recent-onset LADA feature similar whole-body insulin sensitivity as T1D and similar beta-cell function as T2D, placing these patients as a metabolically hybrid phenotype between T1D and T2D.

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Supported by: BMBF, DZD, Ministry of Innovation, Science, Research and Technology NRW
Disclosure: **O.P. Zaharia:** None.

PS 033 Insulin actions in humans II

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Effect of calcidiol on insulin resistance and beta cell function in subjects with pre-diabetes

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Background and aims: Several studies have evaluated extraskeletal effects played by vitamin D on metabolic outcomes but data are not consistent and little evidence is available for calcidiol [25(OH)D]. Aim of this study was to evaluate the effect of calcidiol supplementation on insulin resistance, β-cell function and markers of inflammation and oxidative stress in subjects with pre-diabetes.
Materials and methods: We carried out a double blind placebo-controlled clinical trial enrolling a total of 150 subjects with IGT, IFG (defined by ADA criteria) and 25(OH)D <20 ng/ml, followed up for 6 months. Subjects were either assigned (50 per group) to 1) daily supplementation of 50 mcg of calcidiol (Arm A); 2) 25mcg of calcidiol (arm B); 3) placebo (Arm C). ISOGTT index was used to test insulin resistance while ISSI-2 index was used for beta-cell function. Other parameters included: HbA1c, 25(OH)D, calcium, phosphorus, PTHi, lipid panel, Hs-CRP, TNFα, IL-6, sRAGE
Results: At baseline. subjects were (mean ±SD) 63.8± 2.1 old , BMI of 27.4±1.2; serum glucose 115.1±8.4, HBa1c 6.4±0.6, 25(OH)D 16.3±2.5. There were significant associations of 25(OH)D with ISOGTT (β=0.35; 95% CI, 0.14, 0.46) and β-cell function (ISSI-2; β = 0.15; 95% CI, 0.02, 0.28).At six months, 25(OH)D increased up to 48 ± 3 ng/mL in the A arm (P<0.01) and to 36 ± 5 ng/mL in the B arm (P<0.01); no significant changes in the C arm. Subjects in Arm A had a lower risk of dysglycemia (HR = 0.85, 95% CI, 0.75-0.97 per SD increase) while no significant effects were observed in the Arms B or C. Both ISOGTT and ISSI-2 were improved in Arm A (P<0.05) while no significant changes were observed in Arm B or placebo. Serum levels of sRAGE decreased in Arm A [median 1354 (1069-1680) pg/ml (P<0.01), as compared with levels at study entry, but not in Arms B or C. No significant differences were observed for CRP, IL6, TNFα or lipid panel.
Conclusion: Our finding indicate that high doses of calcidiol improved indices of glucose homeostasis in prediabetic subjects and decreased circulating sRAGE levels, suggesting a positive effect also on oxidative stress.
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Disclosure: **E. Maddaloni:** Grants; Bruno Farmaceutici. Non-financial support; Bruno Farmaceutici.

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Circadian misalignment induces insulin resistance and elevated glucose and FFA levels: a randomised crossover trial

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Background and aims: Circadian misalignment, such as in shift work, has been associated with obesity and insulin resistance. Recently, we demonstrated a day-night rhythm in human skeletal muscle mitochondrial function. It is tempting to suggest that a reduced or a shifted rhythm of mitochondrial function might be involved in the development of insulin resistance by circadian misalignment. However, direct effects of circadian misalignment on peripheral insulin sensitivity have never been investigated.
Materials and methods: Fourteen healthy young lean men (age 22.4 ± 2.8 years; BMI 22.3 ± 2.1 kg/m2 [mean ± SD]) participated in a randomized cross-over study to determine the influence of controlled circadian misalignment on insulin sensitivity, assessed by hyperinsulinemic two-step euglycemic clamping. Participants were studied after a 3-day control period and after a 3.5-day misalignment period performed by a 12-rapid shift in a respiration chamber, isolated from time cues. Whole body indirect calorimetry was performed to assess resting metabolic rate and substrate oxidation. In addition, sleeping metabolic rate (SMR) was measured with whole room indirect calorimetry. Glucose, FFA and triglyceride levels were determined in plasma samples after an overnight fast (either at 7 AM or 7 PM).
Results: Circadian misalignment resulted in a significant decrease in insulin stimulated glucose disposal (Glucose infusion rate: 8.7 ± 1.8 vs. 7.8 ± 1.2 mg/kg/min; control *vs.* misalignment; p=0.04). Fasting glucose and FFA levels were higher in circadian misalignment (Glucose: 5.0 ± 0.2 vs. 5.2 ± 0.2 mmol/l; control *vs.* misalignment; p=0.01; FFA: 390 ± 174 vs. 525 ± 179 μmol/l; control *vs.* misalignment; p=0.006). In addition, participants had higher SMR in circadian misalignment (4.94 ± 0.48 vs. 5.14 ± 0.57 kJ/min; control *vs.* misalignment), while resting metabolic rate tended to increase (5.06 ± 0.58 *vs.* 5.33 ± 0.63 kJ/min; control *vs.* misalignment; p=0.08).
Conclusion: Controlled circadian misalignment leads to decreased peripheral insulin sensitivity, higher glucose and FFA levels and higher SMR. Our findings support a causal role of circadian misalignment in the development of obesity and type 2 diabetes mellitus. Further analysis - including markers of mitochondrial metabolism - is currently ongoing.
Clinical Trial Registration Number: NCT02580513
Supported by: TOP subsidies - ZonMw SA & Dutch Heart Foundation (CVON2014-02 ENERGISE)
Disclosure: **J. Wefers:** None.

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A randomised cross-over study of the acute effects of running 5 km on glucose, insulin and troponin T

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Background and aims: We aimed to study the impact by running 5 km, at maximal speed, on the normal variations of metabolic variables related to glucose, insulin, insulin sensitivity and metabolic rate.
Materials and methods: Five women and 12 men 25.7±5.2 years of age with a body-mass-index of 22.5±2.3 kg/m^2 where recruited to run 5 km at individual maximal speed in the morning, and to a corresponding day of rest, followed by standardized breakfast and lunch meals. Blood sampling and measurement of indirect calorimetry were done before and after meals. The participants were randomized regarding the order of the two trial-days in this cross-over study.
Results: Glucose, cortisol and metabolic rate were higher on race-days compared with days without exercise (ANOVA repeated measures, p=0.030, p=0.007 and p=0.008, respectively) while insulin levels or insulin sensitivity did not show such statistical differences (p=0.31 and p=0.14, respectively). When analyzing specific time-points we found that glucose increased from 5.01±0.37 mmol/l to 6.36 ± 1.3 mmol/l, p= 0.001, by running, while serum insulin concomitantly increased from 42±21 to 90±54 pmol/l, p= 0.003. In accordance, the QUICKI index of serum sensitivity, $1/(\log_{10}\text{insulin}+\log_{10}\text{glucose})$, was lowered by the race, p< 0.0001. Serum cortisol levels increased from 408±137 nmol/l to 644±171 nmol/l, p= 0.001, by the races while serum glucagon was unaffected. Troponin T was detectable post-race in 12 out of the 17 participants and reached or surpassed the clinical reference

level of 15 ng/l in three subjects. Post-race electrocardiograms displayed no pathologies.
Conclusion: Relatively short running-races can apparently induce a temporary reduction in insulin sensitivity that is not fully compensated by concomitantly increased insulin secretion intended to ensure euglycemia. Since also Troponin T was detected in plasma in a majority of the participants, our data suggest that it is possible to induce considerable metabolic stress by running merely 5 km, when striving for maximal speed.
Clinical Trial Registration Number: NCT01274078
Disclosure: **F.H. Nyström:** None.

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Differences in C-peptide levels in gestational diabetes with or without previous bariatric surgery indicate improved insulin sensitivity

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Background and aims: Bariatric surgery (BS) is an increasingly common method to combat obesity. It has been shown that bariatric surgery before pregnancy decreases the risk of developing gestational diabetes mellitus (GDM). However, some women do develop GDM despite weight loss surgery, and this patients group has not been studied extensively before. The aim of this study was to investigate the insulin secretion in patients with GDM with and without prior BS and pregnant women without diabetes who had undergone BS prior to pregnancy.
Materials and methods: All study participants were recruited at a University Hospital. Serum samples from all groups were collected either in the 1st or 3rd trimester of gestation. Patients with GDM (GDM: n=521) were recruited in 2009 - 2017 following a 2h 75 g OGTT. GDM patients that had undergone BS prior to pregnancy (BS-GDM: n=54) were diagnosed 2011 - 2016 following a 3-day plasma glucose monitoring, consisting of 7 capillary blood glucose tests per day (fasting and before and after each meal). The diagnostic criteria for GDM was 2 or more glucose values >8mmol/L. Pregnant women without diabetes that had undergone BS prior to pregnancy (BS-controls: n=34) were recruited 2015 - 2017. The study has been approved by the Regional Ethics Review Board in Lund, Sweden (2014/78, 2014/746). C-peptide levels were analysed in serum that had been frozen within 3 days of sample collection using a commercially available ELISA. Data is reported as median followed by [interquartile range]. Mann-Whitney U-test was used to compared median ranks between the groups. Effect size (ES) was calculated using eta^2 and $p<0.05$ was considered as statistically significant.
Results: The results of this study showed that BS-GDM women had lower C-peptide levels (0.59 [0.39-1.0] ng/mL) than women with GDM and no surgery (1.0 [0.61-1.7] ng/mL; ES=0.20, p<0.0001). There was a trend towards a difference between the groups of BS-GDM and BS-controls (0.44 [0.34-0.67] ng/mL, ES=0.20 p=0.066). There was also a difference between the BS-controls and GDM (ES=0.24, p<0.0001). BMI was highest in the BS-controls (29.9 [27.0-31.0] kg/m^2), followed by the BS-GDM group (26.5 [24.9-29.9] kg/m^2, ES=0.19, p=0.097). The women with GDM had the lowest BMI values, (25.8 [22.4-30.5] kg/m^2), significantly lower than the BS-controls (ES=0.12, p=0.0073), but not the BS-GDM women, (ES=0.075, p=0.091), although there was a trend.
Conclusion: In conclusion, women that have undergone BS and subsequently developed GDM have lower C-peptide levels than women with GDM who have not undergone surgery despite the trend towards higher BMI in the BS-GDM group. This could indicate an improved insulin sensitivity in these women, possibly related to metabolic changes caused by BS.

Supported by: SUS Funding
Disclosure: **J. Dereke:** None.

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Low oxidation of branched-chain amino acids in people with type 2 diabetes may underlie increased systemic levels

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Background and aims: Recent studies implicate that circulating branched-chain amino acids (BCAA) can be involved in the development of insulin resistance, likewise BCAA are elevated in humans at risk to develop type 2 diabetes (T2DM). In the present study we sought to investigate whether systemic BCAA levels differ between patients with T2DM, first-degree relatives (FDR) and control participants (CON), and if BCAA are associated with key metabolic parameters like insulin sensitivity, metabolic flexibility, mitochondrial function and intrahepatic lipid (IHL) content. Also, we investigated whether a low oxidation of BCAA underlies elevated systemic levels of BCAA.
Materials and methods: In total 15 patients with T2DM, 13 FDR and 17 CON (age within 50-65 years) were matched for BMI (31±1, 30±1 and 32±1 kg/m^2, respectively). Hyperinsulinemic-euglycemic clamps combined with D2-glucose and indirect calorimetry were performed to determine whole body insulin sensitivity (ΔRd: μmol/kg/min) and metabolic flexibility (ΔRQ: μmol/kg/min). Proton magnetic resonance spectroscopy (^{1}H-MRS) was used to determine intrahepatic lipid (IHL) content (%) and high-resolution respirometry in vastus lateralis muscle tissue to examine ex vivo mitochondrial function (O_2- flux: pmol/mg wet weight/s). In a sub-group of n=5 T2DM and n=5 CON, clamps were combined with 1-^{13}C leucine isotope to determine leucine oxidation under baseline and insulin stimulated conditions.
Results: Circulating BCAA were higher in T2DM vs. CON and FDR (543 ± 14 vs. 453 ± 16 and 480 ± 18 μmol/l; p<0.05). Negative correlations were found between fasting BCAA plasma levels and ΔRd (r=-0.41; p<0.05), ΔRQ (r=-0.43; p<0.05) and mitochondrial O_2-flux (state 3: r=-0.39; p<0.01 and state u: r=-0.48; p<0.001). In addition, a strong positive correlation was found between BCAA and IHL (r=0.35; p<0.05). Interestingly, leucine oxidation was lower in T2DM vs. CON both at baseline as well as during insulin stimulation (baseline: 0.23 ± 0.02 vs. 0.32 ± 0.02 μmol/kg/min, p<0.05; insulin-stimulated: 0.38 ± 0.03 vs. 0.44 ± 0.02 μmol/kg/min, p<0.05).
Conclusion: BCAA strongly associates with key metabolic parameters and are elevated in patients with T2DM. In patients with T2DM a blunted BCAA oxidation during baseline and insulin stimulation possibly underlies elevated systemic BCAA levels. Our results suggest that altered metabolism of BCAAs is part of the insulin resistant phenotype.
Clinical Trial Registration Number: NTR4181
Supported by: EFSD, NWO Netherlands (VENI)
Disclosure: **E. Phielix:** Grants; Innovational Research Incentives Scheme VENI; Ministry of Education, Culture and Science (OCW) via NWO The Netherlands, European Foundation for the Study of Diabetes (EFSD).

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Glucose homeostasis and whole-body insulin resistance improved 4 weeks after gastric bypass surgery in type 2 diabetes, whereas adipose tissue metabolism was unchanged

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Background and aims: Gastric bypass (GBP) surgery used in severe obesity has also shown beneficial effects to prevent or treat type 2 diabetes (T2D). Markedly improved insulin sensitivity, beta-cell function and reduced hepatic glucose production, visceral and hepatic fat have been reported. Our aim was to explore short-term effects following GBP on glucose tolerance, insulin sensitivity and beta-cell function as well as on adipose tissue metabolic function.
Materials and methods: The data presented are part of a larger study including T2D subjects with BMI 30-45 randomized to either GBP or standard-of-care medical treatment (2:1). For this pilot study, 7 subjects randomized to GBP (age 58.3 ± 9.1; M/F 3/4, diabetes duration 4.1 ± 0.7 yr) were included in the analyses. They were assessed pre-surgery, and 4 weeks post-surgery as follows: 1) oral glucose tolerance test (OGTT) to assess insulin resistance and glucose tolerance, 2) an iv arginine (5g) challenge to assess beta-cell capacity, and 3) adipose tissue biopsies to measure glucose uptake, lipolysis and insulin action in isolated adipocytes.
Results: The in-vivo measurements showed that 4 weeks after surgery, subjects had lower fasting glucose and insulin levels, and glucose levels at 60 and 120 mins of the OGTT were reduced ($p<0.01$). Insulin sensitivity assessed with the Matsuda index was increased ($p< 0.05$). There were no differences pre- and post-GBP in either the disposition index or the insulinogenic index. During arginine challenge insulin levels were consistently lower post-GBP ($p<0.01$). However, the relative peak insulin response to arginine was increased post-GBP ($p<0.05$). In-vitro studies of isolated adipocytes did not show any significant changes in either basal or isoprenaline-stimulated lipolysis rates or glucose uptake, and neither was there any change in the effects of insulin (0.1-1000 μU/mL). However, adipocyte size was significantly decreased 4 weeks post-GBP ($p<0.01$). The first subjects (n=4) of the control group did not display any consistent anthropometric or metabolic changes during a 24-wk follow-up.
Conclusion: These obese patients with type 2 diabetes had a clear improvement in insulin sensitivity and glycemic control already 4 weeks after GBP, but this was not accompanied with any changes in glucose uptake and lipolysis rates or insulin sensitivity in adipose tissue. Therefore, we hypothesize that the mechanisms for rapid improvement of diabetes after GBP occur mainly in other tissues than adipose, like skeletal muscle, liver, and CNS, and that they could involve neuroendocrine pathways and incretin hormones. This will be addressed in the onward analyses of this study.

Clinical and metabolic characteristics	Baseline	4-wks post surgery	Adipocyte in-vitro data	Baseline	4 wks post surgery
BMI (kg/m2)	38.1 ± 1.5	33.6±1.27**	Adipocyte diameter (μm)	110.0 ± 5.3	72.3 ± 10.3**
Weight (kg)	109.5 ±4.1	96.4±3.1**	Glucose uptake (fL/cell/sec)		
Waist hip ratio	0.99 ± 0.03	0.97±0.03	basal	19.5 ± 4.3	16.6 ± 6.7
Body fat (%)	40.3 ± 3.7	33.6±1.3*	25 μU/ml Insulin	23.1 ± 6.3	19.2 ± 7.6
HbA1c (mmol/mol)	48.4 ±6.9	38.5±3.7*	1000 μU/ml Insulin	31.4 ± 4.7	25.7 ± 10.7
HOMA-IR	6.62 ± 0.80	2.84 ± 0.47*	Lipolysis (nmol glycerol/10E5 cells/h)		
Matsuda index	1.53 ± 0.18	2.63 ± 0.31*	basal	12.4 ± 3.5	10.4± 3.9
Disposition index	1.93 ± 0.80	2.60 ± 0.70	0.5 μM Iso	58.4 ± 16.2	53.2 ± 9.3
Insulinogenic index	1.09 ± 0.31	0.95 ± 0.19	0.5 μM Iso + 1μU/ml insulin	33.10 ± 6.5	27.3 ± 6.9
OGTT-glucose (AUC; mmol/L* min)	2950 ± 193	2582 ±228*	0.5 μM Iso + 100 μU/ml insulin	26.4± 4.3	21.6 ± 5.4
Arginine challenge- insulin (AUC; (mU/L*min)	1245 ±189	707 ± 77**			

*p<0.05; **p<0.01, Body fat % measured by bioimpedance. Iso, isoprenaline.

Clinical Trial Registration Number: NCT02729246
Disclosure: **P. Katsogiannos:** None.

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Sam68 mediates insulin and leptin signalling in granulosa cells and is downregulated in polycystic ovary syndrome patients

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Background and aims: Sam68, an RNA-binding protein that participate in signal transduction is expressed in granulosa cells. The Sam68 knockout female mice are subfertile, and present ovulation problems. We have previously found that Sam68 may be recruited to insulin and leptin receptors in different systems including granulosa cells, and Sam68 expression is induced by both insulin and leptin stimulation. In order to assess the relevance of Sam68 in leptin and insulin receptor signaling in granulosa cells, we planned to study the effect of either downregulation or overexpression of Sam68 in the activation of PI3K and MAPK signaling pathways by insulin or leptin. We then wanted to check whether Sam68 expression was altered in granulosa cells from patients with polycystic ovary syndrome, where both insulin and leptin resistance have previously been observed.
Materials and methods: Granulosa cells from control (25) and polycystic ovary syndrome women (25) were obtained obtained as a waste product from follicles recollected getting oocytes for in vitro fecundation, after informed written consent. Downregulation of Sam68 expression was achieved using siRNA method, and Sam68 overexpression was performed using an expression plasmid vector containing the Sam68 cDNA. Signaling was studied by immunoblot of the phosphorylated proteins. The bands obtained in the blots were scanned and analyzed after nrmaization by the PCBAS 2.0 program. The expression level of Sam68, and leptin and insulin receptors are quantified by qPCR and confirmed by immunoblot. Statistical significance was assessed by analysis of variance followed by Bonferroni's post hoc tests. P-value of <0.05 was considered to be statistically significant.
Results: Sam68 down-regulation by Sam68 siRNA prevents the leptin- and insulin-dependent activation of PI3K and MAPK pathways in granulosa cells, whereas overexpression of Sam68 potentiates the activation of PI3K and MAPK pathways in response to leptin or insulin. Moreover, Granulosa cells from polycystic ovary syndrome subjects have significant lower expression of Sam68 than granulosa cells from control women.
Conclusion: Sam68 is necessary for fully activation of leptin and insuln signaling in human granulosa cells, and Sam68 expression is downregulated in granulosa from polycystic ovary syndrome patients. Thus, Sam68 may be an important element in the ovarian insulin and leptin resistance, and may mediate the decreased fertility in women with polycystic ovary syndrome. Therefore, Sam68 could be considered a new target for therapy.
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Disclosure: **V. Sanchez-Margalet:** None.

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Putative miRNA biomarkers of insulin resistance and the effect of metformin: data from the CAMERA trial

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Background and aims: Several circulating miRNAs have been reported to be associated with insulin resistance (IR) and are considered as potential diagnostic biomarkers, although studies have generally been limited by small size. It is also not clear whether these associations are causal. We aimed to 1) validate cross sectional associations of targeted miRNAs within a large study and 2) to explore the effect of metformin on these miRNAs.
Materials and methods: The CAMERA study was a randomised, placebo-controlled double-blinded trial performed in Glasgow, UK (2009-2012). 173 patients aged 35-75 on statins with coronary heart disease and large waist circumferences (but without diabetes) were randomly assigned to metformin or placebo (1:1) and followed for 18 months. miRNAs were extracted from 308 paired stored samples from 154 participants (taken at baseline and after 18 months of treatment). The expressions of mir-221, 222, 144, 155, 192 and 193b (targeted based on previous literature) were measured using real time quantitative polymerase chain reaction (RT-qPCR). Samples from each patient were run simultaneously. Spike-in of cel-miR-39 was used as a quality control. We

used linear regression of CT values for cross sectional analyses, and the $2^{-\Delta\Delta CT}$ method to investigate the randomised effect of metformin.
Results: In cross sectional analysis at baseline, mir-144 showed the strongest association with markers of insulin sensitivity: in models adjusting for age and sex, for every unit increase in HOMA-IR, mir-144 was 10.2% (95% CI 2.5% - 17.2%, p<0.05) lower and for a 1 mmol/L increase in fasting plasma glucose (FPG), mir-144 was 34.9% (95% CI 15.1% - 50%, p<0.01) lower. Other miRNAs showed variable associations with markers of insulin resistance and glycaemia, but no miRNA was associated with BMI. Mir-192 and 193b showed strong associations with liver enzymes. For every 10-unit increase in ALT, mir-192 was 24.7% (95% CI 16.5% - 32.2%, p<0.001 lower while mir-193b was 34.9% (95% CI 28.8% - 40.1%, p<0.001) lower. For every 10-unit increase in GGT, mir-192 (95% CI 2.7% - 8.6%, p<0.001) was 6% lower and mir-193b was 8% (95% CI 5.4% - 10.5%, p<0.001) lower. Adjustment for body fat did not attenuate these associations. Over 18 months, metformin reduced weight by 3.3 kg, FPG by 0.3 mmol/L and HOMA-IR by 0.81 units versus placebo. However, metformin showed no effect on the expression of mir-221, 222, 144, 155, 192 and 193b (p=0.91, 0.51, 0.25, 0.97, 0.89 and 0.69 respectively). From observational data, this effect would be expected to translate into an ~8% increase in miRNA-144, which may not be of clinical relevance.
Conclusion: In this, one of the first studies to investigate the effect of a randomised intervention on miRNA expression, we observed broadly expected cross-sectional associations of targeted miRNAs with biomarkers of metabolic risk in a non-diabetic population with coronary heart disease but without diabetes. These data support the putative utilities of miRNAs as reproducible biomarkers, but more RCTs should explore the effect of interventions on their expression.
Clinical Trial Registration Number: NCT00723307
Disclosure: **T. ALRamah:** None.

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Adipose tissue lipolytic inhibition enhances the glucoregulatory properties of exercise in type 2 diabetes patients

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Background and aims: Exercise combined with adipose tissue lipolytic inhibition augments intramuscular lipid and glycogen use in type 2 diabetes mellitus (T2DM) patients. The present study investigates the impact of adipose tissue lipolytic inhibition during exercise on subsequent postprandial glycemic control in T2DM patients.
Materials and methods: Fourteen male T2DM patients (age: 65±2 years, HbA_{1c} 6.7±0.1%) participated in a double-blind placebo-controlled randomized cross-over study in which subjects performed 60 min of endurance-type exercise (at 45% W_{peak}) after being administered 250 mg of an nicotinic acid analog (acipimox; ACP) or a placebo (PLA). A control experiment was included in which no exercise was performed (CON). Plasma samples were obtained before, during and for up to 7.5 h after exercise.
Results: Sixty min of exercise at 73±6 W did not significantly lower circulating plasma glucose and insulin excursions in PLA when compared with CON (P=0.300 and 0.778 for glucose and insulin, respectively). Acipimox administration strongly reduced circulating plasma FFA concentrations during exercise (175±13 vs 535±47 and 637±58 mmol/L in the ACP vs CON and PLA, respectively; P<0.001). Circulating plasma glucose (tAUC, 3500±124 vs 3794±172 and 3946±183 mmol/L/450min) and insulin (tAUC, 76±7 vs 106±13 and 103±13 nmol/L/450min) excursions were substantially lower during 7.5 h of recovery from exercise (*i.e.* postprandially) in ACP when compared with either CON or PLA (P<0.05 and <0.001, respectively).
Conclusion: Exercise with adipose tissue lipolytic inhibition improves postprandial blood glucose regulation and thus clinical efficacy as compared to exercise alone in male T2DM patients.
Clinical Trial Registration Number: NTR4710
Disclosure: **K. Verboven:** None.

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The effects of feeding a sodium caseinate derived hydrolysate on glycaemic control and insulin sensitivity following an oral lipid load

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Background and aims: Obesity with its associated state of chronic low-grade inflammation with dysregulated metabolism is a major driver of insulin resistance (IR). Dysregulated postprandial insulin and glucose responses, which are hallmarks of IR, are among the main therapeutic targets. Intake of casein and casein derived hydrolysates are associated with increased insulin secretion and reduced glucose levels. Certain casein hydrolysates are also associated with reductions in metabolic inflammation and improvements in insulin signaling. The current study addressed the hypothesis that a sodium caseinate (NaCas) derived bioactive hydrolysate could improve glycaemic control in an obese insulin resistant cohort, using an oral lipid load combined with a hyperinsulinemic euglycaemic clamp technique, using an acute metabolic challenge approach.
Materials and methods: Six obese male volunteers participated in this randomized cross-over controlled trial. Following an overnight fast, participants received an oral lipid load (100 mL soyabean oil) to induce IR. After 2 hours volunteers completed a 4 hour hyperinsulinemic-euglycaemic clamp (30 mU/m^2/min) combined with either 12g sodium caseinate (NaCas), 12g NaCas derived bioactive hydrolysate or a water control (Figure 1). Glucose, insulin, triacylglycerol (TAG), non-esterified fatty acids (NEFA), C-peptide, GIP and GLP-1 responses were determined after the oral lipid load, with or without oral administration of the NaCas derived hydrolysate versus NaCas control and water. Data analyzed by repeated measures ANOVA using SPSS statistical software.
Results: Administration of the NaCas derived hydrolysate did not improve whole-body insulin sensitivity following an oral lipid load, compared to the native NaCas or water controls. Consumption of the NaCas derived hydrolysate did not alter glucose metabolism as measured by glucose, insulin and C-peptide concentrations, compared to NaCas or water controls. NEFA concentrations decreased by 36% following the NaCas derived hydrolysate while triglycerides increased by 30% compared to baseline, however this was not significantly different from the NaCas or water controls.
Conclusion: Ingestion of 12g NaCas or NaCas derived hydrolysate did not improve insulin sensitivity compared to a water control in obese, insulin resistant males following an oral lipid load. Despite the promising finding that NaCas increases insulin secretion and improves insulin signaling this does extend to improved insulin sensitivity in the current study.

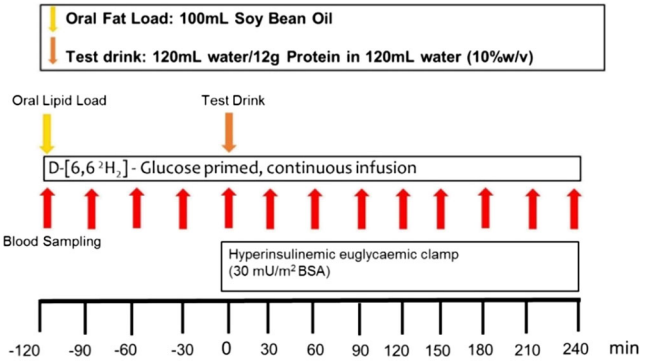

Figure 1. Study Protocol Overview

Supported by: Enterprise Ireland
Disclosure: **E.B. Kennedy:** Grants; Enterprise Ireland.

PS 034 Pharmacological effects on metabolic functions and risk factors

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Exenatide LAR decreases cIMT and improves flow mediated dilation similarly in obese and non-obese patients with type 2 diabetes: an 8-month prospective study

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Background and aims: The increasing prevalence of type 2 diabetes (T2DM) may be explained by increased rate of obesity. Treatment with GLP-1RA, exenatide once-weekly (long-acting release, LAR), after 3 and 5 years, led to sustained body weight loss. Here we investigated whether exenatide LAR may exert different effects on several cardio-metabolic parameters in obese vs. non-obese T2DM patients.
Materials and methods: Sixty subjects with T2DM (41 men and 19 women; 60±10 yrs) naïve to incretin-based therapies, and treated with exenatide LAR as add-on to metformin (from 1500 up to 3000 mg/day) for 8 months, were included in this prospective study. The presence of a previous major cardiovascular event, as well as moderate and severe renal and liver function were the main exclusion criteria. Fasting blood samples were collected at baseline and after 8 months for routine biochemical analysis. The cohort of patients was subdivided in 2 groups: 1) those with body mass index (BMI) ≥30 (n=31) and 2) those with BMI <30 (n=29). The fatty liver index (FLI) was calculated in each subgroup. cIMT was assessed by B-mode real-time ultrasound, while endothelial function by flow mediated dilation (FMD) of the brachial artery.
Results: Statistical analysis was performed using paired t-test and ANOVA. After 8 months of exenatide therapy, improvements in all investigated parameters were seen in both groups (Table). In addition, alanine and aspartate transaminases (AST and ALT) significantly improved in obese patients only (AST: from 22.3±8.0 to 18.4±4.3 U/L, p=0.006 in obese vs. from 22.1±10.2 to 20.7±8.5 U/L, p=0.340 in non-obese; ALT: from 30.1±14.4 to 24.2±12.8 U/L, p=0.004 in obese vs. from 27.9±17.2 to 26.7±18.1 U/L, p=0.609 in non-obese). FLI improved only in obese patients (Table).
Conclusion: This is the first study reporting that exenatide LAR provides significant and equally beneficial cardio-metabolic control, reducing cIMT and improving FMD, in both obese and non-obese T2DM patients. Greater weight loss, decrease in waist circumference and improvement in hepatic parameters were seen in obese vs overweight patients. Whether exenatide LAR use is associated with sustained decrease in obesity rates, in addition to its benefits cardio-metabolic parameters, remains to be established by future studies.

	Baseline *BMI <30*	p=	After 8 months *BMI <30*	Baseline *BMI >30*	*p=*	After 8 months *BMI >30*	p= (between groups)
Weight (kg)	76±9	*0.0661*	75±10	101±16	***0.0009***	97±16	***<0.0001***
BMI (kg/m²)	27±1	*0.0812*	26±2	39±14	*0.1256*	35±5	*0.0939*
Waist circumference (cm)	99±7	*0.1627*	98±8	118±11	***0.0320***	114±11	***<0.0001***
Fasting glycaemia (mmol/l)	8.8±3.3	***<0.0001***	6.8±1.6	8.8±2.3	***0.0271***	7.7±2.6	*0.1855*
HbA1c (%)	8.0±0.3	***<0.0001***	7.0±0.8	8.1±0.4	***<0.0001***	6.9±1.3	*0.5837*
Total cholesterol (mmol/l)	4.4±0.9	*0.1190*	4.2±0.9	4.4±1.0	***0.0019***	4.1±1.0	***0.0256***
HDL-cholesterol (mmol/l)	1.3±0.3	*0.8487*	1.3±0.3	1.2±0.2	***0.0114***	1.3±0.3	*0.0784*
LDL-cholesterol (mmol/l)	2.5±0.7	***0.0462***	2.2±0.8	2.6±0.9	***<0.0001***	2.2±1.0	*0.3407*
Flow mediated dilation (%)	5.7±1.2	***<0.0001***	6.9±1.5	5.8±1.4	***<0.0001***	6.7±1.8	*0.2649*
Carotid IMT (mm)	1.0±0.1	***<0.0001***	0.8±0.2	1.0±0.1	***0.0009***	0.9±0.1	*0.0548*
Fatty liver index	53.8±21.5	*0.2644*	50.1±22.5	88.6±11.4	***<0.0001***	81.0±16.7	***<0.0001***

Clinical Trial Registration Number: NCT02380521
Supported by: AstraZeneca
Disclosure: **G. Castellino:** Other; I have participated in clinical trials sponsored by AstraZeneca and Novo Nordisk.

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Improvement in flow-mediated dilatation and proinflammatory cytokines with exenatide LAR occurred independently of baseline carotid IMT in patients with type 2 diabetes

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Background and aims: The effect of exenatide once-weekly (long-acting release, LAR) on carotid intima-media thickness (cIMT), a surrogate marker of early, subclinical atherosclerosis, is still unknown. We aimed to investigate: 1) whether exenatide LAR may have different effects on cIMT, depending on the value at baseline, and 2) whether this effect is associated with the exenatide modulation of cardio-metabolic parameters, including cytokines potentially involved in endothelial function.

Materials and methods: Sixty subjects with type 2 diabetes (41 men and 19 women; 60±10 yrs) naïve to incretin-based therapies, and treated with exenatide LAR as add-on to metformin (from 1500 up to 3000 mg/day) for 8 months, were included in this prospective study. Exclusion criteria included the presence of a previous major cardiovascular (CV) event, as well as moderate and severe renal and liver function. Fasting blood samples were collected at baseline and after 8 months for routine biochemical analysis. The cohort of patients was subdivided in 2 groups: 1) those with cIMT ≤0.9 mm (considered as a normal value; n=27) and 2) those with cIMT >0.9 mm (considered as an abnormal value; n=33). cIMT was assessed by B-mode real-time ultrasound, while endothelial function was assessed by flow mediated dilation (FMD) of the brachial artery. Cytokines were measured by multiplex analysis using Luminex Magpix®.

Results: Statistical analysis was performed by paired t-test and ANOVA. After 8 months of exenatide therapy, improvements in the majority of assessed cardio-metabolic parameters were seen in both groups of patients (Table). However, improved lipid profile was seen in patients with abnormal cIMT only. Several cytokines improved in the abnormal cIMT group: a decrease was seen in plasminogen activator inhibitor-1 (PAI-1) (from 2.8 ±1.6 to 1.4±0.7 ng/ml; p=0.022), p-selectin (from 11.42±6.8 to 7.1±3.9 ng/ml, p=0.002), interleukins (IL)-2 and 1-B (from 9.3±5.0 to 5.8±2.2 pg/ml, p=0.001 and from 1.2±0.6 to 0.9±0.2 pg/ml, p=0.041, respectively), while a cell adhesion molecule with a key role in endothelial function, L-selectin, increased (from 0.49±0.16 to 0.59±0.23 μg/ml, p=0.018).

Conclusion: This study shows that, in patients with type-2 diabetes, exenatide LAR improves several cardio-metabolic parameters and such effects seem to not be impacted by early, subclinical atherosclerosis. The improvements in investigated cytokines seen only in patients with an abnormal cIMT levels at baseline may, at least in part, explain further protective effects of exenatide in those patients. Whether this finding may be associated with CV prevention remains to be established by future studies.

	Baseline cIMT ≤0.9	p=	After 8 months cIMT ≤0.9	Baseline cIMT >0.9	p=	After 8 months cIMT >0.9	p=(between groups
Weight (kg)	88±17	***0.0008***	85±16	90±19	***0.0227***	87±19	*0.9077*
BMI (kg/m^2)	32±7	***0.0008***	30±6	34±15	*0.1889*	31±5	*0.5817*
Waist circumference (cm)	108±14	***0.0426***	104±11	109±12	*0.1245*	108±14	*0.2944*
Fasting glycaemia (mmol/l)	8.5±2.1	***<0.0001***	7.0±1.5	9.1±3.2	***0.0055***	7.5±2.7	*0.8183*
HbA1c (%)	8.1±0.4	***<0.0001***	6.9±0.7	8.0±0.3	***<0.0001***	6.9±1.3	*0.7442*
Total cholesterol (mmol/l)	4.2±0.9	*0.0998*	4.0±0.9	4.6±0.9	***0.0051***	4.3±0.9	*0.5647*
Triglycerides (mmol/l)	1.4±0.6	*0.8547*	1.4±0.5	1.5±0.7	*0.9950.*	1.5±0.6	*0.6242*
HDL-cholesterol (mmol/l)	1.2±0.3	*0.9263*	1.2±0.2	1.2±0.3	***0.0005***	1.3±0.3	***0.0339***
LDL-cholesterol (mmol/l)	2.3±0.7	*0.0827*	2.2±0.8	2.7±0.9	***0.0147***	2.3±1.0	*0.2852*
Flow mediated dilation (%)	5.9±1.4	***<0.0001***	6.8±1.7	5.6±1.2	***<0.0001***	6.7±1.6	*0.2508*
Carotid IMT (mm)	0.8±0.1	***0.0144***	0.8±0.1	1.0±0.1	***<0.0001***	0.9±0.1	*0.0518*

Clinical Trial Registration Number: NCT02380521
Supported by: AstraZeneca

Disclosure: **A.M. Patti:** Other; I have participated in clinical trials sponsored by AstraZeneca and Novo Nordisk.

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Dulaglutide protects beta cells against cytokine stress by anti-oxidative, anti-inflammatory pathways and modification of membrane remodelling

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Background and aims: Inflammation and oxidative stress are major actors in type 2 diabetes. They prompt pancreatic cell dysfunction associated with membrane remodeling and the release of noxious microparticles (MPs) with pro-apoptotic, pro-inflammatory and pro-coagulant effects. MP are plasma membrane vesicles and procoagulant circulating biomarkers of cell stress and organ damage. In vitro, MPs shed from cytokine or oxidative stress-stimulated β cells act as cellular effectors prompting tissue factor (TF) expression, cytokine release, apoptosis and reducing insulin secretion We investigated whether Dulaglutide (Dula), a GLP-1 analog, could prevent cytokine-driven β cell dysfunction.

Materials and methods: Rin-m5F rat β cells and freshly isolated rat islets were submitted for 24 hours to cytokines (cyt: 1000 IU/ml TNF-α, 50 IU/ml IL1β 1000 IU/ml INF-γ) in the presence of Dula (0.05-1μM). GLP1R-dependent effects were inhibited by 200 nM exendin9-39 (Ex), a GLP1R antagonist. Apoptosis was measured by Propidium Iodide / Annexin 5 staining, MP release by prothrombinase assay, insulin secretion by ELISA. TF activity at β cell surface was measured by tenase assay and reported as fM per 50'000 cells. Islet total ROS amount was quantified by DiHydroEthidium staining and mitochondrial ROS by the MITOSOX fluorescent probe. Inflammatory pathway was explored by study NF-KB pathway by Western blot analysis.

Results: Dula (1-0,05 μM) significantly reduced β cell apoptosis induced by cytokines in a concentration-dependent manner (Cytokines: 17% ± 1 vs Dula 1μM: 8 % ± 1% ; 0.2 μM: 13 %± 1,; 0.05 μM 14% ± 1, vs. p <0.01, n = 4) and 1μM Dula completely abolished the apoptotic response (untreated cells : 8% ± 1). Cell pretreatment with the receptor antagonist GLP1R for 1 hour caused a sharp decline in the protective effect of Dula, indicating a GLP1 receptor-R dependent cytoprotection (Apoptosis: Cyt 17 % ± 1; Dula 1 μM: 9% ± 2; Dula + Exendin: 14 % ± 1, n = 4). Dulaglutide (1μM) limited the release of MP in the supernatant of cytokine- treated and untreated cells (untreated: 8.5 nM± 0.3; Untrd + Dula: 6.3 nM ± 0.5 ; Cyt: 12.2 nM ± 0.5, Cyt + Dula: 10, 6 nM± 0.1; p <0.01, n = 4) and was also dependent on GLP1R (cyt+ Dula +Exe: 12.8 nM ± 0.7, p <0.01, n = 4). Dulaglutide (1μM) decreased TF activity induced by cytokines at β cell surface (Untrd: 95 f. ± .11, Cyt: 211 f. ± 27, cyt+ Dula: 113 ± 5, p <0.05). However, the inhibitory effect of Dula on TF activity seemed independent of the GLP1-R (Cyt+ Dula+ Exendin: 95 fM± 20, p <0.05). Dulaglutide (1μM) decreased the amount of total ROS in islets by 50 % and mitochondrial ROS by 40 % (n=4, p<0.05). Western blot confirmed that expression of ratio pIKB/IKB is altered in cells submitted to cytokine stress and restored by pretreatment with dulaglutide.

Conclusion: Dulaglutide exerts cytoprotection on β cell by drastic reduction of apoptosis and by limiting inflammatory response through GLP-1R dependent- and non-dependent GLP1R pathways prompting membrane remodeling and microparticles release. Cytoprotection against oxidative stress was also obtained in isolated islets. Further studies are ongoing to characterize the underlying signalling pathways. The cytoprotective effect of Dulaglutide on islets will be further investigated in murine models of T2D.

Supported by: Eli Lilly Company

Disclosure: **G. Kreutter:** Grants; Lilly company.

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Changes in lipid concentrations in patients with type 2 diabetes on once-weekly dulaglutide 1.5 mg: post hoc pooled analysis of the AWARD trials

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Background and aims: Glucagon-like peptide (GLP)-1 receptor agonists may improve lipid profiles in patients (pts) with type 2 diabetes (T2D), but few large scale studies exist.
Materials and methods: A post hoc, pooled analysis of 5 studies (AWARD 1, 2, 3, 5 and 6) of once-weekly dulaglutide (DU) 1.5 mg added to oral glucose lowering agents was conducted to evaluate the magnitude of the lipid changes. Associations between lipid changes and baseline covariates were analysed in a multivariable model.
Results: A total of 1424 pts were included: mean (SD) age was 55.6 y (9.9), diabetes duration 6.9 y (5.6), BMI 32.6 kg/m^2 (5.3) and HbA1c 63.9 (12.0) mmol/mol [8% (1.1)], with 683 (48%) on lipid lowering agents (LLAs, 91% statins). DU 1.5-mg treatment for 6 months resulted in modest changes in Total-C and LDL-C (Table). Greater reduction in LDL-C was observed in males (by 0.12 mmol/L vs. females, p<.001) and those on LLAs (by 0.11 mmol/L vs. pts without LLAs, p=.003). Similar associations were observed for Total-C. In addition, pts with higher baseline Total-C, LDL-C and triglycerides had more marked reduction in the respective indices (p<.001 for all) at 6 months.
Conclusion: Treatment with DU 1.5 mg in pts with T2D resulted in modest but statistically significant decreases in Total-C and LDL-C. Preliminary data suggest that these lipid changes are greater in males and those on statin therapy. The clinical relevance of these changes requires further investigation.

Mean Change in Lipids from Baseline to 6 Months with Once-Weekly DU 1.5 mg

Lipid	Baseline (mmol/L) Mean (SD)	Δ From Baseline (mmol/L) LSM (95% CI)	Δ From Baseline (%) LSM (95% CI)	p-value
Total-C	4.7 (1.1)	−0.13 (−0.21, −0.05)	−2.8 (−4.5, −1.0)	.002
LDL-C	2.6 (0.9)	−0.11 (−0.18, −0.04)	−4.2 (−6.9, −1.5)	.002
HDL-C	1.2 (0.3)	0.03 (−0.02, 0.09)	2.6 (−1.9, 7.1)	.26
TG	2.0 (1.3)	−0.07 (−0.18, 0.04)	−3.5 (−8.9, 1.9)	.20

Intent to treat population, without post-rescue visits, last observation carried forward
Abbreviations: HDL-C = high density lipid cholesterol; LDL-C = low density lipid cholesterol; LSM= least squares mean; TG = triglycerides; Total-C = total cholesterol

Clinical Trial Registration Number: NCT01064687, NCT01075282, NCT01126580, NCT00734474, NCT01624259
Supported by: Eli Lilly and Company
Disclosure: **I. Pavo:** None.

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3-month results for GOAL-RCT: a randomised trial comparing colesevelam vs ezetimibe in type 2 diabetes

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Background and aims: Lipid-lowering therapies are often added to statin drugs in patients with type 2 diabetes (T2D) who fail to achieve target LDL cholesterol. Our objective was to compare the efficacy and safety of two second-line LDL lowering options: colesevelam (COL) vs. ezetimibe (EZE) in T2D.
Materials and methods: GOAL-RCT is the first randomized controlled trial comparing COL vs. EZE (NCT02682680). The 6-month, open-label, randomized, parallel-group, multicentre trial enrolled 200 patients with T2D who had uncontrolled A1c (7 - 10%) and LDL cholesterol (>2 mmol/L); and randomized them to either COL (3.75g daily) or EZE (10 mg daily) in a 1:1 ratio. The randomization was stratified for use of Sodium glucose co-transporter 2 inhibitors (SGLT2i). T2D medications as well as statin dose were unchanged during the trial. The primary outcome was the proportion of patients achieving A1c ≤7.0% and LDL ≤2 mmol/L. We present the protocol-specified 3-month results for the randomized trial.
Results: GOAL-RCT enrolled 200 subjects with comparable baseline characteristics: mean age 59 ± 10 years, mean A1c 8.0% and mean LDL 2.5 mmol/L. T2D therapies included metformin (88%), insulin (85%), Dipeptidyl peptidase-4 inhibitor (63%), and SGLT2i (52%). 97% of the enrolled subjects were on statin medications. Table 1 outlines the main results for the trial at 3 months. Three month data were available for 85 COL patients and 98 EZE patients. The proportion of patients achieving A1c ≤7.0% and LDL ≤ 2 mmol/L in the COL arm (14%) was non-inferior to the proportion in the EZE arm (9%) (p <0.01 for non-inferiority; p = 0.28 for superiority). The COL arm had a lesser reduction in both LDL (-0.28 vs -0.65 mmol/L; p<0.01) and non-HDL (-0.32 vs -0.74, P<0.01) but a greater reduction in A1c (-0.4% vs. 0%; p<0.01) compared to EZE, respectively. No significant differences were noted between fasting plasma glucose, triglycerides, HDL and CRP levels. Fifteen COL patients and 6 EZE patients discontinued study treatment (7 and 5 subjects due to adverse effects, respectively). SGLT2i was used by 104 patients, with retrospective analysis suggesting a historical rise in LDL from pre-SGLT2i use of 0.27 mmol/L, a 12% increase (p=0.01). Following randomization, within this SGLT2i subgroup, the COL arm had a lesser reduction in LDL (-0.29 vs -0.81 mmol/l; p=0.05), and a greater reduction in A1c (-0.3% vs. +0.1%; p=0.05) compared to EZE, respectively.
Conclusion: When used in combination with statin therapy, the proportion of patients with T2D achieving A1c ≤7.0% and LDL ≤2 mmol/L was similar for COL and EZE at 3 months. EZE produced greater reductions in both LDL and non-HDL cholesterol whereas only COL led to reduction in A1c.

Table 1 – Comparison of clinical efficacy parameters at 3 months between colesevelam and ezetimibe among subjects with type 2 diabetes

	colesevelam (n=85)		ezetimibe (n=98)		Between-group difference (95% CI) (ezetimibe as reference)
Proportion achieving A1c ≤ 7.0% and LDL ≤2.0 mmol/L	14.0%		9.2%		-5.1% (-13.8 to 3.6%)
Proportion achieving A1c ≤ 7.0%	35.3%		14.3%		-20.0% (-33.7% to -7.8%)
Proportion achieving LDL ≤ 2 mmol/L	43.5%		69.4%		25.9% (10.8% to 39.7%)
	Baseline	3 month Δ	Baseline	3 month Δ	Between-group difference (95% CI)
A1c (%)	8.0 ± 0.9	-0.4 ± 0.92	8.0 ± 0.8	0.0 ± 0.7	-0.4* (-0.6 to -0.1)
LDL (mmol/L)	2.54 ± 0.66	-0.28 ± 0.85	2.50 ± 0.56	-0.65 ± 0.80	0.37* (0.12 to 0.61)
Non-HDL (mmol/L)	3.34 ± 0.78	-0.32 ± 0.98	3.19 ± 0.73	-0.74 ± 0.99	0.42* (0.12 to 0.70)

* significant difference between groups (P<0.05).
Data is presented either as a proportion, or as mean ± standard deviation.

Clinical Trial Registration Number: NCT02682680
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The effects of lobeglitazone on glycaemic control and lipid metabolism in high-fat diet-induced diabetic mice

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Background and aims: The aim of this study was to compare the effects of lobeglitazone, a novel peroxisome proliferator-activated receptor-γ agonist, on glycemic control and lipid metabolism with pioglitazone in mice with type 2 diabetes. We also determined the effects of lobeglitazone and pioglitazone on glucose uptake, translocation of GLUT4 and AMPK activity in 3T3L-1 adipocytes.
Materials and methods: 4-week-old C57BL/6 mice were treated with a high-fat diet for 8 weeks. After 8 weeks of high-fat diet, lobeglitazone or pioglitazone was administered once daily by oral gavage for 6 weeks.
Results: Low-dose lobeglitazone (1 mg/kg) improved fasting blood glucose and insulin levels, HOMA-IR index, and blood triglyceride levels while low-dose pioglitazone did not. In 3T3L-1 cells, low-dose lobeglitazone (1 μM) significantly increased cellular glucose uptake and, GLUT4 translocation. Pioglitzone exhibited similar effects at only high dose treatment (10 μM). High-dose lobeglitazone (10 μM) increased total cellular GLUT4 protein content while pioglitazone did not. In subcutaneous fat and 3T3L-1 cells, relative mRNA expression levels for lipid synthesis were increased in both lobeglitazone and pioglitazone-treated groups compared to vehicle-treated group, but mRNA expression levels of β-oxidation-related genes and the energy expenditure-related genes were significantly increased in lobeglitazone-treated group compared to pioglitazone-treated group. In addition, lobegitazone treatment significantly increased phosphorylation of the AMP-activated protein kinase compared to pioglitazone treatment in 3T3L-1 cells.
Conclusion: These results indicate that low-dose treatment of lobeglitazone might be enough to improve glucose intolerance while pioglitazone dose not. In addition, lobeglitazone could improve lipid metabolism by stimulating β-oxidation and the energy expenditure through activation of AMPK in adipocyte.
Disclosure: **H. Kim:** None.

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DPP4 inhibition by linagliptin prevents the development of heart failure with preserved ejection fraction in the metabolic syndrome ZSF1 rats

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Background and aims: Heart failure with preserved ejection fraction (HFpEF) is the most common HF phenotype. HFpEF is highly prevalent in elderly women and frequently accompanied by a cluster of comorbidities, including obesity, type 2 diabetes mellitus, arterial hypertension, chronic obstructive pulmonary disease (COPD), renal insufficiencies and anaemia. Currently, the pathophysiology underlying HFpEF is not fully elucidated resulting in the absence of prevention and treatment strategies. As the anti-diabetic drug Linagliptin, a dipeptidyl peptidase 4 (DPP-4) inhibitor, has been shown to have cardioprotective effects (e.g. improvement of diastolic dysfunction and cardiac inflammation), we hypothesized that Linagliptin could protect against HFpEF development (hypertrophy, inflammation, fibrosis and diastolic dysfunction) in a chronic metabolic risk-induced HFpEF animal model.
Materials and methods: Sixteen weeks old obese ZSF-1 rats received Linagliptin supplemented diet (83 mg/kg; n=7) or placebo chow (n=7) for four weeks, while hypertensive non-diabetic Lean ZSF-1 rats, which do not develop HFpEF, served as non-diseased controls (n=5).
Results: Linagliptin significantly reduced plasma DPP4 activity by 81.9% and elevated active glucagon-like peptide 1 (aGLP1; inhibited by DPP4) by 271.3% in obese ZSF1 rats proving its therapeutic potential. In line, Linagliptin significantly diminished the fasting glucose levels by 33.5% in diabetic obese ZSF1 rats. Importantly, Linagliptin decreased cardiac hypertrophy as reflected by a significantly diminished left ventricular weight to tibia length (-9%) and a trend towards reduced cardiomyocyte size (-16.2%; p=0.05) in obese ZSF1 rats. Moreover, Linagliptin improved left ventricular diastolic dysfunction as reflected by a 24.1% reduction in deceleration time, an indicator of left ventricular stiffness, in obese ZSF1 rats. In addition to reducing cardiac hypertrophy and improving diastolic function, Linagliptin significantly cardiac leukocyte infiltration, while inducing a trend towards reduced systemic (pro-inflammatory) monocyte levels (-33.9%; p=0.08) in obese ZSF1 rats suggesting that Linagliptin has an anti-inflammatory effect both systemic and cardiac. Furthermore, Linagliptin significantly reduced perivascular fibrosis (-27.7%) and elevated the coronary capillary density (+52.8%) in obese ZSF1 rats proposing a vascular effect. In addition, Linagliptin ameliorated the MetS phenotype as reflected by significantly diminished total body weight (-11.5%), %), spleen (-18.1%), kidney (-19.7%), liver (-34.9%) weight to tibia length and plasma triglycerides (-51.8%) and non-HDL cholesterol levels (-31.2%), while elevating plasma HDL levels (+75.0%) in obese ZSF1 rats.
Conclusion: The anti-diabetic drug Linagliptin diminished cardiac hypertrophy, inflammation, capillary rarefaction and overall diastolic dysfunction in a rat model for diabetes-, obesity- and hypertension-induced chronic HFpEF. In addition, Linagliptin ameliorated the MetS phenotype as reflected by diminished total body and relative organ weight, systemic inflammation, hyperglycaemia, and Therefore, Linagliptin could be a novel treatment for HFpEF.
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PS 035 Novel aspects of metabolic function in animal models

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Exposure to maternal obesity differentially impacts pancreatic islet function in male and female offspring

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Background and aims: Studies have shown that not only does current obesity increase the risk of developing type 2 diabetes (T2D), but obesity during pregnancy can increase the risk of T2D in the offspring. Abnormalities in β-cell function are critical in defining the risk and development of T2D; a defining event in T2D pathogenesis is when functionally impaired β-cells can no longer compensate for insulin resistance in peripheral tissues. In contrast, sustained β-cell adaptation is capable of preventing T2D even in the face of severe insulin resistance. The aim of this study was, therefore, to elucidate whether exposure to maternal obesity (MO) during pregnancy and lactation programs changes in pancreatic islets and whether these changes are different between male and female offspring.

Materials and methods: Mice were generated using a well-established mouse model of MO. Briefly, female mice were fed ad libitum either a chow diet or highly palatable energy-rich obesogenic diet prior to and throughout pregnancy and lactation. Offspring were weaned onto a chow diet and remained on this diet until 8 weeks of age. At this age, offspring had normal body weight thus allowing the investigation of the impact of MO independently of offspring obesity. Pancreatic islets were isolated from male and female offspring. Islets were stimulated with low (2.8mM) and high (16.7mM) glucose ex vivo and insulin and proinsulin secretion as well as content was determined by ELISA. Insulin secretion was also determined upon stimulation with leucine/glutamine (Leu/Gtn; mitochondrial fuel) and potassium chloride (KCl). Mitochondrial (mt) respiration was assessed using Seahorse XF24. Expression of Ins1, Ins2, Tfam and mt encoded genes in islets was determined by qRT-PCR. Data were analysed using an unpaired t-test. A probability level of 5% was taken to be significant.

Results: Glucose-stimulated insulin secretion from islets was increased ($P<0.05$) in both male and female offspring exposed to MO. In females but not males, this was primarily due to increased mt metabolism and exocytotic capacity of islets as reflected by increased Leu/Gtn ($P<0.01$) and KCl-stimulated ($P<0.05$) insulin secretion. Furthermore, islets from female offspring displayed increased mt respiration in response to glucose ($P<0.01$) as well as increased ATP turnover ($P<0.01$). Expression of mt encoded genes mt-Nd5 ($P<0.01$), Cyb ($P<0.001$) and Co1 ($P<0.05$) was increased but the mt transcription factor Tfam was unchanged. Ins1 ($P<0.01$) and Ins2 ($P=0.06$) gene expression was also increased in these islets. In contrast, both mt respiration ($P=0.057$) and ATP turnover ($P<0.05$) were decreased in islets from male offspring. Expression of mt genes and Tfam was unchanged whilst Ins1 expression was increased ($P<0.01$) in these islets. Whilst islet insulin and proinsulin content was unaffected in male offspring exposed to MO, islet proinsulin:insulin content ($P<0.05$) was decreased in female offspring.

Conclusion: Compensatory changes are present in the islets of female offspring exposed to MO that are not present in male offspring. This study is the first to demonstrate sexual dimorphism in the programming of altered pancreatic islet function in the offspring in response to the chronic hyperglycemic environment of MO and may explain differences in time course of the development of offspring glucose intolerance.

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Disclosure: **L.M. Nicholas:** None.

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Reduced insulin causes weight loss without affecting glucose homeostasis

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Background and aims: Obesity is an epidemic affecting more than 500 million people worldwide. Furthermore, it is a risk factor to several diseases, including diabetes, heart disease, stroke and cancer. Obesity is commonly associated with basal hyperinsulinemia and insulin resistance, but the cause and effect relationship between obesity, hyperinsulinemia, insulin resistance and type 2 diabetes remains to be fully elucidated. Some evidence suggests a causal role for hyperinsulinemia in obesity, independently of insulin resistance. Drugs that block hyperinsulinemia have been reported to cause weight loss in humans, but this remains controversial and has been suggested that such drugs work independently of insulin. We previously reported that both life-long and early life transient suppression of basal hyperinsulinemia in mice resulted in protection from diet-induced obesity. These results provide evidence that hyperinsulinemia can play a causal role in mammalian obesity, however it is still unclear whether insulin reduction in adult, obese mice would result in weight loss. To address this critical therapeutic question, we developed a mouse model of acute 50% deletion of the *Ins2* gene.

Materials and methods: We generated an inducible, beta-cell specific insulin gene heterozygous mice ($Pdx1^{CreER}$:$Ins1^{-/-}$:$Ins2^{fl/+}$) to test the hypothesis that diet-induced obesity can be lowered by simply reducing pancreatic insulin production, which would indicate that long-term developmental reprogramming of the insulin system is not required to protect mammals from obesity. Low (10%), moderate (25%) and high fat (58% fat) diets were initiated in male mice at 6 weeks of age. Following 12 weeks on the respective diets, test and littermate control mice were injected with tamoxifen resulting in the inducible loss of one *Ins2* allele in test mice.

Results: Following 12 weeks on a high fat diet, all mice weighed approximately 40 grams. Remarkably, inducible reduction of pancreatic *Ins2* gene dosage in tamoxifen-injected $Pdx1^{CreER}$:$Ins1^{-/-}$:$Ins2^{fl/+}$ mice resulted in a rapid 5% weight loss within 5 weeks. This effect was only noted in mice fed a high fat diet (HFD) and the weight loss observed in these mice was due primarily to significantly reduced gonadal (control 3.3±0.3g vs test 2.2±0.2g; $p<0.05$) and perirenal (control 0.8±0.1g vs test 0.6±0.1g; $p<0.05$) fat mass. Protein levels of the lipodystrophy gene PTRF were ~50% lower ($p<0.05$) and an ~35% reduction in LPL protein levels ($p=0.06$) in the test mice compared to controls, suggesting impaired lipid storage. Weight loss appeared to be unaffected by food intake and was not associated with glucose intolerance in these mice. Furthermore, transcriptional sequencing revealed slight but significant down regulations in several innate immune related genes within gonadal adipose tissue isolated from test mice compared to controls.

Conclusion: Together, these data provide the first evidence in any organism that obesity can be reversed by acutely reducing the production and circulation of insulin. Our results have profound implications for nutritional guidelines and therapeutic efforts to combat obesity.

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Disclosure: **M.M. Page:** None.

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Mutation of OXPHOS NADH-dehydrogenase subunit 2 improves age-dependent adaption of energy metabolism in liver tissue

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Background and aims: The mitochondrial genome of the conplastic B6-mt^{ALR} mouse strain is characterized by a mtDNA encoded *ND2* mutation in complex I of the respiratory chain. OXPHOS mutations in liver are linked to obesity, liver steatosis and diabetes. Liver from B6-mt^{ALR} mouse showed high levels of ROS production only at late life but without susceptibility to hepatosteatosis. In this study we investigated the effect of the mutation on basic parameters of hepatocellular energy metabolism, response to nutrient challenge and AMPK activation in the course of aging.
Materials and methods: Conplastic B6-mt^{AKR} (AKR, control) and B6-mt^{ALR} (ALR, *ND2* mutation) mice were monitored over a period up to 24 months. At defined time-points liver tissue was analysed by Western blot analysis for AMPK activation. Hepatocytes were isolated at the age of 3, 6 and 12 months. In cultured hepatocytes ATP and ADP levels were measured by a luminescence assay. Glucose-induced changes of the ATP/ADP ratios were monitored after transfection of the hepatocytes with the ATP sensor Perceval.
Results: ATP and ADP level were higher in isolated hepatocytes of ALR mice at the age of 3 up to 12 month compared to control mice. Nucleotide levels remained unchanged during aging in the AKR control strain. In contrast to that the mutant strain exhibit significant increased ATP (4.4 vs. 10.9 nmol/mg Protein) and ADP (1.8 vs. 4.5 nmol/mg Protein) amounts at the age of 3 month. Thus, the *ND2* mutation was associated with an increased OXPHOS activity. There was also a lower fat accumulation in liver tissue in ALR compared to AKR mice. The data are in line with higher pAMPK/AMPK ratios in liver of ALR compared to the AKR mice at the age of 3 months. Challenge of isolated hepatocytes by 25 mM glucose resulted in different adaptation of the ATP/ADP ratios (ALR vs. AKR: 3 month, 0.56 AUC vs. 0.35 AUC; 12 month, 0.39 AUC vs. 0.14 AUC, $p < 0.05$) measured after Perceval transfection. Changes of ATP/ADP ratios decreased with aging in hepatocytes but were always lower in liver from ALR mice.
Conclusion: In conplastic ALR mice the mtND2 mutation in complex I of the respiratory chain favored better hepatic energy supply and nutrient sensing during aging. Despite of ROS generation the improved hepatic energy metabolism and AMPK activation in the liver suggesting a protective role against hepatosteatosis in the pathogenesis of diabetes.
Disclosure: **M. Wietzke:** None.

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The loss of the corepressor GPS2 favors "unhealthy" adipose tissue expansion in obesity leading toward the development of type 2 diabetes

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Background and aims: Type 2 diabetes (T2D) and insulin resistance are associated with adipose tissue dysfunction. Adipocyte hypertrophy, i.e. the enlargement of pre-existing fat cells, is thought to increase T2D development independent of the level of obesity per se. Many human studies demonstrate that increased fat cell size is a significant predictor of altered blood lipid profiles and glucose-insulin homeostasis independent of adiposity indices. However, the explanation of why individual (obese and some non-obese) humans differ in their susceptibility to develop dysfunctional adipose tissue and adipocyte hypertrophy are still an issue that is currently not sufficiently addressed. Our recent work discovered the deregulation of the transcriptional coregulator GPS2 (G-Protein Pathway Suppressor 2, core subunit of the HDAC3/SMRT corepressor complex), in adipocytes, which may contribute to the development of insulin resistance and T2D in mice and humans.
Materials and methods: In order to identify the functions of GPS2 in adipocytes, we have generated adipocyte specific Knock-Out (AKO) mice. The WT and AKO mice were fed with a high fat diet and subjected to metabolic and transcriptomic analyses. These in vivo observations were complemented by in vitro experiments to decipher GPS2 actions
Results: Our data suggest that adipocyte-specific GPS2 knockout mice are predisposed to adipocyte hypertrophy, leading upon high fat diet to the rapid development of adipose tissue dysfunction and glucose intolerance. Indeed, the specific loss of GPS2 in adipocytes leads to an epigenomic reprogramming that favors inflammation, inadequate angiogenic remodeling and dysregulation of lipid oxydation.
Conclusion: GPS2 seems to play a protective role in adipocyte, influencing energy metabolism and limiting the progression of obesity towards insulin resistance and T2D. Hence maintaining GPS2 function is essential to have a healthy adipose tissue expansion during obesity.
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Lack of B1 kinin receptor improves glucose metabolism in mice fed with cafeteria diet

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Background and aims: Previously studies have shown that the kinin B1 receptor (B1R), which is involved in inflammatory regulation, activates metabolic processes that modulate weight regulation and glucose homeostasis. Deletion of this receptor in a transgenic mice model resulted in protection for weight gain in mice submitted to a high fat diet in comparison with wild type controls. As a result, this model may be appropriated to test the effect of a cafeteria diet, which has a composition more related to the one consumed in the modern Western society and related to the development of obesity and diabetes. The aim of this study is to investigate whether the genetic deletion of kinin B1 receptor (B1RKO) might influence in the glucose metabolism of mice submitted to cafeteria diet (CAF).
Materials and methods: Two months old male C57Bl/6 wild-type (WT; 24.2 to 28.6 g) or B1RKO mice (21 to 28.6 g) were fed with standard chow (SC: 65% CHO, 22 % PTN, 10 % LIP) or cafeteria diet (CAF: 63% CHO, 12% PTN, 35% LIP) ad libitum for 14 weeks (WT-SC n= 7; 8 B1RKO-SC n= 8; WT-CAF n=7; B1RKO-CAF n = 10). Food intake was evaluated daily and the mice where weighed weekly. For body weight gain (WG), weight was assessed at baseline ($Weight_0$) and after 14 weeks ($Weight_{final}$) and expressed as the % of WG. After 8 hours fasting, glycemia was measured before and after the intraperitoneal injection of 1g/kg (live weight) of glucose in 10% solution, at times -15, 0, 15, 30 60 and 120 min. Glucose tolerance was assessed by the comparison of the area under curve (AUC) for glucose of each group. Two days later, for the Insulin Tolerance Test (ITT), regular insulin (1UI/kg - live weight) was injected intraperitoneally after 2 hours fasting to measure glycemia at 0, 5, 20 and 30 min and estimate insulin sensitivity as the calculation of the glucose decline relative up to 30 min relative to glycemia at 5 min and presented as the glucose decay constant (Kitt; %/min). Values are expressed as mean ± SEM. $P < 0.05$ was considered statistically significant.
Results: While the groups differ for relative weight gain (B1RK0-Caf 73.63 ± 16.10 ,WT-Caf 45.69 ± 16.95, B1RKO-SC 32.56 ± 5.63 , WT-SC 27.01 ± 5.39 mean ± SE % of WG; Diet p = < 0.0001, Genotype p = 0.0005 , Diet vs. Genotype p = 0.0140), B1RKO mice had similar percentage of perirenal and epidimal fat mass when fed with the same diet as the WT controls. However, WT mice had an increased glucose response as showed in the glucose tolerance test (AUC= 46996 ± 2969 vs. 36776 ± 2634; WT vs. B1RKO respectively; p= 0,016 ; and 50768 ± 2874 vs.

33004 ± 2737 Caf vs. SC respectively; p= 0001), suggesting that B1RKO mice had a better tolerance to a glucose load. No interaction was found when compared diet vs. genotype in the AUC of GTT. When analyzing mice fed with Caf, in the WT group there was a stronger positive correlation between glucose response in the GTT test with the percentage of weight gain (r= 0,821; p= 0,023), while weight gain in the B1RKO group was not related with glucose response during GTT test (r= 0,667; p= 0,071 . In the insulin tolerance test no differences were found between groups.

Conclusion: Mice lacking B1kinin receptor presented a higher glucose uptake despite the highest weight gain. Take together these results, it shows that there was a dissociation between the weight gain and glucose metabolism in obese B1RKO mice when submitted to Cafeteria Diet.

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Disclosure: **P.E. Correia:** None.

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Single-gene congenic strain reveals the effect of Zbtb16 gene on dexamethasone-induced insulin resistance and dyslipidaemia

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Background and aims: Glucocorticoid treatment is often accompanied with substantial side-effects, including dyslipidemia, diabetes or obesity. The genetic basis of the interindividual differences in response to glucocorticoids received only limited attention so far. By combining two distinct inbred models of metabolic syndorme, the spontaneously hypertensive rat (SHR) and the polydactylous rat (PD), we have previously isolated an apparently pleiotropic locus on rat chromosome 8 affecting major features of metabolic syndrome. We have established a congenic model SHR.PD5 differing from SHR by a 788kb segment of chromosome 8 of the PD origin containing 7 genes. SHR.PD5 is particularly sensitive to dexamethasone-induced insulin resistance and dyslipidemia. The differential segment contains a glucocorticoid-response gene, Zbtb16. To distinguish the effects of Zbtb16 from other candidate genes, we developed a single-gene congenic subline containing only the Zbtb16 gene and a subline containing 6 remaining genes but not the Zbtb16 gene.

Materials and methods: Adult male rats of SHR.PD(Zbtb16) and PD5(-Zbtb16) strains were fed standard diet (STD) and subsequently treated with dexamethasone in drinking water (0.026 mg/ml) for 3 days. We contrasted their morphometric and metabolic profiles (including oral glucose tolerance test, triacylglycerol content in liver and muscle). Insulin sensitivity of skeletal muscle and adipose tissue was determined by assessment of basal and insulin- stimulated radioactivelly-labeled glucose incorporation into glycogen (isolated soleus muscle) or total lipids of visceral adipose tissue. Isolated genomic DNA was amplified by PCR with specific primers, PCR fragments were analyzed by electrophoresis and sequenced directly using PCR primers and the BigDye Terminator v1.1 cycle sequencing kit.

Results: The single-gene SHR.PD(Zbtb16) congenic subline carries a differential segment spanning 254 kb and contains only the Zbtb16 gene. There is one missense SNP in the Plzf as well as a 3kb deletion of the highly conserved noncoding sequence in intron 2 of Zbtb16 in the congenic subline. The second congenic strain PD5(-Zbtb16) carries in its differential segment the same genes as the original SHR.PD5 strain, i.e. Htr3a, Htr3b, Usp28, Zw10, Tmprss5, and part of Drd2 (promoter, first noncoding exon, and part of the first intron). We identified 2 non-synonymous amino-acid substitutions (H364R in Htr3b and T76S in Usp28). SHR.PD(Zbtb16) showed higher body weight compared to PD5(-Zbtb16) (287±7 vs. 265±4 g, p=0.030), higher glucose levels throughout the first 60 minutes of OGTT (no differences in insulin throughout the test), higher level of serum TG (2.08±0.13 vs. 1.64 ±0.10 mmol/l, p=0.015) but lower content of TG in liver (4.95±0.55 vs. 6.48±0.37 μmol/g, p=0.026). While the insulin sensitivity of the adipose tissue was comparable, both baseline and insulin-induced sensitivity of skeletal muscle tissue was significantly lower in SHR.PD(Zbtb16).

Conclusion: Using the minimal congenic strain approach, we have established the substantial role of Zbtb16 gene in dexamethasone-induced insulin resistance and dyslipidemia.

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Disclosure: **L. Sedova:** None.

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AT2R protect against changes in mitochondrial function in early stages of STZ diabetes

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Background and aims: Diabetic nephropathy (DN) is associated with structural and functional changes of mitochondria and largely influenced by angiotensin II (AngII). The AngII type 2 receptor (AT2R) often counteract the classic AngII effects which are mainly transmitted via the AT1R. The present study addressed the hypothesis, that the AT2R protects mitochondria during DN

Materials and methods: Transgenic rats (TGR) overexpressing the AT2R in renal tubular epithelial cells for more than 30-fold and wildtype littermates (WT) were either untreated or subjected to STZ-diabetes (35 mg/kg body weight) for 7 months and 21 days, respectively. In addition to kidney function and histology, in 21-day STZ-diabetic rats function of isolated mitochondria and expression profile in renal cortex was determined. Co-localization of AT2Rs with a mitochondrial marker was demonstrated by immunohistochemistry (IHC) and confocal microscopy. For quantification, receptor binding studies using radiolabeled 125I[Sar1,Ile8]-Ang II as a ligand were performed on highly pure mitochondrial fractions, which were validated by electron microscopy and western blot analysis.

Results: 7-months of STZ diabetes induced albuminuria in WT but not in TGR. Early STZ diabetes (21-days) did not alter the kidney function. However, increased lipid and glycogen deposits and changes in mitochondrial structure were observed. Isolated mitochondria exhibited increased oxygen consumption and superoxide production, which was blunted in transgenic mitochondria in parallel to enhanced ATP production. Thus, AT2R improved bioenergetics of renal mitochondria in early diabetes. The gene expression profile of renal cortex was differently altered in WT and TGR. Pathway analysis indicated that in TGR important pathways were regulated in a protective manner when compared with WT rats. Oxidative phosphorylation was highly up regulated and fatty acid degradation by β-oxidation as well as fatty acid metabolism and anaerobic glycolysis were significantly down regulated in diabetic TGR relative to diabetic WT rats. Western blotting revealed an increased expression of all 5 ETC complexes in diabetic TGR when compared to controls and diabetic WT rats. Further, we evidenced the existence of high affine mitochondrial AT2R, their density constitutes 60 % of the AT2R density on the cell membrane.

Conclusion: The AT2R protect against DN already at very early stages of diabetes by improving the bioenergetic efficiency of mitochondrial respiration and by a modification of the diabetes induced alterations in the renal expression profile, which seems to allow the cell to switch from preferred FA oxidation to glucose oxidation and as such preventing the harmful "modified Warburg effect ". These protective actions seem to be transmitted at least partly by mitochondrial AT2R.

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Disclosure: **S.C. Hoffmann:** None.

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BACE2 suppression prevents weight gain in mice fed with high-fat diet

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Background and aims: BACE2 (β-site APP-cleaving enzyme 2) is a protease that may play a role in the development of Alzheimer's disease (AD). However, besides the brain, it has also been localized in the pancreas, where it seems to play a physiological role. Nevertheless, despite the potential link between AD and glucose homoeostasis deregulation in humans and rodents, the involvement of BACE2 in metabolic disturbances such as insulin resistance and obesity, has not been explored. Therefore, the aim of the present study was to investigate the effect of BACE2 on whole-body glucose metabolism.

Materials and methods: BACE2-deficient (BACE2-KO) mice and their respective controls were used to analyze the phenotype after 16 weeks of high-fat diet (HFD) feeding. Insulin tolerance test (ITT), glucose tolerance test (GTT) and indirect calorimetry were performed to evaluate metabolic phenotype. mRNA expression of relevant genes from hypothalamus, liver and white adipose tissue was analyzed by quantitative PCR.

Results: BACE2-KO mice fed with HFD showed a 32% reduction in body weight ($p<0.05$), with respect to their wild type counterparts that was accompanied by better glucose homeostasis (28% decrease in the area under curve of GTT, $p<0.01$) and a good insulin sensitivity. Interestingly, BACE2-KO animals in regular diet presented a low respiratory exchange ratio ($p<0.05$) close to the groups of animals fed with HFD. BACE2-KO animals fed with HFD did not show changes in the expression of any of the hypothalamic neuropeptides studied with respect to the animals fed with regular diet. Moreover, regardless the diet, BACE2-KO mice showed an upregulation of the insulin signaling pathway in liver ($p<0.01$) and alterations of fatty acid metabolism markers in liver and white adipose tissue. Furthermore, white adipose tissue from BACE2-KO animals in HFD presented a weight reduction of 25% ($p<0.01$) and a lower expression of the inflammation marker *Ccl2*.

Conclusion: In summary, these results indicate that the absence of BACE2 seems to protect against HFD effects. Thus, targeting BACE2 may represent a good therapeutic strategy to ameliorate the pathological effects of obesity.

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Disclosure: **G. Alcarraz-Vizán:** None.

PS 036 New insight in metabolism from cell models

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Glucose and insulin regulate cholesterol uptake, SR-BI and mRNA levels of genes involved in intestinal lipid transport in polarised CaCo-2 cells

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Background and aims: Diabetic patients have higher and longer post prandial excursion of triglyceride-rich lipoproteins, suggesting abnormal lipid handling at the intestinal level. The aim of this study was to determine the effects of insulin and glucose stimulation on cholesterol uptake and mRNA levels of genes involved in the transport of lipids in a cellular model of intestinal epithelium.

Materials and methods: CaCo-2 cells (ATCC HTB-37) were grown to 80% confluence and then subcultured into 6-well plates with Transwell inserts to induce polarized differentiation. Cells were stimulated with insulin (100 nM) and/or glucose (5 or 25 mM). mRNA levels were quantified by qRT-PCR using Taqman technology. Individual mRNAs were normalized to GAPDH mRNA levels and expressed as fold-change using the $\Delta\Delta$Ct method. Cholesterol uptake was estimated by the amount of NBD-cholesterol taken up by cells in a plate reader (excitation/emission = 485/535 nm). Polarized CaCo-2 cells were fixed with 4% paraformaldehyde and nuclei (H33342), cholesterol (NBD), neutral lipids (BODIPY), SR-BI and Villin were detected by confocal immunofluorescence. Results were expressed as average ± SD of 6 independent experiments. Statistical significance of the differences was determined by one-way ANOVA and Tukey's multiple comparison test.

Results: In polarized CaCo-2 cells, 25 mM glucose increased the mRNA levels of ChREBP, NPC1L1 and ABCA1 (fold-change to vehicle treated cells: 10.9±3.7, 5.8±2.5, 3.5±1.2, respectively, $p < 0.05$), whereas 100 nM insulin increased the mRNA levels of SREBP1c, ABCA1 and SR-BI (fold change to vehicle treated cells: 7.2±2.8, 2.2±0.9, 13.0±1.4, respectively, $p < 0.05$). The abundance of SR-BI was increased by 100 nM insulin in whole cell protein extracts and confocal analysis showed higher levels of SR-BI exclusively at the apical side of CaCo-2 cells. Glucose treatment did not modify SR-BI levels nor its subcellular localization. Cholesterol uptake was increased by 100 nM insulin and 25 mM glucose separately (fold-change to vehicle treated cells: 1.9±0.3 and 1.2±0.2, respectively) but it was even higher with the combined treatment (fold-change: 2.32±0.6). The abundance of lipid droplets and NBD-cholesterol content was increased by 100 nM insulin, preferentially at the basolateral side of the cells. Glucose did not change the abundance nor the subcellular localization of lipid droplets in polarized CaCo-2 cells.

Conclusion: Glucose increases ChREBP, NPC1L1 and ABCA1, whereas insulin increases SREBP1c, ABCA1 and SR-BI, at the mRNA level in polarized CaCo-2 cells. Insulin, but not glucose, increases SR-BI in the apical surface of CaCo-2 cells and increases cholesterol cholesterol uptake and neutral lipids content, at basolateral side, in CaCo-2 cells. These results suggest that insulin promotes cholesterol uptake and intracellular lipid store in a polarized cell model of intestinal epithelium, possibly by increasing SR-BI levels at the apical surface.

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Disclosure: **M.N. Fuentes:** None.

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Effects of fatty acids on GLP-1-producing cells

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Background and aims: Fatty acids acutely stimulate GLP-1 secretion from L-cells *in vivo*. However, a high fat diet has been shown to reduce the density of L-cells in the mouse intestine and a positive correlation has been indicated between L-cell number and GLP-1 secretion. Thus, the mechanism of fatty acid-stimulated GLP-1 secretion, potential effects of long term exposure to elevated levels of different fatty acid species, and underlying mechanisms are not fully understood. In the present study, we sought to determine how long-term exposure to saturated (16:0) and unsaturated (18:1) fatty acids, by direct effects on GLP-1-producing cells, alter function and viability, and the underlying mechanisms.
Materials and methods: GLP-1-secreting GLUTag cells were cultured in the presence/absence of saturated (16:0) and unsaturated (18:1) fatty acids (0.125 mmol/L) for 48 h, followed by analyses of viability and apoptosis, as well as involvement of fatty acid oxidation, free fatty acid receptors (FFAR1) and ceramide synthesis. In addition, effects on the expression of proglucagon, prohormone convertase 1/3 (PC1/3), free fatty acid receptors (FFAR1/2), sodium glucose co-transporter (SGLT) and subsequent secretory response were determined.
Results: Saturated (16:0) and unsaturated (18:1) fatty acids exerted opposing effects on the induction of apoptosis (1.4-fold increase in caspase-3 activity and DNA fragmentation by palmitate versus a 0.3-fold reduction in caspase-3 activity and 0.5-fold reduction in DNA fragmentation in response to oleate; $p<0.01$). Further, co-incubation with oleate abolished the effect of palmitate on caspase-3 activity and DNA fragmentation. Palmitate-induced apoptosis was associated with increased ceramide content and co-incubation with Fumonisin B1 abolished this lipoapoptosis. Oleate, on the other hand, reduced ceramide content, and – unlike palmitate – upregulated FFAR1/2, evoking a 2-fold increase in FFAR1-mediated GLP-1 secretion following acute exposure to 0.125 mmol/L palmitate; ($p<0.05$).
Conclusion: Saturated (16:0), but not unsaturated (18:1), fatty acids induce ceramide-mediated apoptosis of GLP-1-producing cells. Further, unsaturated fatty acids confer lipoprotection, enhancing viability and function of GLP-1-secreting cells. These data provide potential mechanistic insight contributing to reduced L-cell mass following a high fat diet and differential effects of saturated and unsaturated fatty acids on GLP-1 secretion *in vivo*.
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Contribution of obese adipose tissue-derived stem cells to hepato-or breast-carcinoma inflammation, through promotion of Th17 cells and activation of IL-1b by monocytes

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Background and aims: As opposed with lean adipose tissues (AT), obese AT are heavily infiltrated with variety of inflammatory cells such as macrophages and Th17 cells. Obesity-mediated chronic low-grade inflammation is known to contribute to tumor progression in various cancers, including hepatic and breast cancers. Because we have previously demonstrated, using co-culture experiments, that obese AT-derived stem cells (obASC) contribute to AT inflammation through promotion of Th17 cells, and activation of IL-1β-secreting monocytes, we postulated herein that such inflammatory environment could contribute to tumor progression in cancer-suffering obese patients.
Materials and methods: Human ASC were isolated from AT of obese donors. Mononuclear cells (MNC) were collected from healthy blood donors. Co-cultures of obASC and MNC were activated for 48 hours with phytohemagglutinin A (PHA), a T cell mitogen, or not. Conditionned media (CM) were collected, and added for 24h to cultures of HuH7 (hepatocarinoma cell line) or of two breast carcinoma cell lines, i.e MCF-7, or MDA-MB-231. Levels of inflammatory or angiogenic gene expression were evaluated by qRT-PCR. Expression of CXCR4 (a marker of invasiveness) was measured by flow cytometry in the HuH7 cell line.
Results: CM from PHA-activated-obASC/MNC co-cultures enhanced IL-1β, IL-8 and VEGFα mRNA expression in HuH7 cells by 1942.2, 45.7 and 6.1 -fold, respectively, as compared with no treatment. A putative effect of CM on HuH7 invasiveness was supported by a 2- and 3-fold increase in MMP-9, and CXCR4 expression, respectively. In addition, IL-1β, IL-8 and VEGF-α mRNA expression were increased by 34.3, 33.2, and 2.97 fold respectively in MCF-7 cells, and by 85.8, 52.0 , and 1.34 fold respectively, in MDA-MB-231 cells. These results indicated thus a differential sensitivity of cancer cell lines to CM from PHA-activated-obASC/MNC co-cultures, with a preponderant increase of IL-1β mRNA levels in hepatocarcinoma cells versus a similar increase of IL-1β and IL-8 gene expression in breast carcinoma cells.
Conclusion: Our results suggest that in cancer-suffering obese patients, interaction of obASC with AT-infiltrating immune cells contribute to the establishment of an inflammatory environment, propitious to tumor inflammation and/or tumor migration. Whether this inflammation could occur through propagation of obese AT inflammatory environment towards tumors, or through migration of ASC inside tumors and then interaction with tumor infiltrating immune cells, remain to be explored.
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Disclosure: **M. Chehimi:** None.

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Adipose-derived stem cell extracellular vesicles induce inflammatory phenotype in T cells from type 1 and type 2 diabetes patients

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Background and aims: The concept of immune-mediated inflammatory disease is established for type 1 diabetes, but recent evidence indicates type 2 diabetes as an auto-inflammatory disease, with signs of insulitis as well. Adipose tissue is the largest endocrine organ with immune function and adipocyte dysfunction correlates with the development of insulin-resistance type 2 diabetes and with impaired micro- and macrovascular function. On the other hand, studies in animal models of type 1 diabetes reveal that adipocyte dysfunction and high levels of inflammatory cytokines can directly play a role in the onset and progression of the type 1 diabetes. Angiogenesis and cell therapy studies indicate that adipose-derived stem cells (ASCs) hold promise amongst stem cells of mesenchymal lineage, acting through paracrine mechanisms possibly involving the release of extracellular vesicles (EVs), a recognised integral component of the cellular network. Paralleling previous in vitro studies using EVs derived from bone-marrow mesenchymal stem cells (MSCs) indicated the promotion of an anti-inflammatory T cell response. We evaluated whether EVs derived from ASCs may effect inflammatory response in type 1 and type 2 diabetes, acting on T cell.
Materials and methods: EVs were purified from heterologous human adipose mesenchymal stem cells (ASCs) obtained from healthy donors by differential centrifugation. Protein array and gene array analysis showed high expression of some pro-inflammatory factors such as IFN-γ, IL-1, IL-17 and IL-6 and microRNA miR-126 in EVs. PBMCs were obtained from 6 patients with recent onset type 1 diabetes and 6 patients with long standing type 2 diabetes on metformin. Cultures were established with PBMCs and ASC-EVs for 48 hours in type 1 and type 2 diabetic patients. Responses to GAD65 stimulation were assessed by IFN-γ ELISPOT analysis in type 1 patients. Levels of cytokines were measured in the supernatant by ELISA and by intracellular flow cytometry analyses. T helper 17 (Th17) analysis was performed by flow cytometry analyses.

Results: ASC-EVs were internalised by PBMCs, as assessed by confocal microscopy and flow cytometry analyses. ASC-EVs increased IFN-γ spots in GAD65-stimulated PBMCs obtained from type 1 diabetes. Moreover, ASC-EVs increased levels of IFN-γ, IL-6, IL-10, TNF-α, IL-1-β in PBMCs obtained from type 1 and type 2 diabetes. Furthermore, ASC-EVs increased the number of Th17 cells and the levels of IL-17.
Conclusion: ASC-EVs appear to lack the antigen and non-antigen specific anti-inflammatory effects of MSCs, and induce a pro-inflammatory phenotype in T cells. In the context of type 1 diabetes, ASC-EVs may contribute to increased levels of cytokines thus inducing β cell death, inhibit insulin production and possibly contributing to the loss of self-tolerance. In type 2 diabetes may contribute lipotoxicity and exacerbate inflammation, also in the context of pancreatic islets.
Supported by: AIRC
Disclosure: **E. Favaro:** None.

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Studying the proliferation and differentiation of human adipose stem cells: the effects of glucagon

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Background and aims: Obesity is associated with an increase in the fat mass and dysfunctions of the white adipose tissue. Most of the drugs used for the treatment of obesity has limited efficacy in term of stable weight loss: moreover, relevant side effects limit de facto their clinical use. Therefore, a new pharmacological approach based on the use of drugs directly targeting the adipose tissue might be of interest. Glucagon-like peptide 1 (GLP-1) receptor agonists, such as liraglutide, are currently used for the treatment of type 2 diabetes and have been recently proposed as potential anti-obesity drugs, due to their relevant effects on weight loss. Among the products of the expression of the pro-glucagon gene, also glucagon, a key factor in the glucose homeostasis, seems to act on the adipose tissue, promoting lipolysis and energy expenditure. In the present study, we assessed the activity of glucagon in a human adipose stem cell (ASC) model in vitro.
Materials and methods: We assessed the effects of glucagon on ASC proliferation by direct cell count and cytofluorimetric analysis, adipogenesis by specific intracellular lipid staining and apoptosis by cytofluorimetric analysis of annexin V.
Results: We previously demonstrated that both liraglutide and native GLP-1 interfere with proliferation and differentiation ability of human adipose stem cells (ASC). In the present study, we assessed the in vitro effect of glucagon on the same cell model. Glucagon significantly inhibits ASC proliferation, in a dose and time-dependent manner, with a maximum effect at 3 days of culture (14.0%, 25.2% and 37.1%, $p<0.01$ for 1-10-100nM glucagon, respectively). The decrease in cell proliferation in the presence of glucagon is not associated with the activation of apoptosis, unlike what it has previously been observed with liraglutide and GLP-1. Moreover, addition of increasing doses of glucagon (1-10-100nM) inhibits in vitro-induced adipogenesis, lowering intracellular lipid accumulation, similarly to the effects obtained with liraglutide and GLP-1 (-28.82% and -10.2%, $p<0.05$ respectively).
Conclusion: This is the first study that shows glucagon's effects on human adipose cell precursors. Our findings demonstrate that glucagon has an inhibitory action on the proliferation and differentiation ability of these cells. Further preclinical studies are necessary to better elucidate glucagon's action on the body weight and clarify the molecular mechanisms underlying the effects of glucagon and GLP-1 receptor agonists at cellular level.
Disclosure: **G. Cantini:** None.

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Adipose tissue derived stem cells proliferation ability in obese patients with early stage of type 2 diabetes

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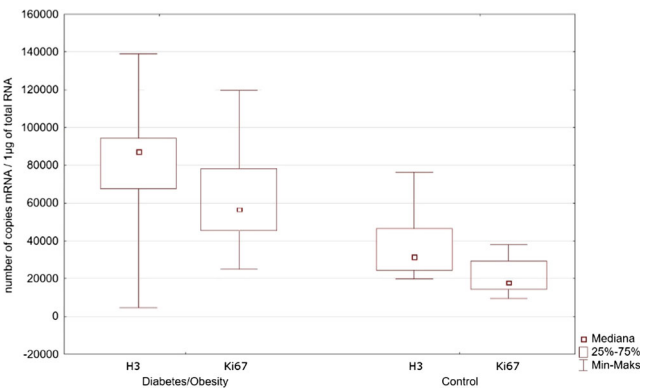
Background and aims: Adipose tissue derived stem cells (ADSCs) emerge as applicable and promising biological material for cell therapy of obesity, diabetes and associated complications. ADSCs are able to differentiate into brown adipose tissue or insulin-producing β-cells. On the other hand, some studies revealed lower ability of survival, regeneration and differentiation of ADSCs isolated from patients with long term type 2 diabetes with cardiovascular complications. It appears, therefore, important to define the group of diabetic patients that would still potentially benefit from autologous ADSCs treatment. The aim of our study was to assess the proliferation ability of ADSCs obtained from obese patients at early stage of type 2 diabetes with no cardiovascular complications.
Materials and methods: Peripheral adipose tissue was acquired by lipoaspiration from 9 obese patients with type 2 diabetes (3M and 6F; aged 45,3±12 years, diabetes duration 4,8±3,3 years, BMI median 39, HOMA-IR median 9,6) treated with metformin, and from 11 healthy control patients (4M and 7F; aged 41,4±16 years, BMI median 26, HOMA-IR median 1,3). The phenotype of ADSCs from each patient was confirmed by flow cytometry with the use of antibodies targeted against CD90, CD105, CD73 (positive markers). The cocktail of antibodies targeted against CD45, CD34, CD11b, CD79α and HLA-DR was used for negative markers analysis to fully confirm the identity of cells. Proliferation ability of ADSCs was measured by LDH, WST-1 and Sulforhodamine B (SRB) assays after 72h cultivation and also assessed by gene expression analysis of histone H3 and Ki67 with the use of RT-qPCR. For statistical analysis the Mann-Whitney U test was used ($\alpha=0.05$).
Results: The expressions of histone H3 and Ki67 were significantly upregulated in ADSCs obtained from patients with diabetes as compared with controls (both $p<0.001$; Fig. 1). In addition, the SRB assay revealed higher density of cellular protein content in the diabetic group ($p<0,05$).
Conclusion: Our results revealed higher proliferation ability of ADSCs obtained from obese patients with short term type 2 diabetes than from healthy subjects. While intriguing and promising, this outcome requires further, larger studies to elucidate its nature and verify its clinical significance.



Disclosure: **A. Witkowska:** None.

PS 037 Dietary effects on metabolism

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Protective effect of Resveratrol against cardiac and endothelial dysfunctions of type 2 diabetic female GK rat heart submitted to ischaemia-reperfusion injury

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Background and aims: Type 2 diabetic women have greater risk of mortality by cardiovascular (CV) disease than non-diabetic women. Particularly, type 2 diabetes (T2D) doubles the risk of myocardial infarction in women, but involved mechanisms are still not clear. Today anti-diabetic treatments allow a reduction in blood glucose but do not decrease patient's CV mortality, this is why new treatments need to be found. Resveratrol (RSV) is a polyphenol found in grapes reported to be beneficial on oxidative stress, endothelial dysfunction and inflammation involved in CV complications of T2D. Consequently we have investigated the effect of RSV on the tolerance to ischemia-reperfusion (IR) injury of type 2 diabetic female Goto-Kakizaki (GK) rat hearts by using multiparametric approach allowing simultaneous measurement of cardiac function, energy metabolism and endothelial function.
Materials and methods: 8-month-old female GK (FGK) rats and their age-matched respective controls female Wistar (FW, n=11) were used. FGK were divided in three groups: one without treatment (FGK-0, n=14) ; one with RSV (FGK-RSV, n=8) at the dose of 1 mg/kg/day in drinking water during 8 weeks ; and one with Placebo (FGK-P, n=9). Then, isolated rat hearts were perfused with 0.4 Mm palmitate, 3% albumin, 11 mM glucose, 3U/L insulin, 0.8 mM lactate and 0.2 mM pyruvate for 24 minutes before switching to 1.2 mM palmitate during 32 minutes low-flow (0.5 mL/min/g wet wt) ischemia. Next, flow was restored with 0.4 mM palmitate buffer for 32 minutes. High-energy phosphates and intracellular pH were measured during the experimental course by ^{31}P magnetic resonance spectroscopy with simultaneous measurement of contractile function. Coronary flow was measured before and after ischemia. Glucose and free fatty acids (FFA) were measured in plasma. Nitric oxide and Sirtuin pathways were studied in freeze-clamped tissues at the end of experiments. Creatine kinase and lactate dehydrogenase activities were also used as markers of myocardial damage.
Results: Glucose was significantly higher in FGK vs. FW ($p<0.0001$) while FFA were similar in the four groups. Heart to body weight ratio was also significantly higher in FGK vs. FW indicating cardiac hypertrophy ($p<0.0001$). Before ischemia, Rate Pressure Product (RPP), index of cardiac performance, was significantly lower in FGK vs. FW ($p<0.0001$), indicating an impaired cardiac function that was not improved by treatment with RSV. During reperfusion, RPP was significantly lower in FGK-0 and in FGK-P vs. FW ($p<0.001$), whereas RSV treatment completely restored cardiac function during reperfusion in FGK-RSV (ns vs. FW). Moreover, baseline coronary flow was similar in the four groups but during reperfusion, coronary flow was impaired in FGK-0 and FGK-P groups vs. FW (respectively $p<0.001$ and $p<0.01$), whereas RSV significantly re-established coronary flow in FGK-RSV to control values (ns vs. FW).
Conclusion: Female type 2 diabetic GK rat hearts exhibit greater sensitivity to IR injury characterized by a decrease of cardiac and endothelial functions. Oral 8-weeks treatment with RSV improved myocardial performance and coronary flow during reperfusion. Consequently, RSV might be an interesting therapeutic approach to improve survival to myocardial IR injury of type 2 diabetic women.
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Disclosure: **M. Desrois:** None.

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HIF1 complex in the hypothalamus: role in the development of obesity

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Background and aims: The balance between food intake and energy expenditure is mainly regulated by the hypothalamus. Dysregulation of this process is critical for the development of obesity. Hypoxia-inducible factor-1 (HIF1) is a transcription factor that activates several genes in response to hypoxia or other harmful conditions. Besides its importance in hypoxia conditions, HIF1 complex also plays a role in the regulation of glucose and energy homeostasis. Dysregulation of HIF1 complex could also be involved in the development of obesity and type 2 diabetes. Previously, it was reported that HIF1 can regulate the expression of POMC modulating food intake. In this work, we hypothesize that the consumption of high fat diet alters the expression of HIF1 in the hypothalamus with changes in the expression of neuronal POMC, and consequently altering the neuronal pathways that control food intake. Our main purpose was to analyze the expression profile of HIF1 in hypothalamus and evaluate whether high fat diet feeding changes the expression of this transcription factor. We also inhibited HIF1 in the arcuate nucleus to evaluate changes on high-fat diet consumption and body composition.
Materials and methods: We used male, 8-week old C57Bl6 mice, fed on chow or a high-fat diet for 1, 3, 7, 14 or 28 days. The expressions of HIF-1α and HIF-1β were measured by PCR and western blot and their hypothalamic distribution were evaluated by fluorescence microscopy. Inhibition of HIF-1β in arcuate nucleus of hypothalamus was performed using stereotaxic injection of shRNA lentiviral particles and animals were grouped under chow or high-fat diet for 14 days. Body mass, food intake and glycemia were evaluated throughout the experiments.
Results: HIF-1α and HIF-1β were mainly localized in the arcuate nucleus of the hypothalamus, and they were colocalized with POMC and with ACTH, but not with AgRP. These results indicate that HIF-1α and HIF-1β were only present in POMC neurons. As expected, we also observed that both proteins were colocalized with microglia and glial cell markers. The expression of mRNA of HIF-1α and HIF-1β significantly decreased after 3 and 7 days of high-fat feeding, returning to baseline levels after 14 and 28 days. However, the protein levels of HIF-1α significantly increased after 7, 14 and 28 days on high-fat diet consumption, with a decreased in the protein levels of VHL (E3ligase) which indicates an increase in HIF-1α stabilization. Mice with inhibition of HIF-1β in the arcuate nucleus and fed on chow, had an increase in body mass compared with control animals. This effect was more pronounced when animals were maintained on high-fat diet. However, food intake and glycemia were not affected by the inhibition of HIF-1β.
Conclusion: In summary, HIF1 complex is mainly expressed in POMC neurons in the arcuate nucleus of the hypothalamus. Although the mRNA expression of HIF-1α and HIF-1β decreased with high-fat diet feeding, its protein levels are increased indicating an increase in HIF-1α stabilization. These results point to a dysregulation of hypothalamic HIF1 complex associated with the consumption of high-fat diet, which can, at least in part, impact on changes in body mass. The increase in body mass without change in caloric intake in mice with hypothalamic inhibition of HIF complex, suggest that HIF1 complex could be involved in the regulation of energy expenditure. The role of HIF1 complex in the regulation of energy expenditure will be addressed in future studies.
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Disclosure: **J.M. Gaspar:** None.

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The role of the liver in the adaptibility to high dietary lipid intake in C57BL/6J mice

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Background and aims: Exposure to an 'obesogenic' environment results in body weight gain (BWG), which is however quite variable among individuals. While obesity and related insulin resistance are linked to changes in lipid metabolism in the liver, the differences in mitochondrial energy efficiency may also represent a substantial cause of the variability in BWG. Our previous study has revealed heterogeneous responses to obesogenic high-fat diet (HFD) in C57BL/6J (B/6J) mice. In spite of the identical genetic background, large variations in BWG, glycemic control and lipid profiles were observed. Therefore, B/6J mice represent a suitable model for studying the mechanisms underlying the propensity to obesity and associated disorders. In this study, B/6J mice were used to examine the adaptability of liver metabolism to HFD feeding and its dependence on gender.
Materials and methods: Both male and female B/6J mice (n=30 per gender) were fed HFD (lipids ~32 wt %) for 12 weeks, while BWG and changes in glucose tolerance were assessed. At the end of HFD intervention, liver samples were collected for further analyses. Mitochondrial metabolism was characterized in liver homogenates using Seahorse XF24. Levels of acylcarnitines (AC) were quantified by FIA-ESI MS/MS. Hepatic gene expression was analyzed by qPCR. Within each gender, mice with either the lowest (LG, low gainers) or the highest (HG, high gainers) BWG (n=8 per group) were selected for comparisons. Data evaluation was performed using t-test ($p< 0.05$). Both univariate (Spearman pairwise correlations) and multivariate (PLS-DA) statistical analyses were used to examine relationship between measured variables. Data are presented as mean±SE or fold change.
Results: BWG significantly differed between LG and HG group in both males (LG, 2.8±0.8 vs HG, 14.7±1.0 g) and females (LG, 5.4±0.6 vs. HG, 15.8±0.7 g). Despite higher overall BWG in females, only in males a strong relationship between BWG and obesity-related metabolic disturbances such as fasting blood glucose (r_s=0.86) and hepatic lipid content (r_s=0.61) was observed. Gene expression analysis identified changes related to BWG (e.g. upregulation of *Srebp-1c* and downregulation of *Pepck* in HG as compared to LG), which were similar in both genders. In contrast, only in males, propensity to obesity was associated with a decrease in expression of genes related to lipid catabolism (*Atgl*, 1.5-fold; *Crat*, 1.5-fold; *Vlcad* 1.2-fold in HG as compared to LG). Moreover, liver AC profiles, primarily reflecting catabolism of fatty acids, distinguished HG and LG groups in males but not in females, while the most discriminant metabolites were C20:4; C0; C5-OH; C3 and C5. In females, HG group exhibited upregulation of lipogenic genes, namely *Fasn* (1.4-fold) and *Hmgcs-1* (1.7-fold) in comparison to LG, which is not associated with increase of intrahepatic triglyceride content. Only in females, ADP-stimulated respiration in liver increased with BWG (1.3-fold), while the maximal respiration capacity remained unchanged.
Conclusion: Our results document that female B/6J mice are less susceptible to HFD-induced impairment of glucose homeostasis when compared with male mice. This difference is not caused by BWG, which is lower in male mice. Hepatic metabolism could be involved, since HFD-fed females exert relatively high adaptability of both the lipogenesis and mitochondrial oxidative phosphorylation in the liver.
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Disclosure: **J. Hansikova:** None.

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Differential contribution of glucose to triglyceride lipogenesis in visceral adipose tissue and liver following glucose/fructose supplementation

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Background and aims: Sugar consists of glucose and fructose monosaccharides which can both be utilized for triglyceride (TG) synthesis via *de-novo* lipogenesis (DNL) and high sugar intake is implicated in the development of non-alcoholic fatty liver disease (NAFLD). Under these conditions, increased liver TG (L-TG) is also strongly associated with expansion of visceral adipose tissue TG (VAT-TG). While DNL is considered to play a major in elevated L-TG, its contribution to VAT-TG is less clear. Liver and VAT are considered to have different capacities for utilizing glucose and fructose hence we hypothesized that they would contribute differently to the synthesis of new fatty acids in each tissue. We used a combination of deuterated water (^2H_2O) and [U-^{13}C]glucose to monitor the specific contribution of the glucose component of a glucose/fructose mixture to TG-fatty synthesis in VAT and liver.
Materials and methods: Five adult male C57BL/6 mice were kept on a 12/12h dark/light cycle and fed with standard chow and drinking water supplemented with 17.5% glucose and 17.5% fructose (w/v) for 24 weeks. At the start of the final night, mice were injected with 3g/100g body weight 99.9% ^2H_2O and the drinking water was replaced with 17.5% fructose and 17.5% glucose enriched to 50% with [U-^{13}C]glucose and 5% ^2H_2O. Mice were then allowed to feed naturally overnight. At the end of the dark period, mice were sacrificed, VAT and livers were freeze-clamped and VAT-TG and L-TG extracted and purified. Triglyceride positional ^{2}H enrichments and ^{13}C isotopomers were measured by ^{2}H and ^{13}C NMR. These data were integrated to yield the specific contribution of [U-^{13}C]glucose to the newly-synthesized FA components of VAT-TG and L-TG.
Results: In liver, [U-^{13}C]glucose contributed 9±3%, of newly-synthesized oleate, 10±3% of newly-formed palmitoleate and 19±5% of newly-synthesized C16 and C18 saturated fatty acids (SFA). In VAT, [U-^{13}C]glucose contributed 16±4% of newly synthesized oleate ($p<0.02$ *vs.* liver); 23±5% of newly-synthesized palmitoleate ($p<0.04$ *vs.* liver) and 57±10% of newly-synthesized SFA ($p<0.02$ *vs.* liver).
Conclusion: We demonstrate that in mice whose diet was supplemented with equimolar amounts of glucose and fructose in the drinking water, the glucose component was utilized to a greater degree for the synthesis of TG-fatty acids in VAT compared to the liver. This could reflect increased competition of the unlabeled dietary fructose for supplying lipogenic carbons in liver compared to VAT. Also, given that VAT supplies fatty acids to the liver, these data indicate that a portion of L-TG derived from [U-^{13}C]glucose may in fact been synthesized in VAT.
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Disclosure: **J.G. Jones:** None.

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A dietary switch from saturated to monounsaturated fat is protective against metabolic dysregulation, hyperinsulinaemia and beta cell dysfunction

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Background and aims: Saturated fatty acids (SFA) promote metabolic-inflammation and insulin resistance. Conversely, feeding a high-fat diet (HFD) enriched with monounsaturated fatty acids (MUFA), rather than SFA-HFD, induces less IL-1β mediated inflammation, insulin resistance and hyperinsulinemia. The purpose of this study was to determine if switching from a SFA-enriched HFD to a MUFA-HFD affected pancreatic islet function.
Materials and methods: Male C57BL/6 mice were fed one of three diets: 1) a low fat diet for 32 weeks (LFD; 10% kcal), 2) a SFA-enriched HFD for 32 weeks (SFA-HFD; 45% kcal) or 3) a SFA-enriched diet for 16 weeks followed by a MUFA-enriched diet for a further 16 weeks (MUFA-HFD; 45% kcal). In vivo glucose-stimulated (1.5g/kg) insulin secretion response was examined at baseline and

following the diet, where plasma insulin was obtained via tail vein bleed and analyzed by ELISA. HOMA-IR and HOMA-%β were calculated from fasting glucose and insulin levels. Pancreatic islets were isolated and gene expression was examined by real time RT-PCR with β-actin as the housekeeping gene. Glucose-stimulated insulin secretion from isolated islets was measured by ELISA. Pancreatic immunostaining assessed macrophage infiltration, insulin and IL-1β expression. Data was analyzed by 1-way or 2-way ANOVA, where appropriate with Bonferroni post-hoc comparisons.

Results: MUFA-HFD mice displayed attenuated hyperinsulinemia compared to the SFA-HFD group ($p<0.001$). Whilst HOMA-IR was elevated by both HFDs, the degree of insulin resistance was significantly lower following the MUFA-HFD at 20, 24 and 32 weeks. The insulin stimulatory index from islets isolated after the SFA-HFD was significantly (~50%) lower, compared to LFD mice. This decline was partially reduced in MUFA-HFD mice. Immunostaining demonstrated elevated pancreatic macrophage infiltration and IL-1β content in SFA-HFD mice compared to MUFA-HFD and LFD mice. Interestingly, markers of β-cell development and function (e.g., Nkx6.1, Pdk1, insulin) were significantly reduced (36%, 15% and 27%, respectively) in SFA-HFD mice compared to LFD and MUFA-HFD mice. Additionally, Ampk mRNA expression was reduced in islets isolated following the SFA-HFD but maintained in MUFA-HFD islets, which coincided with a 57% reduction in IL-1β in MUFA-HFD islets compared to both LFD and SFA-HFD fed mice. Expression of the disallowed gene Ldha was unaltered by diet.

Conclusion: While SFA-HFD mice may have somewhat compensated for the dietary challenge through hyperinsulinemia, our results suggest that a SFA-HFD induces the early-stages of islet and β-cell dysfunction. Importantly, switching to a MUFA-HFD partially prevented pancreatic dysfunction, further highlighting the importance of the type of dietary fat for overall health.

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Disclosure: **J.C. Ralston:** None.

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Differential fractional synthetic and elongation rates of hepatic saturated fatty acids and oleate from dietary [U-13C]glucose in healthy mice

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Background and aims: Diet-derived glucose and fructose are considered to be potent lipogenic substrates and may explain the association between increased sugar intake and soaring NAFLD incidence rates. However, there is little information on the precise contributions of these substrates to hepatic de novo lipogenesis. Current stable-isotope tracer methods quantify the fractional synthetic and elongation rates (FSR, FER) of liver fatty acids (FA) from all precursors but cannot resolve contributions from individual substrates. We combined deuterated water (^2H_2O) and [U-^{13}C]glucose to measure FSR and FER from this substrate under natural feeding conditions.

Materials and methods: At the start of the dark period in a 12h/12h dark/light cycle, four adult male C57/BL6 mice fed with standard chow were given an intraperitoneal injection of 99.9% ^2H_2O/0.9% NaCl to raise body water ^{2}H-enrichment to 3.5%. The drinking water was supplemented with 5% ^2H_2O, 17.5% w/w unlabeled fructose and 17.5% w/w glucose enriched to 50% with [U-^{13}C]glucose. Animals were allowed to feed naturally overnight and then sacrificed at the end of the dark cycle. Livers were freeze-clamped and triglycerides were extracted and purified from other lipid species and analyzed for ^{2}H and ^{13}C-enrichment by ^{2}H and ^{13}C NMR at 11.7T and 14.1T, respectively.

Results: ^{2}H-enrichment analysis indicated that the fraction of newly-synthesized FA from all lipogensis precursors was 33 ± 6% (mean ± S.D.). ^{13}C NMR provided resolved signals for methyl and carboxyl carbons of oleate, and saturated FA (palmitate + stearate) as well as their position in the glyceryl esterification sites (1,3-flanking or central). [U-^{13}C]glucose accounted for 18 ± 3% of newly-synthesized saturated FA, but only 9 ± 2% of newly synthesized oleate ($p<0.01$ *vs.* SFA). Its contribution to the elongation of saturated FA was negligible (0 ± 2%) while it made small but significant contribution to oleate formation via elongation (5 ± 2%, $p = 0.016$ *vs.* SFA). The distribution of FA between the glyceryl 1,3-flanking and central esterification sites was highly non-homogenous with oleate being the principal fatty acid occupying the center site, while the 1,3-sites were occupied by a ~40/60 mixture of oleate and SFA.

Conclusion: The integration of ^2H_2O and ^{13}C-enriched substrates coupled to ^{2}H/^{13}C-NMR analysis of triglyceride allows contributions of specific substrates to fatty acid synthesis and elongation rates to be determined. Glucose, in the presence of fructose, contributed different FSR and FER for oleate and SFA. Moreover, these FA were not evenly distributed among the glyceryl sites.

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Disclosure: **J. Silva:** None.

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The role of small intestine in differential metabolic effects of various lipid forms of dietary n-3 fatty acids

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Background and aims: *n-3* polyunsaturated fatty acids (Omega-3) are known to exert hypolipidemic and anti-inflammatory effects. Previous studies have suggested that metabolic responses to Omega-3 depend on the lipid form of their administration, with phospholipids showing better efficacy than triacylglycerols, especially with regard to the effect on glucose homeostasis. The aim of this study was to investigate in dietary obese mice, whether this difference in efficacy might result already from changes in cellular and metabolic processes at the level of small intestine.

Materials and methods: Four groups of male C57BL/6NCrl mice were fed for 8 weeks a corn oil-based high-fat diet (cHF; lipids ~35% wt/wt), as well as cHF diet supplemented with Omega-3 (~30g EPA+DHA/kg diet) in the form of fish oil (i.e. triacylglycerols; TG diet) or Krill oil (i.e. phospholipids; PL-H diet). Moreover, Krill oil was also supplemented in the amount matching that of fish oil in the TG diet (i.e. PL-L diet), thus containing only ~10g EPA+DHA/kg diet. At week 7 of the study mice underwent intraperitoneal glucose tolerance test. At the end, total RNA was isolated from samples of small intestine, reverse transcribed, and hybridized to SurePrint G3 Mouse Gene Expression v2 8x60K Microarrays (Agilent).

Results: Mice in the PL-H group gained less body weight when compared to cHF (PL-H, 5.8±1 vs. cHF, 14.4±0.6 g; $p<0.05$ by ANOVA) and both PL groups had improved glucose tolerance assessed as incremental area under the glucose curve (AUC; PL-H, 899±77 vs. PL-L, 2260±193 *vs*. cHF, 2670±186 mmol/180min; $p<0.05$ vs. cHF for both PL groups). Blood glucose levels in the fed state were decreased only in PL-H group (PL-H, 9.43±0.42 vs. cHF, 11.98±0.81 mmol/l; $p<0.05$). In the small intestine, the most regulated pathways, as compared to cHF, included: (i) lipid metabolism (all groups), (ii) cytoskeleton remodeling (TG and PL-L), (iii) leptin signaling and macrophage regulation (TG), (iv) gastrin and VEGF signaling and immune response signaling pathways including NFkB and IL-16 (PL-L), and (v) peroxisomal fatty acid oxidation, retinol and glutathione metabolism and ketone bodies biosynthesis (PL-H).

Conclusion: Dietary Omega-3 efficiently regulated intestinal lipid metabolism and cellular processes depending on their lipid form. Our findings support the idea that changes of intestinal lipid metabolism might underlie differential metabolic efficacy of various lipid forms of Omega-3 at the whole-body level.
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Disclosure: **P. Kucharikova:** None.

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Vitamin-D improves insulin sensitivity in high fat diet induced diabetic mice by downregulating fetuin-A gene expression

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Background and aims: Vitamin-D(VD) deficiency is associated with a chronic low grade inflammation (metaflammation) in adipose tissue that leads to insulin resistance. While VD supplementation improves insulin sensitivity and may prevent progression from prediabetes to T2DM, the underlying mechanism by which VD ameliorates insulin resistance(IR) is not clear. Fetuin-A (FetA), a hepato-adipokine expressed through NF-kB pathway, induces adipose tissue inflammation and IR by proinflammatory polarization of resident macrophages that leads to production of inflammatory cytokines. Our recent unpublished data shows a significant inverse correlation between serum levels of fetA and VD in insulin resistant human subjects with various degrees of glucose intolerance. This observation prompted us to check whether VD has any regulatory influence on FetA gene expression that might impact insulin sensitivity.
Materials and methods: In the in vitro study, preincubated hepatocytes isolated from Balb/c mice (n=6) for 1hr with incremental doses of calcitriol (50, 75 & 100 nM), followed by addition of FFA (0.75mM) for 4hrs by keeping one positive control(only palmitate) & one negative control(only vehicle). For the in vivo study, three groups of high fat diet treated (HFD) Balb/c mice were taken (n=6). Group-A comprised of six HFD mice treated with intraperitoneal injection of 25-hydroxy cholecalciferol (25OHD) at a dose of 5 μg/kg body weight every third day for 9 weeks. Group-B comprised of six HFD mice not treated with 25OHD& a third group of Balb/c mice treated with standard diet (Group-C). Insulin sensitivity was assessed from all the three groups of mice by oral glucose tolerance test (OGTT), insulin tolerance test (ITT) and ^{14}C-2-DOG uptake. Gene expression and protein levels of FetA, proinflammatory cytokines and insulin signaling molecules were determined by qPCR and immunoblot analysis from all the groups. NF-kB binding to FetA promoter was determined by ChIP and promoter-reporter activity assay.
Results: VD treatment of hepatocytes abrogated palmitate induced increase in the mRNA ($p<0.01$) and protein levels of FetA and proinflammatory cytokines as revealed by qPCR analysis and immunoblot respectively. VD induced FetA suppression in group A appeared to be due to NFκB deactivation as evidenced by immunofluorescenece and immunoblot analysis. VD markedly reduced phosphorylated NFκB levels in HFD mice (Group A). ChIP assay and NFκB promoter-reporter activity analysis showed, hepatocytes and adipocytes isolated from mice belonging to Group A have greater depletion in Fet-A and proinflammatory cytokine expression compared to Group B. VD also significantly augmented adiponectin gene expression ($p<0.01$) and protein levels in adipocytes isolated from Group A mice. The rise in plasma adiponectin levels($p<0.01$) was associated with improved insulin sensitivity, leading to significant reduction in serum levels of triacylglcerol, nonesterified fatty acids (NEFA) and cholesterol in Group A mice.
Conclusion: Our investigation shows VD induced down regulation of Fet A gene through NF-Kb pathway resulted in significant improvement in insulin sensitivity, probably due to upregulation of adiponectin and suppression of anti inflammatory cytokines in VD treated HFD mice.
Supported by: West Bengal DST
Disclosure: **S. Ali Mondal:** None.

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Dietary fat oxidation is elevated in middle-aged type 2 diabetes

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Background and aims: Older individuals have increased delivery of endogenous fat to skeletal muscle, which may predispose to insulin resistance if fat oxidation is reduced. Likewise, insulin resistance increases the delivery and storage of dietary and endogenous fat in skeletal muscle. The aim of the present study was to investigate the effect of type 2 diabetes (T2D) and age on dietary fat oxidation.
Materials and methods: Seven middle age (46.0 ± 1.1 y) and seven older (63.4 ± 0.6 y) patients with T2D (metformin and/or diet control for 4.8 ± 1.1 y) were matched with seven middle age (46.1 ± 2.9 y) normoglycaemic controls for BMI (31.1 ± 1.5; 29.6 ± 1.1; 29.6 ± 1.3 kg/m^2, respectively), % body fat by DXA (28.5 ± 2.3; 28.2 ± 4.0; ± 30.6 ± 1.2 %, respectively), and habitual physical activity (measured and self-reported). Subjects were prescribed a eucaloric diet (Henry equation) for 72 h preceding a fasted oral glucose tolerance test (OGTT). During this time, interstitial glucose was continuously measured using a subcutaneous probe. Twenty-four h before the OGTT, subjects consumed a meal replacement drink (330 kcal: 44g carbohydrate, 11g fat, and 14g protein) containing 15 mg/kg [$^2H_{31}$]palmitate and 0.2 g/kg [^{18}O]water and were asked to collect all passed urine for the following 10 h. Indirect calorimetry was performed to determine respiratory exchange ratio (RER) before and at the end of the 120 min OGTT. Blood samples were taken every 15 min throughout the OGTT for measurement of blood glucose, serum insulin and free fatty acid (FFA) concentration. Urine samples were analysed for ^{2}H/^{1}H and ^{18}O/^{16}O isotope ratios by infrared spectroscopy in order to determine dietary fat oxidation and total body water, respectively. Two-way and one-way ANOVA was used to detect any differences in the blood and urine measurements, respectively. Data are presented as mean ± SEM.
Results: Average blood glucose during the OGTT was greater ($P<0.001$) in T2D (11.9 ± 0.9 and 10.7 ± 0.8 mmol/L for middle age and older, respectively) than control (7.6 ± 0.5 mmol/L), and average serum insulin was almost half (50.3 ± 5.05 mIU/L and 56.0 ± 7.15 mIU/L vs. 93.9 ± 11.5 mIU/L, respectively; $P=0.09$). Average 24 h interstitial glucose was also higher in T2D (9.9 ± 0.18 and 7.6 ± 0.14 mmol/L for middle age and older, respectively) compared to control (6.4 ± 0.07; $P<0.001$). There was a trend ($P=0.08$) for 10 h dietary fat oxidation to be greater in middle age T2D (22.6 ± 1.6 % recovery of dose) than older T2D (13.9 ± 3.3 %) and control (13.4 ± 2.7 %). Furthermore, fasting plasma FFA in middle age T2D (0.39 ± 0.05 mmol/L) was lower ($P=0.05$) than older T2D (0.48 ± 0.05 mmol/L) and control (0.49 ± 0.05 mmol/L), but higher ($P<0.05$) at the end of the OGTT (0.10 ± 0.03 vs. 0.05 ± 0.01 and 0.04 ± 0.01 mmol/L, respectively). RER increased during the OGTT in all groups ($P<0.001$) from similar fasted values (0.74 ± 0.05, 0.75 ± 0.04, and 0.74 ± 0.03 for controls, middle age T2D, and older T2D, respectively) to similar fed values (0.82 ± 0.03, 0.83 ± 0.02, and 0.84 ± 0.03, respectively).
Conclusion: Despite similar fasting fat oxidation, dietary fat oxidation was increased in middle age T2D compared to age matched controls. Combined with an inability to suppress plasma FFA with feeding, this suggests excessive postprandial fat delivery to skeletal muscle in T2D. The lower dietary fat oxidation in older T2D does not appear to exacerbate insulin resistance.
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Disclosure: **F.B. Stephens:** None.

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Higher serum levels of omentin-1 are associated with increases in glycaemia and incident type 2 diabetes: KORA F4/FF4 study

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Background and aims: Cross-sectional studies found that higher levels of the novel adipokine omentin-1 were associated with higher adiponectin and lower levels of risk factors for type 2 diabetes, but its relevance for incident type 2 diabetes is currently not understood. Therefore, this study investigated the associations between serum omentin-1, changes in glycaemia (fasting glucose, 2-hour glucose, HbA1c) and incident type 2 diabetes and compared them with adiponectin.
Materials and methods: The study was based on the population-based Cooperative Health Research in the Region of Augsburg (KORA) F4/FF4 cohort. Associations of baseline serum omentin-1 and adiponectin with changes in glycaemia were assessed in 471 non-diabetic participants, associations between both adipokines and incident type 2 diabetes in 76 cases and 430 non-cases (follow-up 6.5 years). Multivariable linear and logistic regression models were adjusted for multiple potential confounders.
Results: Higher serum levels of omentin-1 were associated with increases in fasting glucose, 2-hour glucose and HbA1c (all $p<0.001$) and with incident type 2 diabetes (adjusted OR (95% CI) 1.40 (1.03; 1.90) per SD of log2-transformed omentin-1; $p=0.032$). These associations were independent of potential confounders including adiponectin. In contrast, adiponectin levels showed inverse associations with changes in 2-hour glucose ($p=0.046$) and HbA1c ($p=0.041$), and were also inversely associated with the risk of type 2 diabetes (OR (95% CI) 0.60 (0.42; 0.85) per SD of log2-transformed of adiponectin; $p=0.004$).
Conclusion: Thus, the direction of the longitudinal associations between omentin-1 and glycaemia differed markedly from the direction observed in previous cross-sectional studies. It can be hypothesised that elevated omentin-1 levels at baseline indicating a higher risk of type 2 diabetes are the results of a counterregulatory mechanism in the face of metabolic and/or proinflammatory risk factors of type 2 diabetes.
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Disclosure: **C. Herder:** None.

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Inverse association between fasting insulin levels and postprandial changes of plasma asprosin concentration in patients with type 2 diabetes

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Background and aims: Asprosin, a fasting-induced protein secreted by adipose tissue, has recently been discovered as a glucogenic hormone that promotes hepatic glucose release in mice and humans. While circulating Asprosin levels are reported to physiologically decrease after feeding, the pathophysiological role of Asprosin in patients with glucose intolerance remains poorly understood. The aim of this study was to evaluate the relationship between Asprosin postprandial concentration kinetics and other biomarkers.
Materials and methods: 11 healthy subjects and 23 type 2 diabetes mellitus (T2DM) patients underwent a 2-h meal tolerance test in the morning after an overnight fast; the meal consisted of 460 kcal of total caloric load with 56.5 g of carbohydrates, 18 g of protein and 18 g of fat. Blood samples were collected immediately before and 2 hrs after meals. HbA1c, fasting plasma glucose (FPG), total cholesterol, triacylglycerol, and other biomarkers were measured in all subjects. Plasma Asprosin levels was determined by enzyme-linked immunosorbent assay according to the manufacturer's protocol (Eiaab, Catalogue No.E15190h). Correlations were evaluated by Spearman's rank test. P values <0.05 were considered statistically significant.
Results: Fasting Asprosin levels in healthy subjects were significantly less than those in T2DM patients ($p < 0.05$). In T2DM patients, postprandial reduction of Asprosin levels showed a significant negative association with fasting insulin levels ($r = -0.41$ $p < 0.05$). No significant correlation was found between Asprosin levels and other biomarkers including FPG.
Conclusion: To our knowledge, this is the first report of a negative correlation of fasting insulin levels with postprandial reduction in Asprosin levels and higher fasting Asprosin levels in T2DM patients than healthy controls. Our results suggest that insulin resistance could be associated with the regulation of circulating Asprosin levels.
Disclosure: **S. Tokumoto:** None.

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Lipolysis-associated tumor necrosis factor induces Syndecan 4 upregulation in the subcutaneous adipose tissue one year after bariatric surgery

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Background and aims: Syndecan 4 (SDC4) is a membrane-bound extracellular matrix protein relevant for inflammation, focal adhesion, and LDL-uptake. We recently showed that tumor necrosis factor (TNF) is highly upregulated in the subcutaneous adipose tissue one year after bariatric surgery, in the context of increased lipolysis, whereas other inflammatory cytokines were downregulated. Since TNF was described to induce SDC4 in an endothelial-like cell line, we wanted to investigate whether TNF and lipolysis may influence SDC4 expression in the adipose tissue after weight-loss.
Materials and methods: We analyzed subcutaneous adipose tissue (SAT) gene expression from 31 non-diabetic morbidly obese patients (BMI>40 kg/m^2) shortly before and one year after bariatric surgery and compared these to a lean control group. Genes relevant to lipid metabolism, insulin sensitivity and inflammation were analyzed by RT-qPCR. Independent predictors were determined by regression analysis. Primary adipocytes were stimulated in vitro for 24 hours and SDC4 gene expression was determined.
Results: Compared to the lean group, SDC4 was upregulated by 170 % ($p<0.05$) before and by 2000 % ($p<0.001$) one year after bariatric surgery. TNF expression and serum TNF were positive predictors for the postoperative SDC4 expression. Furthermore, TNF significantly upregulated SDC4 expression in cultured preadipocytes.
Conclusion: The TNF-induced upregulation of SDC4 in SAT from patients one year after bariatric surgery points to an important role of SDC4 and TNF in the context of weight-loss and lipolysis.
Disclosure: **T.M. Stulnig:** None.

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The role of acyl ghrelin in human visceral adipose tissue in relation to the LXR-ABC pathway and the metabolic state

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Background and aims: Peroxisome proliferator-activated receptor gamma (PPAR-γ) induces the removal of cellular lipids by high density lipoproteins via the activation of liver X receptor isoform β (LXRβ) and in turn ATP binding cassette G1 (ABCG1). Centrally acting acyl ghrelin has been implicated as having a detrimental effect on the transcription of the PPAR-LXR-ABC pathway, resulting in increased white adipose tissue depots. However, the relationship between ghrelin, lipid retention and lipid biosynthesis genes are disputed amongst published studies. Visceral adiposity increases hepatic free fatty acid oxidation which has been implemented in triggering insulin resistance and increased glucose output. The aim of the present study was to clarify and translate contradictory theories about acyl ghrelin as a mediator of lipid homeostasis and insulin resistance within human visceral adipose tissue (hVAT).

Materials and methods: 30 hVAT biopsies taken from the greater omentum and EDTA blood samples were collected during routine abdominal surgery (Non-obese (NO)=10, Obese (O)=10 and Obese with Type 2 diabetes (OT2D)=10). RNA extracted from hVAT underwent Real Time PCR and analysed using the $2^{-\Delta\Delta CT}$ gene expression method, normalised against a β-actin housekeeper. Lipid profiles were analysed in fasted plasma samples via a standard colorimetric assay. Acyl ghrelin was measure in plasma samples that were treated with a protease inhibitor, 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride, using a human ghrelin ELISA.

Results: Samples were split into two arms for data analysis to enable investigation of metabolic state (i) obesity effect ([OT2D+O] v NO) and (ii)diabetes effect ([O+NO] v OT2D. The analysis of obesity effect gene expression data indicates no overall gene expression alterations. However, analysis of the diabetes effect data indicates a marked down-regulation in LXRβ, ABCG1 and ghrelin (2.2, 2.2 and 2.1-fold change, respectively). Fasted acyl ghrelin levels were significantly decreased in OT2D samples compared to both O (OT2D v O: 228.5 [98-439] pg/mL v 515.5 [309-701] pg/mL, $p<0.05$) and NO individuals (OT2D v NO: 228.5 [98-439] pg/mL v 467.2 [326-508] pg/mL, $p<0.05$). Plasma acyl ghrelin was negatively correlated with plasma glucose levels ($r_s(29)=-0.37$, $p<0.05$) and body weight ($r_s(29)=-0.42$, $p<0.05$). A positive correlation was demonstrated between plasma acyl ghrelin levels with total cholesterol ($r_s(29)= 0.38$, $p<0.05$) and LDL ($r_s(29)= 0.39$, $p<0.05$).

Conclusion: Correlation of acyl ghrelin with key lipid profile markers allows us to determine that in a low acyl ghrelin environment there is a decrease in plasma lipid profiles and a decline in expression of the LXR-ABC pathway. OT2D had a significantly decreased level of circulating ghrelin, which was dependent upon plasma glucose levels and independent of octanyol modification genes. With increased endogenous glucose levels present in OT2D already being shown to increase cellular lipid concentrations, data suggests that the cellular export mechanism to counterbalance this lipid increase is being stalled due to a decline in acyl ghrelin levels. These findings correspond to the observed low plasma lipid concentrations due to hypertrophy trapping lipids within the cell and lowering the rate of release into the circulation independent of statin usage.

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Reduction of superficial subcutaneous adipose tissue thickness, mitochondrial efficiency and stearic-to-palmitic acid ratio in type 2 diabetes

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Background and aims: Human whole abdominal subcutaneous adipose tissue (WSAT) is divided by Scarpa's fascia in two putative metabolically distinct subdepots, referred to as deep (DSAT) and superficial (SSAT) adipose tissue layers. Previous studies showed that whole body insulin sensitivity (IS) correlates negatively with abdominal DSAT/WSAT thickness and positively with both muscle mitochondrial function and lipogenesis in WSAT of glucose-tolerant humans (CON). This study aimed to characterize differences between the two WSAT layers by assessment of SSAT/WSAT thickness, mitochondrial activity/efficiency and stearic-to-palmitic acid ratio (18:0/16:0), as marker of lipogenesis, in SAT of male patients with type 2 diabetes (T2D).

Materials and methods: We included 14 T2D and compared them to 14 CON matched for body mass index (BMI), age and WSAT thickness (32 ±1 kg/m^2, 53±2 yrs, 29±2 mm vs. 31±1 kg/m^2, 53±2 yrs, 27±3 mm). All participants underwent euglycemic-hyperinsulinemic clamp tests to assess M-values, as a measure for IS, as well as ultrasound imaging of SSAT and DSAT thickness at the level of rectus abdominis muscle, validated by single-slice magnetic resonance imaging. Ultrasound guided biopsies were performed to obtain targeted samples of SSAT and DSAT to assess 18:0/16:0 by gas chromatography-mass spectrometry. Furthermore, maximal mitochondrial oxidative capacity and mitochondrial efficiency, reflected by respiratory control ratio (RCR=state 3/state 4), was assessed via high-resolution respirometry in biopsy samples of both depots.

Results: T2D had lower 18:0/16:0 in SSAT than CON (0.15±0.01 vs. 0.18±0.01, $p<0.05$). The comparison of both depots in T2D showed lower 18:0/16:0 in SSAT than in DSAT (0.15±0.01 vs. 0.17±0.01, $p<0.01$). IS was 41% lower and SSAT/WSAT thickness was 33% lower in T2D compared to CON ($p<0.001$ respectively). However, 18:0/16:0 and SSAT/WSAT did not correlate with M-value. RCR, as marker of mitochondrial efficiency, was 33% lower in SSAT of T2D compared to SSAT of CON ($p<0.001$). Furthermore, maximal oxidative capacity of SSAT correlated positively with M-value ($r=0.80$, $p<0.001$) in CON upon adjustment for age and BMI, whereas maximal oxidative capacity was similar in both depots.

Conclusion: In T2D abdominal WSAT is composed of two distinct subdepots. The decrease of SSAT thickness, mitochondrial efficiency and stearic-to-palmitic acid ratio in T2D compared to CON point the presence of an unfavorable subphenotype in SSAT of T2D.

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Differences in abdominal and femoral adipose tissue oxygen tension contribute to the adipose tissue phenotype in overweight and obese women

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Background and aims: Upper and lower-body adipose tissue (AT) depots exhibit opposing associations with obesity-related cardiometabolic diseases. AT oxygen tension (pO_2) may be a key player in AT dysfunction. We compared *in vivo* abdominal (ABD) and femoral (FEM) subcutaneous AT pO_2 in overweight/obese women, and investigated *in vitro*

depot-specific effects of physiological AT pO_2 on adipocyte metabolism and inflammation.

Materials and methods: ABD and FEM subcutaneous AT pO_2 were assessed in 8 well-phenotyped overweight/obese (BMI 34.4±1.6 kg/m^2) post-menopausal women with impaired glucose metabolism using an optochemical measurement system. AT blood flow (ATBF) was measured (^{133}Xe wash-out), and ABD and FEM AT biopsies were collected to determine adipocyte morphology and AT gene expression. The effects of prolonged exposure (14d) to physiological human AT pO_2 on adipogenic differentiation, adipokine expression/secretion, mitochondrial respiration and glucose uptake was investigated in differentiated human multipotent adipose-derived stem cells, derived from the same individuals.

Results: AT pO_2 was significantly higher in ABD than FEM AT (62.7 ±6.6 vs. 50.0±4.5 mmHg, P=0.013), whereas ATBF was comparable between depots (1.8±0.3 vs. 2.8±0.5 ml•100g tissue^{-1}•min^{-1}, respectively, P=0.122). Oxygen consumption was significantly lower in differentiated ABD than FEM human adipocytes for all pO_2 exposure regimens. Low physiological pO_2 (5% O_2) significantly decreased pro-inflammatory gene expression, increased basal glucose uptake and altered adipokine secretion in differentiated ABD and FEM adipocytes.

Conclusion: We demonstrated for the first time that AT pO2 is higher in ABD than FEM subcutaneous AT in overweight/obese women, likely due to lower oxygen consumption in ABD adipocytes. Moreover, physiological pO2 exposure markedly affects adipocyte functionality in a depot-independent manner, suggesting that altered pO2 may contribute to AT dysfunction in human obesity.

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CDKN2C expression is low in type 2 diabetes and associated with reduced lipid storage capacity in subcutaneous adipose tissue and elevated free fatty acid levels

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Background and aims: We have recently reported that impaired adipose tissue (AT) lipid storage, but not altered lipolysis, contributes to increased fatty acid levels in type 2 diabetes (T2D). CDKN2C (Cyclin Dependent Kinase Inhibitor 2C) is a cell growth regulator that controls cell cycle progression and has previously been associated with beta-cell differentiation, adipogenesis and obesity. A recent integrative genome-wide analysis study (GWAS) also implicates CDKN2C as a putative effector gene associated with insulin resistance phenotypes, risk for T2D and impaired peripheral AT storage capacity. This study aims to explore CDKN2C as a possible mediator for lipid storage defects in T2D subjects.

Materials and methods: 20 control and 20 metformin-treated T2D (HbA1c 6.6 ± 0.3%, mean±SD) subjects were matched for gender (10M/10F), age (58 ± 11 vs 58 ± 9 y) and BMI (30.8 ± 4.6 vs 30.7 ± 4.9 kg/m^2). Subcutaneous AT (SAT) samples were obtained by abdominal needle biopsies to measure mRNA of genes related to lipid storage and adipogenesis. In vivo lipolysis was assessed during a 3 h-OGTT with plasma glycerol and free-fatty-acids (FFA) levels. Magnetic resonance imaging (MRI) was performed for body fat measurements.

Results: *CDKN2C* mRNA expression in SAT was down-regulated in the T2D group compared with the control group (P<0.05). In the T2D group, *CDKN2C* expression was negatively correlated with FFA AUC during OGTT (r=-0.57, P<0.01) and with glycemia and insulin resistance (HbA1C, glucose AUC during OGTT, QUICKI; all P<0.05). In addition, there was a negative association with WHR, visceral AT (VAT) volume, VAT/SAT ratio and liver fat percentage (P<0.01) in the T2D group, but no association with BMI was found. In the control group, only VAT volume (P=0.001) and VAT/SAT (P<0.05) were negatively correlated with *CDKN2C* expression. In multivariate analyses, VAT/SAT (st beta coeffi= -0.674, P<0.001) and FFA AUC (st beta coeffi = -0.486, P<0.001) were significantly associated with *CDKN2C* expression (P<0.001, adjusted R^2 for model=0.79) in the T2D group, suggesting a link between CDKN2C and higher circulating levels of FFA, as well as preferential fat deposition in visceral rather than subcutaneous stores. Furthermore, *CDKN2C* expression positively correlated with the expression of genes promoting lipid storage (e.g. *CIDEC*, *FASN*, *DGAT1/2*, *FABP4*, *PC, PPARG*, *LPL*, *LIPE*, *CD36*; all P<0.01). Look-up in GWAS databases showed that a common variant in *CDKN2C* (rs12855, C-allele) was nominally associated with lower body weight (P<0.001), BMI (P<0.01), but with higher HbA1c (P<0.001), WHR adjusted for BMI (P<0.01), and risk for T2D (P<0.001) and coronary artery disease (P<0.01). In publicly available datasets, CDKN2C is highly expressed in AT compared to other tissues (GEO database), and shows increasing expression during adipocyte differentiation (FANTOM5 database).

Conclusion: *CDKN2C* gene expression is down-regulated in T2D and is associated with elevated plasma fatty acids and visceral adiposity. Our findings suggest that CDKN2C might be an important regulator of lipid storage and fatty acid turnover. Its downregulation in T2D might contribute to insulin resistance, impaired SAT lipid storage and to redistribution of AT from subcutaneous to visceral depots.

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Disclosure: **M.J. Pereira:** None.

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Global protein profiling of visceral adipose tissue among type 2 diabetic and non-diabetic morbidly obese patients

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Background and aims: The accumulation of visceral fat has been shown to be highly associated with increased risk of obesity, metabolic dysregulation and type 2 diabetes (T2D). Proteomic analysis of visceral fat from obese non-diabetic (Non-T2D) and obese T2D subjects might identify factors in visceral adipose tissue linked to diabetes.

Materials and methods: In this study, we used a straightforward label-free quantitative proteomics approach to analyze visceral fat tissue in type 2 diabetic and non-diabetic participants that are morbidly obese with body mass index above 40 kg/m2. A total of 18 biopsies, including 9 Non-T2D and 9 T2D, age, sex and BMI matched were analyzed.

Results: Proteomics analysis identified more than 1500 unique protein groups in our study with FDR at 0.01 and minimum 2 unique peptides. One hundred thirty-three protein groups show at least 2-fold differences between Non-T2D visceral fat and T2D visceral, including 53 protein groups with significant change (pglt 0.05). Of particular interest, we identified 6 proteins (Proteasomal ubiquitin receptor ADRM1, Signal recognition particle 14 kDa protein, Fatty acid synthase, Proteasome inhibitor PI31 subunit, Serine/threonine-protein kinase OSR1, and Switch-associated protein 70) with 4-7 fold increase in the visceral adipose tissue of patients with T2D when compared to viscera fat from non-diabetic controls. Pathway analysis for these 53 proteins indicated that multiple pathways in involved in oxidative stress, proten synthesis and degradation are significantly enriched, such as NRF2-mediated Oxidative Stress Response, mTOR Signaling, EIF2 Signaling and Protein Ubiquitination Pathway.

Conclusion: We have performed proteomics analysis on visceral fat tissues in non-T2D and T2D subjects, and identified new abnormality in multiple proteins and pathways in T2D visceral fat, which may increase our understanding of the pathogenesis of type 2 diabetes and insulin resistance.

Disclosure: **B. Seyoum:** None.

PS 039 Pharmacological and non-pharmacological effects on metabolic phenotypes

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Negative effect of exogenous methylglyoxal on metabolic changes in visceral adipose tissue of metabolic syndrome model

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Background and aims: Methylglyoxal (MG) is a highly reactive metabolite excessively produced in connection with metabolic disoders such as hyperglycaemia and dyslipidemia. Excessive dicarbonyl production contributes to metabolic and vascular changes in many tissues through participation in inflammatory processes, disturbed regulatory mechanisms and contribution to the development of organ complications. Recent studies suggest that MG accumulation in adipocytes may lead to structural and functional changes in adipose tissue. Here, we used transcriptomic analysis to investigate the effect of MG on metabolic changes in visceral adipose tissue of Hereditary Hypetriglyceridemic (HHTg) rats, a non-obese metabolic syndrome model with dyslipidemia, insulin resistance and ectopic lipid deposition.
Materials and methods: 5-month-old male HHTg rats were fed standard diet and treated with MG intragastrically (0.5 mg/kg b.wt., 3x/week) for 4 weeks. Commercial/ELISA kits, Multiplex Assay, HPLC-method and gass chromatography were used for analysis. Transcriptomic analysis was performed using microarray, gene expressions by RT-PCR.
Results: MG administration was associated with increased glyceamia (+23.8 %, $p<0.001$), insulinaemia (+109.3 %, $p<0.05$), cholesterolaemia (1.19 ± 0.07 *vs* 1.59 ± 0.99 mmol/l, $p<0.05$), and impaired glucose tolerance ($AUC_{0\text{-}120}$: + 8.2%, $p<0.05$). Compared to controls, MG increased serum levels of pro-inflammatory factors MCP-1 and TNF-alpha, while serum adiponectin and leptin levels were not affected. Adipose tissue insulin sensitivity, measured *ex vivo* according to basal and insulin-stimulated ^{14}C-U-glucose incorporation into lipids, was markedly impaired ($p<0.05$) after MG administration. MG-treated rats exhibited strongly impaired fatty acid composition in visceral adipose tissue phospholipids. The proportion of saturated fatty acids, especially palmitic (16:00) and myristic (14:00) acid, was significantly increased together with decreased proportion of PUFA n-3 ($p<0.001$) suggesting a possible negative influence on the membrane fluidity and insulin signaling. MG also significantly reduced relative mRNA expression of transcription factor *Nrf2* (-16.7%, p<0,01) which controls antioxidant and lipogenic genes and increased relative mRNA expression of pro-inflammatory factor *Mcp-1*. Comparative transcriptomic analysis identified 61 differently expressed genes (FDR<0.05) in visceral adipose tissue after MG administration. Network analyses revealed these genes to be overrepresented in insulin signaling regulation (*Irs1, Igf2, Ide*), lipid metabolism (*Nr1d1, Lpin1, Lrpap1*) and apoptosis (*Bcl6, Irs1, Tp53inp1*).
Conclusion: In a rodent model of metabolic syndrome, methylglyoxal administration markedly impaired insulin sensitivity and fatty acid composition of white adipose tissue and increased inflammatory parameters on both transcriptomic and metabolic level suggesting a possible role of MG in the induction of the features of metabolic syndrome.
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Disclosure: **M. Hüttl:** None.

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7-ketocholesterol regulates inflammation in mesangial cells: potential role in diabetic nephropathy

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Background and aims: In type 2 diabetes, it is known that oxysterol is greatly involved in various organ disorders and also in diabetic nephropathy. The concentration of 7-ketocholesterol (7-KCHO), which is representative of oxysterols, is reported to increase in the blood of patients with type 2 diabetes, and it is also suggested that 7-KCHO is involved in the development of diabetic nephropathy. However, molecular mechanism of 7-KCHO for the development of diabetic nephropathy has not been sufficiently elucidated. Interestingly, lipoxygenase (LOX) and cyclooxygenase (COX), those are enzymes of arachidonic cascade, are also reported to be involved in the development of diabetic nephropathy. In order to clarify the roles of 7-KCHO in diabetic nephropathy, we studied the effects of 7-KCHO on cytotoxicity, inflammatory gene expression of IL-1β and Il-6, and arachidonic cascade gene expression of LOX and COX using human mesangial cells (MCs).
Materials and methods: Human MCs were purchased from Lonza (Basel, Switzerland). We used the cells between the three and eighth passages. Cell viability was determined by the WST-8 assay. Reverse transcription polymerase chain reaction was performed to examine the expression of each message. Detection of intracellular ROS production was evaluated by flow cytometry.
Results: Although 10 or 30μM of 7-KCHO did not affect cell proliferation of MCs at 24 and 48 hours, COX-2, 12-LOX and IL-6 mRNA expression were significantly stimulated by 30μM of 7-KCHO at 24h, and the increment of IL-1β mRNA expression was observed 48h after the treatment. Importantly, 5-LOX and 15-LOX mRNA expression were not detected with or without the treatment of 7-KCHO. Reactive oxygen species (ROS) in MCs was assessed by flow cytometry. 7-KCHO 30 or 50μM induced a dose-dependent increment of ROS. In contrast, addition of N-acetylcysteine (NAC) 5mM caused a decrease in ROS production to inhibit the mRNA expression of COX-2, 12-LO, IL-6, and IL-1β.
Conclusion: These data demonstrated that 7-KCHO induces the increment of inflammatory gene expression of IL-1β and Il-6, and arachidonic cascade gene expression of LOX and COX in a ROS-dependent manner in MCs suggesting that ROS, 12-LOX, and COX-2 are related to interactivity and may also be partly involved in the mechanism of development of nephropathy. These findings may lead to elucidation of the new etiology of diabetic nephropathy.
Disclosure: **Y. Watanabe:** None.

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Treatment with 4-phenylbutyrate prevents pancreatic amyloid formation in obese mice overexpressing human islet amyloid polypeptide

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Background and aims: Loss of β-cell mass and function during Type 2 Diabetes (T2D) has been associated to several stressors suffered by the pancreatic β cell, including misfolding and aggregation of human islet amyloid polypeptide (hIAPP) that leads to amyloid deposits. It has been previously demonstrated that the administration of the chemical chaperone 4-phenylbutyrate (PBA) prevents amyloid formation in islets overexpressing hIAPP cultured at high glucose concentrations. Furthermore, PBA improves glucose metabolism abnormalities in a mouse model of obesity. The aim of the present work was to determine whether in vivo administration of PBA was able to prevent amyloid deposition and ameliorate glucose metabolism in obese mice overexpressing hIAPP, which present impaired fasting glucose and amyloid deposits, two hallmarks of T2D.
Materials and methods: Obese and insulin-resistant A^{vy} mice were crossed with mice overexpressing hIAPP. Mice were treated with PBA

dissolved in drinking water (1g/kg/day) during 12 weeks. Glucose and insulin tolerance tests were performed before and after treatment. Insulin plasma levels were determined by ELISA. After sacrificing mice, pancreas was removed, fixed and stained against amyloid deposits with Thioflavin S.

Results: PBA treatment started at 8 weeks of age, when A^{vy}-hIAPP transgenic mice presented impaired fasting glucose, and were glucose intolerant compared to wild-type, A^{vy} and hIAPP littermates. After 12 weeks of treatment, PBA decreased severe fasting hyperglycemia (320 ±20.6 mg/dL *vs*. 180±22.5 mg/dL, p<0.0001) and showed a tendency to decrease hyperinsulinemia (1.83±0.44 ng/mL *vs*. 0.68±0.23 ng/mL) in A^{vy}-hIAPP transgenic mice. Furthermore, both impaired glucose tolerance and insulin resistance observed in double transgenic mice were improved when mice were treated with the chemical chaperone. Finally, PBA administration also prevented amyloid accumulation (25.24±1.28 % *vs*. 9.00±0.88 %, p<0.0001) and prevalence (80.18±7.49 % *vs*. 52.42 ±4.06 %, p<0.05) in pancreatic islets of A^{vy}-hIAPP transgenic mice.

Conclusion: PBA treatment decreased fasting glucose, improved glucose tolerance and insulin sensitivity and prevented amyloid deposition in pancreatic β cells in obese mice overexpressing human IAPP. These results suggest that PBA could be considered as a therapeutic strategy to treat islet dysfunction in T2D.

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Visceral adipose tissue of healthy male mice shows active de novo lipogenesis during both short-term and long-term high-sugar feeding

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Background and aims: High sugar feeding promotes NAFLD through the stimulation of de novo lipogenesis (DNL). Visceral adipose tissue (VAT) is also implicated in promoting NAFLD by delivering high amounts of free-fatty acids (FFA) into the liver via the portal vein. To date, it has been assumed that the majority of DNL activity fuelling NAFLD is hepatic, with only minor contributions from adipose tissue. We hypothesized that VAT DNL is upregulated by high sugar feeding thereby contributing to increased FFA spillover into the liver. To determine if this occurs during short or long-term sugar feeding, we measured DNL in mice whose diet was supplemented with sugar for a single night (short-term, ST) or for 24 weeks (long-term, LT).

Materials and methods: Five adult male C57BL/6 mice fed on standard chow over a 12/12 hr light/dark cycle were injected intraperitoneally with 3g/100g body weight 99.9% ^2H_2O containing 0.9% NaCl w/w at the start of the dark period. The drinking water was supplemented with 17.5% fructose (w/v) and 17.5% glucose and the mice then fed naturally overnight. At the end of the dark period, mice were sacrificed, visceral and epididymal adipose tissues (EAT) and livers were freeze-clamped and triglyceride from each tissue extracted and purified. A second group of 5 mice that had been given the same amount of glucose/fructose in their drinking water for a period of 24 weeks prior, were likewise administered with ^2H_2O. Body water and FA methyl ^2H enrichments and were measured by ^2H NMR. Fractional rates of fatty acid synthesis (FA-FSR) were calculated from the ratio of FA methyl ^2H-enrichments to body water.

Results: For ST mice, the FSR of VAT was 7.4±2.9%, while that of EAT was 1.1±0.3%, both being significantly less than that of liver (33.0 ±3.6%). For LT mice, the FSR of VAT, EAT and liver were identical to that of ST (7.4±2.8%, 2.3±0.3% and 31.6±4.8%, respectively). While VAT and EAT FSR were not significantly different from each other within each group, when compared over both ST and LT groups, FSR values for VAT were significantly higher compared to EAT (p = 0.028).

Conclusion: Following ^2H_2O administration, triglyceride fatty acids of VAT, EAT and liver became enriched with ^2H. Enrichment of VAT was 2-3 fold higher than that of EAT following both short-term and long-term sugar feeding. These results suggest that compared to EAT, VAT has an inherently higher capacity for DNL from dietary sugar. Thus, an increase in visceral fat depots during obesity may represent a significant extrahepatic capacity for DNL from dietary sugar that may significantly contribute to NAFLD.

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Disclosure: **C. Marques:** None.

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Effect of exercise training on insulin resistance and adipose tissue macrophages in elderly women

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Background and aims: Aging is associated with a progressive increase of fat mass (FM) to fat free mass (FFM) ratio as well as redistribution of adipose tissue (AT). Such changes contribute to the development of low grade systemic inflammation and increased risk of metabolic diseases such as diabetes mellitus type 2. The studies performed with elderly people suggest that the risk of metabolic diseases may be ameliorated by physical activity, however the effects of exercise training on AT remain largely unexplored. Therefore, we aimed to determine whether regular physical activity improves peripheral insulin sensitivity and inflammatory status of AT expressed as relative content and phenotype of adipose tissue macrophages in elderly women.

Materials and methods: Two groups of elderly women (mean age 68 years ± 3 years) differing in physical activity level, i.e. a group of trained women (n= 12) and a group of sedentary women (n= 12) were recruited for the study. Fitness status was assessed by senior fitness tests (chair stand, arm curl, sit and reach and back scratch test) and a bicycle ergometry with determination of VO_2 max. We compared anthropometric parameters, biochemical profile, and the degree of insulin sensitivity (using a hyperinsulinaemic euglycemic clamp (HEC). The samples of subcutaneous abdominal AT obtained by needle biopsy were used for isolation of stromal vascular fraction cells that were subjected to flow cytometry analysis.

Results: Compared to sedentary group, the group of trained women had higher physical performance expressed by VO_2max (29.7 vs. 18.2 ml/kg/min, p<0.001) and in senior fitness tests trained women scored more often significantly above normal average values for senior population. Trained women had lower BMI (23.4 vs. 27.5, p = 0.003), percentage of FM (33.9 vs. 39.9%, p = 0.001), and higher FFM percentage (66 vs. 60 % p = 0.001). In the biochemical analysis the only significant difference was higher total and LDL cholesterol in trained group (total 6.3 vs. 5.4 mmol/l, p= 0.038 and LDL 4.0 vs. 3.0 mmol/l, p= 0.005). Insulin sensitivity determined as Glucose Disposal during HEC was higher in trained group (6.7 vs 4.9 mg/kg/min, p=0.035).The monocyte/macrophage population defined as CD45+/14+ cells, and its subpopulations expressing either CD40+, TLR2 or TLR4 antigens did not differ among the investigated groups, while the content of CD45/14/206+ macrophages was higher in AT of the sedentary women (66 vs. 47%, p= 0.013).Negative correlation between FM and Glucose Disposal (r = -0.695, p<0.001) and VO_2max (r = -0.669, p = <0.001) was observed. Percentage of CD45/14/206+ macrophages showed trend to positively correlate with fat mass (r = 0.416, p=0.06) and negatively correlate with insulin sensitivity (r = -0.423, p=0.056).

Conclusion: The higher physical performance of trained elderly women was associated with higher insulin sensitivity and lower abundance of

FM. However, our findings suggest that inflammatory status of AT is not markedly influenced by exercise training in elderly and it is more dependent on the percentage of FM.
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Disclosure: **M. Stepan:** None.

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The influence of physical activity on risk of cardiovascular disease in metabolic healthy obese people

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Background and aims: The metabolic outcomes of metabolically healthy obesity (MHO) remain controversial. The aim of the present study was to determine the effect of physical activity on the cardiovascular disease (CVD) outcomes of MHO.
Materials and methods: The study included participants who were followed for 10 years and recruited from the Korean Health and Genome Study (KoGES), a population-based cohort study. Participants with previously recorded CVDs or cancer, or who had received steroids or anticoagulants at baseline were excluded.
Results: A total of 8144 participants (3,942 men and 4,202 women) fulfilled inclusion criteria. In a multivariate Cox regression model adjusted for age and sex, MHO participants were not at elevated risk of CVD compared with their metabolically healthy non-obese (MHNO) counterparts (HR, 1.28; 95% CI, 0.96- 1.71), although both the non-obese (HR, 1.50; 95% CI, 1.19-1.90) and obese (HR, 1.85; 95% CI, 1.48- 2.30) participants with metabolic abnormalities were at elevated risk. However, in the subgroup analysis by physical activity, physically inactive MHO participants had a significantly higher HR for CVD events compared to active MHNO participants (HR, 1.54; 95% CI, 1.03-2.30), while active MHO participants were not at elevated risk (HR, 1.15; 95% CI, 0.70-1.89)
Conclusion: In MHO participants, only physically inactive participants had a significantly increased risk of CVD compared to physically active MHNO participants.

Hazard ratios for CVD events according to obesity, metabolic health status and physical activity.

	Model1		Model2	
	HR (95% CI)	P	HR (95% CI)	P
MHNO with active PA	1 (Reference)		1 (Reference)	
MHNO with inactive PA	1.129 (0.794-1.607)	0.498	1.112 (0.781-1.582)	0.557
MHO with active PA	1.147 (0.697-1.886)	0.590	1.147 (0.697-1.888)	0.590
MHO with inactive PA	1.536 (1.026-2.298)	0.037	1.524 (1.018-2.281)	0.041
MUNO with active PA	1.598 (1.081-2.361)	0.019	1.438 (0.970-2.133)	0.071
MUNO with inactive PA	1.640 (1.160-2.320)	0.005	1.467 (1.034-2.081)	0.032
MUO with active PA	1.890 (1.304-2.741)	0.001	1.608 (1.101-2.349)	0.014
MUO with inactive PA	2.051 (1.475-2.853)	<0.001	1.767 (1.262-2.474)	0.001

Abbreviations: MHNO, metabolically healthy nonobese; MHO, metabolically healthy obese; MUNO, metabolically unhealthy nonobese; and MUO, metabolically unhealthy obese; PA, physical activity; HR, hazard ratios; CI, confidential interval.

Model 1 was adjusted for age and gender.
Model 2 was adjusted for the variables in Model 1, plus drinking, smoking, physical activity and medication of hypertension, diabetes mellitus and dyslipidemia.

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Cognitive behavioural group therapy to prevent weight regain in type 2 diabetes: a randomised controlled trial

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Background and aims: Current weight loss programs for overweight and obese patients with type 2 diabetes are not effective in the long term due to regain of weight. The aim of this study was to determine the 2-years efficacy of cognitive behavioral group therapy (CBGT) in preventing long-term weight regain after a very low-calorie diet in patients with type 2 diabetes. The secondary objective was to determine the effect of CBGT on cardiovascular risk factors and psychological wellbeing.
Materials and methods: The Prevention Of Weight Regain (POWER) trial was a single center, parallel-group, randomized controlled trial, conducted from March 2010-May 2015. Participants were recruited from the outpatient diabetes clinic of a university hospital. Of the 276 eligible patients with type 2 diabetes and BMI ≥27 kg/m^2, 206 agreed to participate and started with the very low-calorie diet. The 158 participants who achieved ≥5% weight loss by 8 weeks of dieting were included in the trial. Subsequently, the participants were randomly assigned to the intervention or control group. Participants in the control group received usual care only, which consisted of regular scheduled visits to the treating internist and diabetes nurse. Participants in the intervention group received CBGT on top of usual care. The CBGT consisted of 17 group sessions led by a trained psychologist, aimed at restructuring dysfunctional cognitions on lifestyle, weight, body perception and relapse. The primary outcomes were the between-group differences in (1) body weight at two years and (2) weight regain from randomization to two years of follow-up. Secondary outcomes were the between-group differences in HbA1c, insulin dose, lipids, depression, anxiety, self-esteem, quality of life, fatigue, eating disorders and physical activity. Data were analyzed by linear mixed modeling (intention to treat as well as per-protocol).
Results: The between-group difference in body weight at two years was -1.2 [95%CI, -7.7 to 5.3] kg (p=0.717). The between-group difference in weight regain was -0.7 [95%CI, -3.1 to 1.6] kg (p=0.556). Similar results were found in the per-protocol analyses. None of the secondary outcomes differed between the two groups.
Conclusion: CBGT after diet-induced weight loss did not prevent weight regain or improve cardiovascular risk factors or psychological wellbeing better than usual care alone in obese patients with type 2 diabetes. Our results provide no scientific justification to offer CBGT on top of usual care to optimize the effect of weight loss dieting in obese patients with type 2 diabetes.
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Disclosure: **K.A.C. Berk:** None.

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Beneficial effects of carotid sinus nerve resection on obesity co-morbidities

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Background and aims: Obesity is a major cause of cardiovascular and metabolic disturbances, contributing to significant morbidity and mortality worldwide. The therapeutic options to treat this pandemic are clearly scarce. Our group has recently proposed a new role for the carotid body (CB), a chemoreceptor organ classically defined as an O_2 sensor, in the pathogenesis of metabolic syndromes. We demonstrated that abolishment of CB activity, through the resection of its sensitive nerve, the carotid sinus nerve (CSN), prevents and reverses insulin resistance and glucose

intolerance in animal models of metabolic diseases. Herein, we investigated the effects of carotid sinus nerve resection on weight loss and the pathophysiological mechanisms that can contribute to these beneficial results.

Materials and methods: Experiments were performed in 9 weeks male *Wistar* rats. Animals were submitted to 10 weeks of high-fat diet (HF) (5.1Kcal/g) or to a standard diet (2.85Kcal/g). After 10 weeks of diet, animals were submitted to bilateral carotid sinus nerve (CSN) resection. A sham procedure was applied to control groups. CSN resection was confirmed by the absence of ventilatory responses to hypoxia by pletismography in conscious animals. After CSN resection, animals were kept under the respective diets and insulin sensitivity, glucose homeostasis, caloric intake and body weight were monitored. At a terminal experiment animals were anaesthetised with pentobarbital (60mg/kg i.p.) and white and brown adipose tissue were collected, weighted and store for analysis of proteins involved in adipose tissue metabolism.

Results: As expected, HF diet produced an increase in weight gain (weight controls=296.5±64.3 g, weight HF=365.8±87.2 g). CSN resection decreased weight gain by ≅13% in control animals and by ≅ 40% in HF animals, an effect that is not due to a decreased caloric intake (control sham=254.57±21.78 Kcal/day/Kg; control with CSN resection =262.50 ±62.11 Kcal/day/Kg; HF sham=301.74±49.12 Kcal/day/Kg; HF with CSN resection=287.20±84.65 Kcal/day/Kg). HF diet induced whole-body insulin resistance (K_{ITT} controls=4.52±1.18 %glucose/min; K_{ITT} HF=1.49±1.26 %glucose/min) and glucose intolerance (AUC glucose excursion curve controls=17059±1506 mg/dlxmin; AUC glucose excursion curve HF =22036±3206 mg/dlxmin). CSN resection restored insulin sensitivity (K_{ITT} before surgery=1.49±1.26 %glucose/min; K_{ITT} 3 weeks after CSN resection= 4.974±1.30%glucose/min) and glucose homeostasis (AUC HF before surgery=22036±3206 mg/dlxmin; AUC HF 3 weeks post-CSN resection =19423±1498 mg/dlxmin). Also, CSN resection in HF animals decreased the total fat amount of the animals by 38% and perinephric and subcutaneous adipose tissues by, 49 and 39%, respectively.

Conclusion: We can conclude that abolishment of CB activity through CSN resection positively impacts weight gain, an effect that was accompanied by a decrement in total, perinephric and subcutaneous adipose tissues and not attributable to alterations in caloric intake. We suggest that modulation of CB activity can be a therapeutic target for the treatment of obesity.*Both authors have contributed equally to this work.

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The effect of transcranial direct current stimulation associated with hypocaloric diet in subjects with different degrees of glucose tolerance

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Background and aims: Non-adherence to lifestyle modifications is an important determinant of failure to treat obesity. The dorsolateral prefrontal cortex (DLPFC) plays an important role in appetite and food intake regulation and may be a target for electric brain stimulation, a new treatment modality that has been used in diseases such as depression and drug addiction. The aim of this study was to test the effect of active anodal tDCS (a-tDCS) over the right DLPFC (rDLPFC) associated with a hypocaloric diet on weight loss in overweight or obese adults with different degrees of glucose tolerance.

Materials and methods: In this randomized, placebo-controlled, double-blind pilot study, 9 overweight or obese adults with different degrees of glucose tolerance (NGT: normal glucose tolerance, IGM: impaired glucose metabolism, T2D: type 2 diabetes), aged 20-50 years, completed a 4-week (20 sessions) of fixed-dose tDCS (2mA, 20 min). Subjects were randomized in a 1:1 ratio to receive one of two types of intervention: (1) active a-tDCS + hypocaloric diet (*Active*), or (2) sham a-tDCS + hypocaloric diet (*Sham*), both delivered over the rDLPFC. To determine body mass index (BMI, in Kg/m²), body weight (BW, in kg) and height (m) was assessed at baseline (t_0), and BW at visits 5 (t_5), 10 (t_{10}), 15 (t_{15}), 20 (t_{20}), and at the last visit of the study (t_F); blood samples was collected for an 2-h OGTT, a standard 4-h meal tolerance test ([MTT], glucose and insulin measurements) and HbA_{1c} determination at t_0 and t_F. The self-reported Beck Depression Inventory ([BDI], score range 0-63, higher scores indicating worse mood) questionnaire was administered at t_0 and t_F. Changes in BMI and BW were analyzed with generalized estimating equations (GEE) and Bonferroni post-hoc testing for normally distributed continuous variables; descriptive statistics are reported as means±SD or %.

Results: 9 subjects completed the study on this interim analysis (female 66.7%, obese 66.7%, mean age 38.3±4.8 years, BMI 30.9±2.5 kg/m², IGM 11.1%, T2D 11.1%). Reduction of BW and BMI was respectively greater in the *Active* than in the *Sham* group (GEE, p=0.009 and p<0.001). Weight changes over the time (t_F-t_0; mean±SE) were -2.8±0.5 Kg for *Active* and -1.7±0.8 Kg for *Sham*. Changes over the time in BMI (t_F-t_0; mean±SE) were -1.0±0.2 kg/m² in the *Active* group and -0.6±0.3 kg/m² in the *Sham* group. Although there was a greater reduction in the AUC for glucose (GEE: p=0.091) and insulin (GEE: p=0.062) from t_0 to t_F in the *Active* vs *Sham* group during the MTT, these changes did not reach statistical differences.There was a greater reduction in *Active* compared to *Sham* in BDI scores over the 4-week intervention (mean±SE; *Active*: t_0=10.0±2.2; t_F=2.2±1.2 vs *Sham*: t_0=6.2±1.4; t_F=6.0±1.6, GEE, p=0.016).

Conclusion: This preliminary analysis suggests that repetitive active a-tDCS may be a promising non-invasive technique that could be used to increase weight reduction and improve BDI scores in overweight or obese individuals with different degrees of glucose tolerance on a low-calorie diet.

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Disclosure: **C. de Araujo:** None.

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Effect of T cell specific PPARβ overexpression on diet-induced obesity and its associated inflammation and insulin resistance

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Background and aims: It is now recognized that obesity-induced chronic inflammation is central to the development of many obesity-associated pathologies such as type-2 diabetes. Obesity-induced T cell changes in adipose tissue (AT) has been linked to metabolic phenotypes of obese subjects. Interestingly, it has also been shown that T cell differentiation and responses may be altered by modulating their metabolism. Accordingly, we have recently shown that Peroxisome-Proliferator-Activated-Receptor beta (PPARβ) increases lipid metabolism in T cells, which changes T cell homeostasis. Our objectives were to study the consequences of changes in T cell populations induced by T cell-specific PPARβ overexpression on obesogen high fat diet (HFD)-induced AT inflammation and the subsequent development of insulin resistance (IR).
Materials and methods: We created transgenic mice that overexpress PPARβ specifically in T cells (Tg T-PPARβ mice) using the Cre-Lox system. 12-week old male mice (16 Tg T-PPARβ and 17 control Lck-Cre mice) were fed with HFD (60% of calories derived from fat) during 16 weeks. Insulin- and glucose-tolerance tests (ITT/GTT) were performed at week 11 and 13 of the diet, respectively. At the end of the 16-week diet, AT (subcutaneous and epididymal) stromal vascular cells were isolated for analysis of the presence of macrophage and T cell populations using flow cytometry. Liver steatosis was analyzed by histology and triglyceride measurements.
Results: HFD-induced weight gain was 8% less in Tg T-PPARβ compared to control mice (P<0.001). ITT/GTT data showed that Tg T-PPARβ mice develop less IR than control mice, even in weight-matched mice. Total AT macrophage numbers were decreased predominantly due to a decrease in pro-inflammatory M1 macrophages. Furthermore, AT T cells numbers were also decreased due to a decrease in αβ T cells, while γδ T cell numbers remained unchanged. Together, this led to an increase in the proportion of γδ T cells in AT. Liver weight and triglyceride content was decreased.
Conclusion: T cell-specific overexpression of PPARβ results in: 1) a partial protection against HFD-induced obesity, insulin resistance, and liver steatosis, 2) a reduction in AT inflammation, 3) an increase in the proportion of γδ T cells in AT. Our hypothesis is that the beneficial effects observed are the consequence of a decrease in αβ T cells (leading to an increase in γδ T cell prevalence) as the result of changes in T cell metabolism induced by PPARβ overexpression.
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Disclosure: **G. Le Menn:** None.

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Loss of fractalkine-CX3CR1 signalling exacerbates obesity-induced inflammation and insulin resistance

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Background and aims: Adipose tissue macrophage (ATM) accumulation via C-C chemokine receptor 2 (CCR2) and its ligand monocyte chemoattractant protein-1 (MCP-1) is pivotal for the development of insulin resistance. We previously reported that a different C-C chemokine receptor, CCR5, promotes obesity-associated adipose tissue inflammation and insulin resistance. Fractalkine (also known as CX3CL1) is a CX3C chemokine involved in cell adhesion, recruitment, and survival upon binding to its receptor, CX3CR1. Fractalkine-CX3CR1 is a chemokine system that exerts both negative and positive influences on disease pathogenesis and progression. However, it is unclear how the fractalkine-CX3CR1 axis impacts adipose inflammation and insulin resistance in the development of obesity. In the present study, we show that fractalkine-CX3CR1 signaling plays a crucial role in obesity-induced insulin resistance by suppressing ATM recruitment and adipose tissue inflammation.
Materials and methods: Comprehensive DNA microarray analyses of chemokines and their receptors in epididymal white adipose tissue (eWAT) of high-fat diet (HFD)-induced obese (DIO) mice or lean controls were performed. To determine whether fractalkine-CX3CR1 is required for obesity-induced ATM recruitment and insulin resistance *in vivo*, we examined the metabolic phenotype of *Cx3cr1*$^{-/-}$ mice. In addition, we performed bone marrow (BM) transplantation of *Cx3cr1*$^{-/-}$ and wild-type (WT) C57Bl/6J mouse donor cells into irradiated WT recipient mice to generate myeloid cell-specific chimeric mice.
Results: Fractalkine mRNA expression was persistently downregulated in eWAT of DIO mice compared to lean controls. Consistently, DNA microarray analyses of eWAT of DIO mice demonstrated decreased fractalkine gene expression despite increased expression of other chemokines systems, such as MCP-1-CCR2 and CCR5. Interestingly, downregulation of fractalkine preceded ATM recruitment in DIO mice. Furthermore, immunofluorescence analyses of eWAT in DIO mice revealed that both fractalkine and CX3CR1 were expressed by F4/80^{+} macrophages in crown-like structures. *Cx3cr1*$^{-/-}$ mice fed normal chow developed slightly worsened glucose tolerance. Compared to WT mice, *Cx3cr1*$^{-/-}$ mice fed an HFD exhibited increased macrophage infiltration and formation of crown-like structures in eWAT, despite similar body weight and adipocyte size. HFD-induced glucose intolerance, hyperinsulinemia (WT vs. *Cx3cr1*$^{-/-}$; 0.5 ± 0.1 ng/ml vs. 1.3 ± 0.2 ng/ml, $p < 0.05$), and insulin signaling were exacerbated in eWAT of *Cx3cr1*$^{-/-}$ mice. These findings were associated with increased plasma TNFα levels (WT vs. *Cx3cr1*$^{-/-}$; 47.9 ± 4.7 pg/ml vs. 73.2 ± 9.1 pg/ml, $p < 0.01$), JNK amplification, and NF-kB activation in eWAT, as well as progression of hepatic steatosis. Importantly, HFD-induced adipose inflammation and hyperinsulinemia were aggravated in chimeric mice lacking CX3CR1 in BM cells (BM transplantation of *Cx3cr1*$^{-/-}$ donor cells into WT mice), compared to BM transplantation of *Cx3cr1*$^{+/+}$ donor cells into WT mice.
Conclusion: Fractalkine gene expression is persistently decreased in WAT, and its downregulation precedes ATM recruitment in obese mice. Furthermore, loss of fractalkine-CX3CR1 signaling increases ATM recruitment, thereby exacerbating obesity-induced inflammation and insulin resistance. Thus, fractalkine-CX3CR1 signaling plays a critical role in obesity-induced insulin resistance by decreasing ATM recruitment and adipose tissue inflammation.
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Disclosure: **M. Nagashimada:** None.

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Impairment of intestinal barrier integrity in human obesity: involvement of dietary lipids and links with bioclinical phenotypes

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Background and aims: Obesity is associated with a low-grade inflammation, which could be promoted by intestinal barrier impairment as suggested by studies in fat-fed rodents. Intestinal permeability (IP) is poorly described in human obesity. We characterized IP in obese subjects and evaluated the impact of acute lipid supplies
Materials and methods: IP was evaluated ex vivo in Ussing chambers using FITC-labeled tracers at baseline and after exposure to lipid micelles, on jejunum samples of obese and non-obese subjects obtained from surgical waste of gastric bypass and surgeries performed in non-obese subjects. In vivo, IP was assessed by lactitol/mannitol urinary excretion ratio (L/M). Tight junction proteins were studied by immunofluorescence. LPS activity and zonulin levels were measured in serum. Effect of lipid micelles on epithelial permeability was studied in Caco-2/TC7 cells
Results: In fasting condition, P to 0.4 and 4 kDa tracers was not increased in obese as compared to non-obese subjects. Occludin and tricellulin labelling was reduced at tight junction. L/M ratio and seric LPS levels were comparable but increased zonulin serum levels were observed in obese subjects ($p=0.02$). In Caco-2/TC7 cells, lipid micelles increased paracellular permeability through a Src kinase-dependent mechanism. After lipid supply, higher values of ex-vivo IP were recorded for the jejunum of obese compared to non-obese subjects (+92%; $p<0.05$), with decreased tricellulin intensity. Ex vivo IP to 0.4 kDa tracer was positively correlated with systemic inflammation (Haptoglobin, CRP; $p<0.01$); IP to 4 kDa tracer was negatively correlated with insulin-resistance surrogate (adiponectin; $p<0.05$) and Fiber consumption ($p<0.01$). After lipid challenge, IP to 4kDa positively correlated with systemic (CRP), intestinal (calprotectin) inflammation ($p<0.01$) and negatively with Fruit and vegetable consumption ($p<0.05$)
Conclusion: Increase of jejunum permeability in obese subjects is exacerbated after an acute lipid supply and associated with tight junction impairments. Jejunum permeability is linked with systemic and local inflammation as well as dietary profile
Clinical Trial Registration Number: NCT02292121
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Disclosure: **L. Genser:** Grants; Fondation Nestlé, SFCD, Obélisque, Appert.

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Myeloid-specific deletion of SIRT1 aggravates hippocampal inflammation in a high-fat diet-fed mice

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Background and aims: Obesity-induced peripheral inflammation is associated with hippocampal inflammation. Sirtuin 1 (SIRT1) regulates cellular metabolism and inflammatory response. Nuclear factor kappa B (NF-κB)-mediated inflammation contributes to the development of insulin resistance and diabetes, however, the myeloid-specific SIRT1 function in the context of neuroinflammation is largely unknown.
Materials and methods: Myeloid-specific SIRT1 knockout (KO) mice were fed a high-fat diet (HFD) or normal diet (ND) for 40 weeks.
Results: HFD-fed SIRT1 KO mice had an increase in hepatic inflammation and macrophage infiltration of adipocytes compared to HFD-fed wild type (WT) mice. Hippocampal expression levels of acetylated NF-κB were increased in HFD-fed KO mice compared to HFD-fed WT mice. In particular, HFD-induced lipocalin-2 was increased in liver, adipose tissue, and hippocampus of WT mice. However, their expressions were decreased in KO mice compared to WT mice. SIRT1 deletion increased hippocampal iba1 and amyloid precursor protein expression in HFD-fed mice.
Conclusion: These results suggest that myeloid-specific SIRT1 deletion may contribute to the reduced secretion or production of LCN2 and lead to suppression of anti-inflammatory responses against HFD-induced obesity.
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Disclosure: **K. Kim:** None.

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CXCL16: a novel regulator of insulin signalling in C2C12 myotubes

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Background and aims: Insulin resistance often arises in the context of obesity and can lead to the development of type 2 diabetes. Chronic low-grade inflammation has emerged as a contributing factor to skeletal muscle insulin resistance, with several immune cells and cytokines being implicated. Macrophages infiltrate skeletal muscle in obesity and pro-inflammatory cytokines secreted locally impact insulin sensitivity. *In vitro*, the saturated fatty acid (FA) palmitate polarises macrophages towards a pro-inflammatory phenotype. However, the effects of secreted cytokines and chemokines on skeletal muscle, particularly those recently identified, such as CXCL16, have not been defined. In this study, we evaluated the secretion of cytokines and chemokines by palmitate-treated macrophages with the aim of identifying novel paracrine mechanisms impacting skeletal muscle insulin sensitivity.
Materials and methods: J774 macrophages were incubated with or without 500μM palmitate ± 500μM unsaturated FA (palmitoleate) for 8 hours, followed by incubation of cells with fresh media, which was collected after 16 hours. These conditioned media were analysed using a mouse cytokine array. The results of the array analysis were validated in separate experiments using specific ELISAs, including lipopolysaccharide (LPS) as a positive control. Differentiated C2C12 myotubes were treated with 750μM palmitate, murine recombinant soluble CXCL16 (sCXCL16), combined treatment, or control medium. The expression and activation state of insulin signalling pathway intermediates was determined by western blotting, while the overall response to insulin was assessed by measuring glycogen synthesis.
Results: Treatment of J774 macrophages with palmitate or palmitate plus palmitoleate led to secretion of a modified cytokine profile versus control. Of the 62 cytokines examined, 7 were significantly differentially expressed versus control. Intriguingly, secretion of CXCL16, a relatively recently described cytokine that has not been studied in the context of insulin resistance, was down-regulated by palmitate treatment (51.6 % decrease; $p<0.01$). Reduced CXCL16 secretion by palmitate-treated J774 macrophages was verified by ELISA (palmitate: 690 ± 147 pg/mL *vs* control: 1572 ± 258 pg/mL; $p<0.05$). Separate incubation with LPS also diminished CXCL16 secretion (836 ± 116 pg/mL; $p<0.05$ *vs* control). Short-term exposure (<1 hour) of C2C12 myotubes to sCXCL16 resulted in a concentration-dependent stimulatory effect of the cytokine on the phosphorylation state of Akt and ERK1/2. Treatment of C2C12 myotubes with 750μM palmitate (16 hours) led to the expected reduction of Akt phosphorylation upon insulin (100 nM) stimulation, which was attenuated by simultaneous treatment with 100ng/mL sCXCL16.
Conclusion: These data demonstrate that sCXCL16 modulates the insulin signalling pathway in murine skeletal muscle *in vitro* and ameliorates the negative effect exerted by palmitate. The attenuated secretion of CXCL16 by palmitate- or LPS-treated macrophages may contribute to diminished skeletal muscle insulin sensitivity, identifying CXCL16 as a putative novel mediator of skeletal muscle insulin signalling under conditions of chronic low-grade inflammation.
Supported by: Royal Veterinary College PhD Studentship
Disclosure: **S. Bitsi:** None.

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Exploring GPR120-mediated regulatory actions on lipid metabolism, inflammation and autophagy in islet beta cells: its potential as a therapeutic target for obesity and type 2 diabetes

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Background and aims: The current therapies for obesity-related type 2 diabetes mellitus (T2DM) are suboptimal. In this regard, G protein-coupled receptor 120 (GPR120) is a novel potential therapeutic target for the prevention and treatment of T2DM. Recent studies have shown that GPR120 activation in pancreatic islet β cells promotes insulin secretion; however, there have been no report to further examine the modulatory roles of resident GPR120 in islet β-cell survival and function under lipotoxicity-induced diabetic conditions. In the present study, we therefore aimed to investigate into the potential roles of islet β-cell GPR120 in regulating lipid metabolism, inflammation and autophagy under lipotoxic conditions.
Materials and methods: Rat β-cell line INS-1E and isolated C57/BL6J mouse islets were used as in vitro and ex vivo models, respectively. GPR120 knockout (KO) mouse islets were used to validate the roles of GPR120. To induce lipotoxicity, β cells and islets were exposed to palmitic acid; GPR120 agonism was achieved by two agonists, namely docosahexenoic acid (DHA) and GSK137647 (GSK); the regulatory roles of GPR120 in β-cell/islet lipid metabolism and inflammation, as well as autophagy were then elucidated; the related signaling pathways involved in the GPR120-mediated protective actions were further examined.
Results: GPR120 agonists DHA and GSK inhibited the mRNA expression of the lipid uptake/synthesis-related genes, such as SREBP-1c (1.69 ±0.11 vs 0.71±0.10, p<0.001; 1.69±0.11 vs 1.19±0.08, p<0.01), in mouse islets and INS-1E cells under lipotoxic conditions. In addition, DHA reversed the lipotoxicity-induced mRNA expression of pro-inflammatory genes in both mouse islets and INS-1E cells, of which these inhibitory effects were abrogated in mouse islets with GPR120 KO (18.42±3.97 vs 5.34±1.29, p<0.05; 40.81±8.18 vs 45.57±13.80, p>0.05). Moreover, the GPR120 KO mouse islets exhibited alterations in the expression of autophagy-related genes (1.01±0.03 vs 0.80±0.04, p<0.05; 1.01±0.07 vs 1.3±0.06, p<0.05). On the other hand, INS-1E cells with GPR120 knockdown exhibited reductions in insulin expression and intracellular insulin content; consistently, GPR120 KO mice were characterized with higher fasting blood glucose levels (7.34±0.36 vs 10.56 ±0.59, p<0.01). Our mechanistic studies further showed that the gene expression related to β-cell proliferation, function and insulin synthesis were reduced in GPR120-knocked-down INS-1E cells. In corroboration, the stimulatory effects of DHA on protein kinase B (also known as Akt) and extracellular signal-regulated kinases (ERK) phosphorylation were abolished in GPR120 KO mouse islets (1.00±0.01 vs 2.11±0.28, p<0.05; 1.00±0.01 vs 1.04±0.27, p>0.05; 1.08±0.08 vs 1.35±0.05, p<0.05; 1.00 ±0.04 vs 1.12±0.07, p>0.05).
Conclusion: These data indicate that GRP120 agonism in β cells/islets may have therapeutic potential in regulating lipid accumulation, lipotoxicity-induced inflammation, and autophagy, as well as being associated with β-cell proliferation and insulin synthesis, probably via the mediation of Akt/ERK signaling pathway.
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Disclosure: **D. Zhang:** Grants; Research Grants Council of Hong Kong (CUHK470413).

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Fetuin A disrupts energy homeostasis through cleavage of SIRT1 in lipid induced inflamed adipocyte

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Background and aims: Lipid is associated with two critical metabolic diseases, type 2 diabetes and cardiovascular disease, together called as metabolic syndrome which is posing a major threat to global human health. Adipose tissue inflammation is in the centre stage of this disease. Morphology and functional status of adipocyte dramatically changes during obesity induced insulin resistance, it becomes an inflamed tissue with increase in number of hypertrophied adipocytes that stores excess amount of lipid and produces proinflammatory cytokines. Result is more of energy stored than expenditure; this misbalance could be sensed by energy sensor, SIRT1 which act as gatekeeper of the master regulator of mitochondria, PGC1α. SIRT1 activate PGC1α through deacetylation and this enhances the expression of plethora of genes which regulates lipid mobilization for energy yield and that effects insulin sensitization. In hypertrophied adipocytes, this pathway is disrupted and that produces insulin resistance. However, underline mechanism of excess lipid induced negative signals that disrupts energy homeostasis is still incompletely understood. Whether excess lipid is directly involved in producing these metabolic defects or there are some other mediators which are/is involved here is not yet clear.
Materials and methods: Abdominal adipose tissue and serum were collected from standard diet (SD), high fat diet (HFD), FetA gene knocked down HFD mice ($FetA^{KD}$HFD) and FetA reinforced $FetA^{KD}$HFD mice and OGTT, ITT, FetA, pro-inflammatory cytokines, inflammasome markers, SIRT1 and its downstream targets were investigated. In vitro effects of FetA on SIRT1 were assessed in 3T3L1 adipocytes and TNFα knocked down (TNFα siRNA) 3T3L1 adipocytes. Further, mitochondria were isolated from mice as well as 3T3L1 adipocytes and mitochondrial bioenergetics and biogenesis parameters were investigated.
Results: In the present study we have observed that inactivation of SIRT1 through its cleavage and subsequent decrease of acetylated PGC1α (p<0.05), mitochondrial mass (p<0.01) and ATP synthesis (p<0.05) coincided with the higher Fetuin A (FetA) level in high fat diet (HFD) induced obese diabetic mice. Knock down of FetA gene ($FetA^{KD}$) markedly recovered SIRT1 and its downstream targets in HFD mice, while reinforcement of FetA into $FetA^{KD}$HFD mice reduced SIRT1 activity significantly (P<0.01). Interestingly, there was concurrent increase in TNFα expression (p<0.05) and caspase1 activity (p<0.01) in HFD mice adipocytes when FetA level was high. Overexpression of TNF α by FetA augmented inflammasome formation (increased levels of NLRP3, ASC) and caspase1 release (p<0.01). FetA failed to increase caspase1 and cleave SIRT1 when TNFα gene was silenced in 3T3L1 adipocytes. These results suggest that FetA mediate its inhibitory effect on SIRT1 through TNFα-caspase1 pathway.
Conclusion: Our results indicate that FetA is obligatory for defects in SIRT1 and PGC1α pathway that cause impairments in mitochondrial biogenesis, bioenergetics, which in turn adversely affect insulin sensitivity.
Supported by: UGC-DS Kothari Postdoctoral Fellowship
Disclosure: **M. Chattopadhyay:** None.

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cd36 acts as an important mediator of FFA, FetA and TLR4 induced adipose tissue inflammation

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Background and aims: Type 2 diabetes (T2DM) currently affects more than 382 million people worldwide and by 2035 the prevalence may go

up to 592 million (Diabetes Atlas, 2015). Lipid induced insulin resistance (IR) plays an important role in the pathogenesis of T2DM and 'Diabetes Lipidius" is believed to precede 'Diabetes Mellitus'. Elevated plasma levels of free fatty acids (FFAs) induce production of proinflammatory cytokines from liver and adipose tissue. We recently reported that FetuinA (FetA), a hepato-adipokine, acts as an adaptor protein between FFA and TLR4 to initiate adipose tissue inflammation. However, how FFA-FetA-TLR4 complex trigger adipose tissue inflammation is yet unclear. Available reports suggest that cd36, a scavenger receptor, binds with TLR4 and TLR6 to activate NLRP3-inflammasome formation and participates in LPS- TLR4 mediated inflammatory signals. As TLR4 mediated inflammatory response also requires FetA, we went on to see if FetA also operates through cd36 to trigger adipose tissue inflammation.
Materials and methods: We preincubated 3T3L1 cell line and adipocytes from Balb/c mice with cd36 recombinant protein at increasing concentration along with either FFA or FFA+FetA for 4 h followed by ELISA and immunoblot analysis of proinflammatory cytokines (TNFα, IL6 and IL1β). Insulin sensitivity was estimated by ^{14}C-2DOG uptake and immunoblot of pGLUT4, pAKT and pIR from both the cell line and primary adipocyte culture. To investigate whether cd36 induced activation of inflammation requires direct binding of FetA, we generated high fat diet (HFD)-diabetic mice and performed co-immunoprecipetation experiment from the adipocyte cell lysates of standard diet (SD) and HFD mice. We also immunodepleted FetA and TLR4 and then immunobloted the adipocyte lysates with cd36 antibody to confirm cd36 interaction with FetA.
Results: On ELISA and immunoblot analysis of cd36 immunodepleted adipocytes, FFA+FetA+TLR4 trimer failed to elicit a proinflammatory cytokine response (TNFα,IL6 and IL1β). Cells treated with FFA alone, FetA alone or FFA +FetA showed significant increase in the proinflammatory cytokines (2-4 folds; n=5). No notable stimulation in mRNA expression and activation of NFkB was noted in cd36 immunodepleted cells. Adipose tissue inflammation induced by FFA-FetA-TLR4 trimer impaired insulin sensitivity as observed by underexpression of insulin sensitive molecules (pIR, pAKT,pGlut4)and reduced ^{14}C-2DOG uptake, while suppression of cd36 in these cells abrogated these defects. Co-immunoprecipetation experiment involving adipocytes from SD and HFD mice confirmed cd36 binding to FetA.
Conclusion: The present investigation establishes cd36 is an important mediator of FFA-FetA-TLR4 trimer induced adipose tissue inflammation that impairs insulin sensitivity. Since targeting TLR4 in T2DM patients may leads to unwanted inhibition of immune function, cd36 could be a potentially useful drug target to treat T2DM.
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Disclosure: **S. Mukherjee:** None.

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Alpha1-antitrypsin prevents cytokine-induced beta cell death in mouse islets and improves glucose homeostasis in human islet amyloid polypeptide transgenic mice

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Background and aims: Islet inflammation has emerged as a key factor for the loss of functional β-cell mass in both type 1 (T1D) and type 2 diabetes (T2D). Alpha1-antitrypsin (AAT) is a circulating serine protease inhibitor that exerts anti-inflammatory effects, and AAT therapy offers great promise in protecting islets from inflammation. Human islet amyloid polypeptide (hIAPP) aggregation is associated with β-cell death in type 2 diabetes (T2D) and induces islet inflammation and β-cell dysfunction in transgenic mice overexpressing hIAPP. The aim of this study was to determine the protective effects of human AAT on β cells from cytokine-induced death in mouse islets and in transgenic mice overexpressing hIAPP.
Materials and methods: Isolated mouse islets were treated with cytokines (50U/mL Il1β, 1000U/mL TNFα, 1000U/mL IFNγ) and AAT (0.5mg/mL). Apoptosis and cell death was determined by *in toto* staining of cleaved caspase-3 and propidium iodide, respectively. Clodronate liposomes (1mg/ml) were used to deplete islet macrophages. Global gene expression analysis of isolated islets was performed and selected genes were validated by qPCR. 8 week-old wild-type and hIAPP transgenic mice were treated with intraperitoneal injections of AAT (2-6 mg/mouse) for 4 weeks. Glucose tolerance tests were performed before and at the end of the treatment.
Results: Low doses of pro-inflammatory cytokines induced the activation of a transcriptional inflammatory program and β-cell death in mouse islets. AAT abolished 90±5% of islet cell death and 83±9% of β-cell apoptosis induced by exogenous cytokines. Clodronate liposome-mediated depletion of islet macrophages blocked cytokine-induced β-cell apoptosis, indicating that resident macrophages are required for the cytotoxic action of cytokines in islets. A global transcriptomics analysis revealed that AAT treatment did not globally block the inflammatory program induced by cytokines, but interestingly decreased the expression of pro-inflammatory genes, including *Ccl2*, and increased by two-fold the expression of *Hspa1*, a stress-induced gene known to protect β cells. Finally, we also explored the effect of AAT *in vivo* in hIAPP transgenic mice. AAT treatment was started at 8 weeks of age, when these mice were already glucose intolerant. After 4 weeks, non-treated animals presented a more exacerbated phenotype. Remarkably, AAT treatment prevented the impairment of glucose intolerance and normalized glucose homeostasis in hIAPP transgenic mice.
Conclusion: Our results demonstrate that AAT prevents macrophage-mediated β-cell death induced by cytokines and normalizes glucose homeostasis in a mouse model of islet inflammation and dysfunction, highlighting the potential of AAT treatment as a therapeutic strategy for the treatment of T1D and T2D.
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Disclosure: **J. Rodríguez-Comas:** None.

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Circulating lipopolysaccharide and gut permeability in obese subjects with type 2 diabetes: the influence of surgical and endoscopic interventions

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Background and aims: Low-grade inflammation is considered one of the key mechanisms linking obesity to type 2 diabetes mellitus (T2DM) with translocation of gut microbiota being suggested as its potential initiator. The aim of our study was to assess the effect of selected interventions on parameters of intestinal leakage along with systemic and adipose tissue inflammation in obese subjects with T2DM.
Materials and methods: Thirty obese T2DM subjects (15 undergoing gastric plication (GP) and 15 endoscopic duodenal-jejunal by-pass liner implantation (DJBL)) and 10 healthy lean controls (C) were examined at baseline, 1 month and 6 (GP) and 10 months (DJBL) after intervention. Lipopolysaccharide binding protein (LBP) was used as a marker of circulating lipopolysaccharide levels and fatty acid binding protein 2 (FABP-2) as an indicator of intestinal leakage. Subcutaneous adipose tissue macrophages (ATMs) were quantified using flow cytometry.
Results: At baseline, both GP and DJBL groups had increased hsCRP (3.3±1.1 and 3.5±0.8 vs. 0.7±0.3 mg/l for GP and DJBL vs. C, p=0.002) and LBP (15.4±1.1 and 14.0±1.5 vs. 9.8±0.7 μg/ml, p=0.002) which positively correlated with ATMs percentage (R=0.463, p=0.035). Baseline FABP-2 did not differ between groups (1.81±0.29 and 1.65 ±0.25 vs. 1.24±0.97 ng/ml for GP and DJBL vs. C, p=0.478). Postprocedurally, except of reduced body weight (BMI 42.4±1.3 vs. 36.0±1.3 kg/m^2, p<0.001 for GP and 42.5±1.0 vs. 38.7±1.2 kg/m^2, p<0.001 for DJBL), HbA_{1C} (60.1±4.5 vs. 43.9±2.0 mmol/mol, p<0.001 for GP and 72.1±5.0 vs. 52.6±3.8 mmol/mol, p<0.001 for DJBL) and hsCRP (3.3±1.1 vs. 1.3±0.3 mg/l, p=0.040 for GP and 3.5±0.8 vs. 2.0 ±0.3 mg/l, p=0.069 for DJBL) both groups showed decreased amount of ATMs (26.8±2.7 vs. 15.4±2.0 %, p=0.008 for GP and 21.0±2.1 vs. 13.3 ±1.9 %, p=0.027 for DJBL), while no effect was seen on LBP (15.4±1.1 vs. 13.3±0.8 μg/ml, p=0.059 for GP and 14.0±1.5 vs. 12.7±1.3 μg/ml, p=0.104 for DJBL) and FABP-2 levels (1.81±0.29 vs. 1.91±0.47 ng/ml, p=0.109 for GP and 1.65±0.25 vs. 2.15±0.32 ng/ml, p=0.161 for DJBL).
Conclusion: Both interventions were associated with the reduction of systemic as well as subcutaneous adipose tissue inflammation. Modification of circulating lipopolysaccharide levels or gut leakage do not seem to be involved in these effects although circulating lipopolysaccharide might play a role in the increased ATM content in obese T2DM subjects.
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Early remission and later progression of type 2 diabetes after gastric bypass are related to changes in intestinal glucose transport

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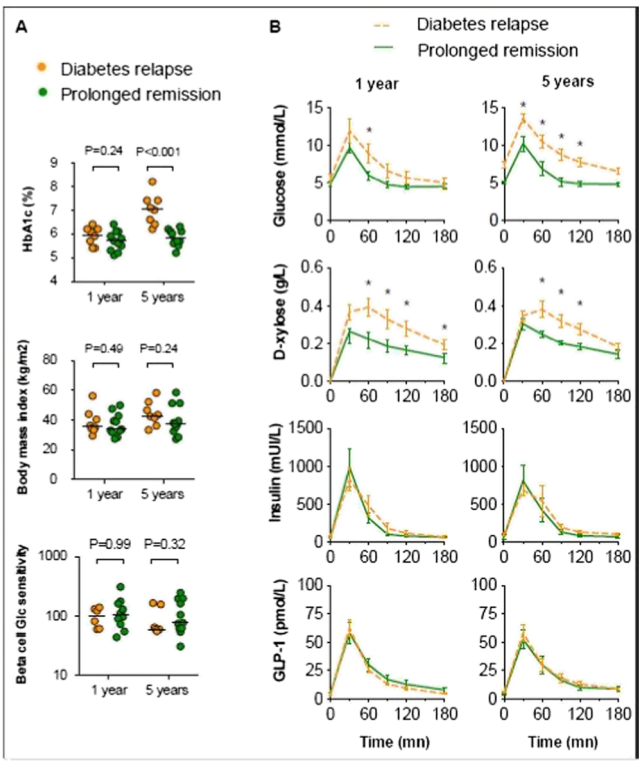
Background and aims: Understanding the intestinal mechanisms responsible for the metabolic benefit of Roux-en-Y gastric bypass (RYGB) may open novel strategies to treat type 2 diabetes. We recently showed in minipigs that RYGB modulates postprandial glycemic response by reducing intestinal sodium-glucose cotransport. The aim of this study was to confirm the clinical relevance of this mechanism in patients with type 2 diabetes submitted to RYGB.
Materials and methods: In this longitudinal study, we studied during 5 years obese patients with type 2 diabetes submitted to RYGB (n=26) or calorie restriction alone with adjustable gastric banding (n=20). Postprandial glycemic response, insulin and glucagon-like peptide 1 secretion as well as intestinal D-xylose absorption were evaluated during 180 min after a standardized mixed meal prior to surgery, and again during 3 postoperative visits: after the loss of 10 percent of initial body weight, after one year, and after five year.
Results: The postprandial glycemic response decreased significantly after RYGB independent of weight loss while it remained unchanged after adjustable gastric banding. This decrease in postprandial glycemic response after RYGB coincided with a reduction of intestinal D-xylose absorption and an increase in beta cell function and GLP1 postprandial response. In patients experiencing diabetes remission one year after RYGB (N=19), later disease progression and diabetes relapse at 5 years (N=8) were associated with higher D-xylose absorption (but not with lower BMI, beta cell function, nor GLP1 postprandial response
Conclusion: Conclusion: The decrease in intestinal glucose transport plays a central role in the metabolic benefit of Roux-en-Y gastric bypass in type 2 diabetes.



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Roux-en-Y gastric bypass results in rapid postprandial mixing of bile and foods and augmented enterohepatic bile acid retention

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Background and aims: Bile acids are major metabolic regulators with important effects on glucose homeostasis. After Roux-en-Y gastric bypass (RYGB) plasma concentrations of bile acids are increased, which could explain some of the metabolic improvements observed after surgery. To elucidate the mechanisms responsible for this increase, we assessed flow of bile and foods using direct scintigraphic techniques in RYGB operated patients and non-surgical matched controls.
Materials and methods: 15 RYGB patients (P) (age 46.5±10.8 yrs [mean ±sd], BMI 29.4±3.0 kg/m^2, time from surgery 3.8±1.2 yrs) and 15 non-surgical controls (C) (46.8±10.0 yrs, BMI 29.4±3.6 kg/m^2) underwent cholescintigraphy using an intravenous radiolabelled bile marker (^{99m}Tc-mebrofenin) combined with a standardized test-meal also containing a radiolabelled marker (^{111}In-DTPA). This method allowed concomitant visualization of foods and bile in the gastrointestinal tract, including assessment of the timing and extent of the mixing phase. Bile acid retention was estimated using a standard ^{75}Se-HCAT method (a radiolabelled taurine conjugated bile acid analog), which measures the fraction of ^{75}Se-HCAT retained in the enterohepatic bile circulation 7 days after oral ingestion.
Results: Prior to the meal, peak gallbladder filling was decreased in RYGB patients (25.0±3.9 vs 37.9±4.2%, p=0.029) and more bile marker had passively passed into the small intestine (median 58.1% [IQR 32.9;65.0] vs 17.4% [5.2;26.4], p=0.005). Gallbladder emptying in response to the meal was seen in both groups, but the total content of bile marker in the small intestine remained elevated over time in RYGB patients (at 30 min 86.7±10.8 vs 68.9±13.8%, p<0.001). By the end of the meal, pouch retention of the meal marker in RYGB patients was negligible compared to gastric retention in controls (9.5% [7.3;16.4] vs 94.7% [89.5;100], p<0.001). Consequently, almost complete mixing of foods with bile was seen immediately after the end of meal in RYGB patients, compared to a slow and gradual mixing in controls (percent of foods mixed with bile by end of meal: 80.0% [66.9; 89.7] vs 5.7% [0.0;9.6], p<0.001). After 7 days, retention of ^{75}Se-HCAT was increased in RYGB patients compared to controls (54.0±24.6 vs 31.7±21.1%, p=0.016). Further, in RYGB patients, bile acid retention was positively correlated with the amount of bile marker in the small intestine prior to meal intake during the cholescintigraphy (r=0.57, p=0.034).
Conclusion: RYGB patients are characterized by reduced gallbladder filling, increased bile flow from the liver into the small intestine in the fasting state, and augmented enterohepatic retention of bile acids. Combined with a negligible meal retention in the gastric pouch in these patients, rapid mixing of foods and bile is seen after RYGB. These alterations in bile circulation after RYGB likely affect plasma bile acid concentrations and nutrient absorption, in particular fat, and might thereby contribute to the postoperative metabolic improvements seen after RYGB surgery.
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Disclosure: **A. Eiken:** Grants; Novo Nordisk Foundation, Research Foundation of Amager and Hvidovre Hospitals.

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Metabolic control and bone turnover after Roux-en-Y gastric bypass in response to weight loss and weight stabilisation

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Background and aims: Metabolic improvements after Roux-en-Y gastric bypass (RYGB) are attributed to altered nutrient absorption and gut-islet cell cross talk in addition to major weight loss. Bone mineral density (BMD) is decreased, but whether RYGB induces changes in bone turnover beyond adaptation to major weight loss is not known. We investigated metabolic control and bone turnover during weight loss and after weight stabilization from 1 week (wk) post-RYGB and throughout the first 4 years (y).
Materials and methods: Ten subjects with preoperative type 2 diabetes (T2D) and ten with normal glucose tolerance (NGT) were investigated before (pre), 1 wk, 3 months (mo) and 1 y after RYGB with 16 subjects returning after 4 y. Plasma markers of metabolic control and bone turnover were obtained at fasting at all visits, during oral glucose tolerance tests (OGTTs) at all but 1 wk and DXAs (lumbar spine, hip and forearm) before, at 1 y and 4 y.
Results: Weight loss was maximal at 1 y post-RYGB with weight stabilization until 4 y (BMI pre: 39.1±4.2 kg/m^2 [mean ±SD], 1 year: 29.6 ±5.4, 4 year: 30.9±5.6, all p<0.01 vs pre). Good glycemic control was obtained early and maintained for 4 y despite cessation of antidiabetic agents in 9/10 T2D subjects (HbA1c pre: 7.0±1.0 %, 3 mo: 5.9±0.6, 4 y: 5.9±0.5, all p<0.01 vs pre). In both groups, insulin sensitivity (OGIS index from OGTT) increased during weight loss and remained stable from 1 y to 4 y. Early insulin secretion (IGI) increased in T2D only. Low glucose (<3.9 mmol/L) was observed in 7/16 (6 NGT) post-OGTT at 4 y contrasting 0/20 pre-RYGB, but neuro-glycopenic symptoms occurred only in 1 NGT. Fasting CTX, P1NP and osteocalcin were similar in T2D and NGT; unchanged at 1 wk, but increased by 60-100% at 3 mo, 110-150% at 1 y and remained 40-100% elevated at 4 y. CTX suppression during OGTT increased in both groups ($iAUC_{0\text{-}120}$ pre: −8±5 mg · min/mL, 3 mo: −32±11, 1 y:−29±14, 4y:−23±11, all p<0.01 vs pre), while suppression of P1NP and osteocalcin was largely unchanged post-RYGB. Changes in BMD were similar between groups with 6-8% decreases in hip and lumbar BMD during weight loss (pre - 1y) and 3-5% after weight stabilization (1y - 4y). In contrast, the largest decrease in forearm BMD was observed during weight stabilization, particularly in trabecular (pre-1y: −4% [95CI:−6, −2]; 1-4y:−11% [−14, −8]) but also in cortical bone (pre-1y: −0.6% [−3, +2]; 1-4y:−4% [−7, −1]).
Conclusion: Metabolic control in T2D is obtained early after RYGB and maintained for 4 years, whereas bone turnover is increased both during and after weight loss. Continued BMD loss is observed after weight stabilization, especially in non-weight bearing bone suggesting effects beyond adaptation to decreased mechanical load. Benefits in terms of long term stable glycemic control vs risks of accelerated bone loss must be considered when referring patients with type 2 diabetes to RYGB. Additionally, glucose tolerant patients are at particular risk of developing postprandial hypoglycemia after RYGB.
Clinical Trial Registration Number: NCT 01202526/NCT 03046147
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Disclosure: **K.N. Bojsen-Møller:** Grants; Danish Council of Independent Research.

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Soluble urokinase plasminogen activator receptor (suPAR) and high-sensitivity C-reactive protein (hsCRP) levels after bariatric surgery

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Background and aims: Low-grade inflammation in obesity can contribute to the development of cardiovascular disease, type-2 diabetes mellitus, cancer and mortality. The urokinase-mediated plasminogen activation system, including the urokinase-type plasminogen activator and its cellular receptor (uPAR) is connected to fibrinolysis, as it converts plasminogen to plasmin, angiogenesis, and shows promitogenic and proinflammatory qualities. uPAR's soluble form (suPAR) shed from the cellular surface and presents analogous levels in plasma to the anchored receptor. Although several studies have been conducted regarding biomarkers in severely obese patients, and the possibly favorable effects of weight reduction, to our knowledge only few series have examined the uPA/uPAR system with regard to inflammation following obesity. In this study we examined changes in plasma concentrations of suPAR amid other measurements, among a small cohort of severely obese subjects, undergoing two different types of bariatric surgery.
Materials and methods: Sixteen non-obese healthy subjects and 32 severely/morbidly obese patients who had already settled upon bariatric surgery [either Roux-en-Y gastric by-pass (RYGB) or sleeve gastrectomy (SG)] were included in the study. Non-obese patients were examined once, at study baseline; obese patients were examined pre-operatively and at 3, 6 and 12 months postoperatively. Fasting (FBG) and 2-h postprandial (PPG) serum glucose, triglycerides (TG), total cholesterol (Tchol), high-density lipoproitein cholesterol, fasting insulin, high-sensitivity C-reactive protein (hsCRP) and suPAR (using ELISA) levels were measured.
Results: Plasma suPAR concentrations at baseline were higher in obese than in lean participants (2.68 ± 0.85 vs. 1.85 ± 0.34 ng/ml, p=0.001). However, plasma levels of suPAR following bariatric surgery did not change and no differences were found between those undergoing SG and RYGB. Overall differences in levels between surgical arms were noted for FBG (p=0.013), PPG (p=0.008), Tchol (p=0.004) and LDLc (p=0.010), with SG patients presenting higher mean levels than RYGB patiens. Tchol (r=- 0.006, p=0.01) and LDLc levels (r= -0.008, p=0.007) were negatively, while log-transformed hsCRP (r=0.345, p=0.032) were positively associated with suPAR levels. One-year change in BMI was negatively associated with suPAR levels (r= -0.106 p=0.046).
Conclusion: Our findings strongly support association of obesity with inflammatory biomarkers and significant post-operational effects on levels of such biomarkers; however, a differential pattern of change was noted between suPAR and hsCRP levels.
Disclosure: **C. Liaskos:** None.

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Reductions in circulating Fetuin B and TGFβ1 contribute to higher hepatic insulin sensitivity after gastric bypass surgery compared to sleeve gastrectomy

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Background and aims: Duodenum exclusion via gastric bypass surgery has been suggested to contribute to higher type 2 diabetes remission rates compared to sleeve gastrectomy. Whether improvements in hepatokine and adipokine profiles as well as in hepatic and adipose tissue insulin sensitivity differ between surgery types remains unclear.
Materials and methods: Obese patients (OBE, n=36, age 40±11 yrs, BMI 51±7 kg/m^2) underwent hyperinsulinemic-euglycemic clamps with [6,6-$^2H_2$]glucose to assess hepatic and adipose tissue insulin sensitivity measured from insulin-mediated EGPsuppr (endogenous glucose production suppression) and AUC_{FFA} (free fatty acid area under the curve) during clamp, respectively. Measurements were performed before, 2, 12, 24 and 52 weeks after gastric sleeve (GS) or gastric bypass (GB) surgery. Covariance pattern models were used for statistical analysis. Normal-weight humans were studied at baseline (CON; n=7, age 38±4 yrs, BMI 24.9±1.1 kg/m^2).
Results: OBE had higher AUC_{FFA} at baseline (733±245 vs 358±28 AU in CON, p<0.05), which increased ca. 2-fold at 2 weeks, but was improved at 24 and 52 weeks after both surgical procedures. Impaired suppression of lipolysis at 2 weeks related positively to increases in fibroblast growth factor 21 (FGF21), cytokeratin 18 and interleukin-1 receptor antagonist (IL-1ra) by 112%, 12% and 286%, respectively, while improved AUC_{FFA} at 52 weeks associated with decreases in FGF21 and transforming growth factor β 1 (TFGβ1) and increase in adiponectin. Hepatic insulin sensitivity was comparable between CON and OBE at baseline (90±24 vs 89±9 % in CON, p>0.05). Only bypass surgery resulted in continuous improvements of EGPsuppr from 24 to 52 weeks in OBE. Of note, gastric bypass surgery, but not sleeve gastrectomy, improved both fetuin B (2.2±0.3 vs 2.6±0.6 μg/ml at baseline, p=0.03) and TGFβ1 levels (26.9±6.5 vs 30.8±5.5 ng/ml at baseline, p<0.05) 52 weeks after surgery.
Conclusion: The unrestrained adipose tissue lipolysis occurring after bariatric surgery is linked to altered hepatokine and cytokine release. Reduction in fetuin B and TGFβ1 likely contribute to the better metabolic outcomes after gastric bypass compared to gastric sleeve surgery.
Clinical Trial Registration Number: NCT01477957
Disclosure: **S. Gancheva:** None.

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Increased albuminuria and its determinants in non-diabetic and diabetic patients with morbid obesity and the effect of weight loss following bariatric surgery

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Background and aims: Patients with morbid obesity carry a high cardiovascular risk, which can only be partially explained by classic cardiovascular risk factors. In this study we wanted to evaluate (a) the prevalence of microalbuminuria and its determinants in non-diabetic and diabetic patients and (b) the effect of weight loss following bariatric surgery.
Materials and methods: We included 1307 patients (77% women, mean age: 40 ±12 years, mean BMI: 45.6 ± 6.6kg/m²) in a cross-sectional study. A subgroup of these patients (n=318) was followed up for two years after bariatric surgery. Apart from weight and cardiovascular risk-markers (blood pressure, lipids), a glucose tolerance test (75g oGTT), renal and inflammation parameters were assessed. HOMA-IR was calculated. Albuminuria was assessed by collecting 24h urine on three consecutive days.
Results: In the cross sectional study, prevalence of microalbuminuria was 16.0% (n=209), and of macroalbuminuria 3.1% (n=41). The Chi square for the association of albuminuria and diabetes was 31.937; p-value <0.001. However, of all patients with albuminuria 42.3% had a normal glucose tolerance. In a multivariate linear stepwise backward regression analysis, systolic blood pressure (beta= 0.236; p<0.001), log fasting

insulin (beta= 0.309; p<0.001) and log 2hour postprandial insulin (beta= -0.173; p=0.033) remained the most predicting parameters for albuminuria. In the longitudinal study, we found a significant decrease of albuminuria: 11.1 mg/24h (6.4, 18.4 mg/24h) to 7.8 mg/24h (4.9, 13.0 mg/24h; p< 0,001) in the whole group (n=318). In the group with albuminuria preoperatively, albuminuria decreased from 65.7 mg/24h (38.2, 147.08 mg/24h) to 13.5mg/24h (8.4, 36.8 mg/24h; p<0.001). After adjusting for age, sex and baseline albuminuria, patients with a lower creatinine clearance showed a smaller decrease of albuminuria (beta= 0.177; p= 0.021).
Conclusion: A substantial portion of patients with morbid obesity exhibits microalbuminuria, nearly half of those present with normal glucose tolerance. After significant weight loss induced by bariatric surgery we found a significant decrease of albuminuria, potentially indicating or even contributing to the known reduction of cardiovascular mortality after bariatric surgery.
Disclosure: **J.M. Brix:** None.

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A comparison of the effects of Roux-en-Y gastric bypass on glucose tolerance in Zucker diabetic fatty rats

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Background and aims: Roux-en-Y gastric bypass (RYGB) is the most efficacious intervention for the treatment of obesity and type 2 diabetes. Improvements in glycaemic control begin prior to significant weight loss and are characterised by improved hepatic insulin sensitivity and oral glucose tolerance (OGTT), the latter occurring secondary to enhanced incretin responses. We previously showed that in Zucker Diabetic Fatty rats (ZDF), RYGB equivalent weight loss achieved through food restriction could not in isolation recapitulate the glycaemic improvements seen after RYGB. The aim of the current study was to compare intravenous and oral glucose tolerance between Zucker Diabetic Fatty rats 7 weeks after either RYGB or a multimodal diet and pharmacotherapy protocol aimed at mimicking RYGB induced weight loss and improvements in fasting glycaemia.
Materials and methods: At 12 weeks of age, ZDF *fa/fa* rats underwent RYGB (n=15) or Sham surgery (laparotomy, n=25). Sham operated rats were assigned to a control untreated group (8) or "medical bypass" group (MB) (17). The MB group was calorie restricted to mimic weight loss in the RYGB group and received medication including GLP-1 analogue to control hyperglycaemia, hyperlipidemia and blood pressure. Intraperitoneal and oral glucose tolerance tests (IPGTT and OGTT) were carried out at 19 weeks in all groups and values obtained compared against non-diabetic, non-obese *fa/+* control rats. For the OGTT, half of the medical bypass group were withdrawn from GLP-1 analogue therapy for 24 hours.
Results: RYGB resulted in 20-30% weight loss and normalized fasting glycemia. The MB protocol successfully matched both the weight loss and improvement in fasting glycaemia of the RYGB group without the requirement for ongoing insulin administration. Glucose tolerance as assessed by IPGTT and OGTT was improved versus Sham by both RYGB and MB, but reductions in 2-hour area under the curve (AUC) for plasma glucose IPGTT were greater after MB (RYGB 1669 ± 353 v MB 1335 ± 269, p=0.02). Elevations in AUC for plasma glucose relative to control rats only persisted following OGTT after RYGB (*fa/fa* 1145 ± 142 v *fa/+* 721 ± 142 p=0.03).
Conclusion: In the ZDF rat, multimodal diet and pharmacotherapy resulting in RYGB matched weight loss and reductions in fasting glycaemia, improved both intravenous and oral glucose tolerance to extent to a greater degree than observed in RYGB operated rats, even when GLP-1 analogue was omitted from RYGB. Thus, despite the increased clinical efficacy of RYGB, enhanced improvements in glycaemic control can be obtained non-surgically when equivalent weight loss is paired with comprehensive metabolic control measures. Future studies may identify a lesser threshold of weight-loss feasibly achievable in the clinic which when accompanied by comprehensive medical control of the metabolic milieu may prove as effective as RYGB in the treatment of obesity and type 2 diabetes
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Disclosure: **L. Shakerdi:** None.

PS 042 Brown adipose tissue functioning and dysfunctioning

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Inhibitory action of secretin on food intake depends on UCP1-mediated thermogenesis in brown adipose tissue

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Background and aims: Secretin was discovered as the endocrine stimulus of pancreatic fluid production more than 100 years ago, but since then was also reported to have other effects including the inhibition of food intake. Like many other gut hormones, secretin levels in the circulation rise after a meal. We found that the secretin receptor is highly expressed in brown fat, which harbors a remarkable capacity to evoke energy expenditure through uncoupling protein 1 (UCP1) dependent thermogenesis in brown adipocyte mitochondria. A role of brown fat for meal-induced thermogenesis, defined as the increase of energy expenditure induced by meal ingestion, was suggested in the past. According to the thermostatic theory, meal-induced thermogenesis may serve as a feedback signal for satiation thereby controlling meal size. Therefore, the aim of this study was to investigate the effect of the gut hormone secretin on brown fat thermogenesis and food intake.
Materials and methods: Microplate respirometry of murine primary brown adipocytes was applied to investigate oxygen consumption, which is a key readout for thermogenic UCP1 activity. Reverse transfection with siRNAs was applied to knock down the secretin receptor. *In vivo*, oxygen consumption of mice was measured via indirect calorimetry after i.p. injection of either secretin or vehicle (PBS). Furthermore, the impact of exogenous secretin on food intake was investigated in both UCP1 wildtype and knockout mice after i.p. injection following ad libitum refeeding after an 18h fasting period.
Results: Stimulation of primary brown adipocytes with secretin acutely increased cell respiration comparable to isoproterenol, a β-adrenergic receptor (AR) agonist known to activate uncoupled respiration mediated by UCP1 (3.8±0.1 vs. 4.1±0.7-fold above basal proton leak, p=0.89, n=5). The β-AR antagonist propranolol abolished the effect of isoproterenol, but not the thermogenic effect of secretin. Secretin induced respiration was abolished knockdown of the secretin receptor. Mice injected with secretin showed a larger increase in oxygen consumption compared to vehicle injected controls (4376 ±661 vs. 3691±354 ml O_2/h, p=0.011, n=8-9). Furthermore, exogenous secretin injection reduced food intake in fasted mice compared to vehicle injection (0.56±0.30 vs. 0.89±0.19 g/h, p=0.0008, n=6-7). Notably, these effects of secretin on thermogenesis and food intake were attenuated in UCP1 knockout mice.
Conclusion: Our results reveal secretin as a novel non-adrenergic activator of brown fat that potentially stimulates meal-induced thermogenesis. The inhibitory action of secretin on food intake depends on UCP1-mediated thermogenesis in brown fat. In line with the thermostatic theory of feeding, we suggest that meal-induced thermogenesis is triggered by a post-prandial rise in circulating secretin and activation of thermogenesis in brown fat, which promotes meal termination. This proposed mechanism does not necessarily require central action of secretin in the brain, as secretin directly stimulated thermogenesis in cultured brown adipocytes. Notably, brown fat activity is negatively associated with BMI and age, and largely attenuated in obese and diabetic patients. Thus, secretin might hold promise for developing novel obesity therapies as the activation of brown fat exhibits negative impact on energy balance through both, increasing energy expenditure and decreasing energy intake.
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Disclosure: **K. Braun:** None.

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Ambient air pollution exposure promotes insulin resistance via changes in white and brown adipose tissues in mice

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Background and aims: Increasing evidence implicates ambient fine particulate matter (less than 2.5 mm in aerodynamic diameter, PM2.5) air pollution as a risk factor that are associated with systemic diseases globally, especially with cardiovascular morbidity and mortality, such as atherosclerosis and obesity. Adipose tissues, which includes mainly white and brown adipose tissues, endure important endocrine and metabolic functions, especially with recent demonstration of considerable amounts of metabolically active brown adipose tissue in adult humans. We therefore hypothesized that exposure to ambient PM2.5 exposure would induce inflammation and oxidative stress with adipocyte-specific gene changes in adipose tissues, which result in mitochondrial dysfunction and insulin resistance, and promote type 2 diabetes and metabolic disorders.
Materials and methods: To demonstrate the impact of PM2.5 exposure on insulin resistance and metabolic disorder via adipose tissues, mice were exposed to concentrated ambient PM2.5 or filtered air (FA) (6 hours/day, 5 days/week) for up to 10 months in Columbus, OH. Major examinations were performed, which primarily include energy-dispersive X-ray fluorescence (ED-XRF) for elemental analysis on the filters collected, intraperitoneal glucose tolerance test before and after the exposure, blood samples for the measurement of circulating inflammatory biomarkers, ultrastructure of adipose tissues by transmission electron microscopic (TEM), Dihydroethidium (DHE) staining to detect superoxide, immunohistochemical staining and Western blot for protein examination in the adipose tissues, and quantitative real-time PCR for adipose tissue gene analysis.
Results: Exposure to PM2.5 increased reactive oxygen species (ROS) production, decreased mitochondrial size in brown adipose tissue while mitochondrial number was significantly reduced in both white and brown adipose tissues. PM2.5 exposure also down-regulated brown adipocyte-specific genes while white adipocyte-specific genes up-regulated. In addition, PM2.5 exposure induced insulin resistance and decreased glucose tolerance compared with the FA exposure. Circulating adiponectin and leptin were significantly decreased in PM2.5-exposed group although there were no significant differences in circulating cytokines between PM2.5- and FA-exposed groups. Additionally, PM2.5 exposure decreased mitochondrial count in visceral adipose and mitochondrial size in interscapular adipose tissue, which were associated with reduction of uncoupling protein 1 (UCP1) expression and downregulation of brown adipocyte-specific gene profiles.
Conclusion: These findings suggest that ambient PM2.5 exposure triggers oxidative stress that results in mitochondrial gene expression and mitochondrial alterations in brown adipose tissue, which causes imbalance between white and brown adipose tissue functionality, impaired glucose tolerance and insulin resistance and thereby predispose to metabolic dysfunction and a risk factor for the development of type 2 diabetes.
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Disclosure: **Q. Sun:** None.

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Zbtb16 affects impact of maternal high-sucrose feeding on the metabolic and transcriptomic profiles of the offspring

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Background and aims: Over-nutrition in pregnancy and lactation affects fetal and early postnatal development, which can result in metabolic disorders in adulthood. Previous studies have shown the effects of improper

maternal diet and associated them with altered fetal development, however, little is known about involvement of genetic factors in this process. We suggested that a significant energy metabolism regulator, Zbtb16 gene, could be important for pathogenesis of metabolic syndrome and tested a hypothesis that variation of Zbtb16 gene modulates the programming effect of high-sucrose diet (HSD) on metabolic and transcriptomic profiles of the offspring.

Materials and methods: We established a new minimal congenic rat strain containing only a single gene, Zbtb16, from a metabolic syndrome model, the polydactylous rat (PD/Cub) strain, within the spontaneously hypertensive rat (SHR) strain genomic background. 16-week-old SHR and SHR-Zbtb16 rat dams were fed either standard diet during pregnancy and 4 weeks of lactation (control groups) or a high-sucrose diet (HSD, 70% calories as sucrose) during the same period. We assessed comprehensively the metabolic profiles of the four groups of dams as well as their adult offspring fed standard diet including glucose tolerance tests, levels of insulin, cytokines and hormones as well as concentrations of triglycerides and cholesterol in 20 lipoprotein fractions. Furthermore, we have assessed the transcriptome (GeneChip Rat Gene 2.1 ST Array Strip) in liver, brown and white adipose tissue. Two-way ANOVA with STRAIN and MatDIET as major factors was used for metabolic profile, transcriptomic data were processed in Partek Genomics Suite and resulting differentially expressed transcripts (FDR < 0.05) were analyzed by Ingenuity Pathway Analysis.

Results: In dams of both strains we observed an increase of cholesterol and triglyceride concentrations in large particles (chylomicrons, VLDL) and decrease of cholesterol and triglyceride concentrations in medium to very small LDL particles when fed HSD. We identified significant STRAIN*MatDIET interactions for levels of VEGF (p=0.01), PYY (p=0.03) and area under te glycemic curve (p=0.02). In offspring, exposure to maternal HSD substantially increased brown fat weight in both strains (MatDIET p = 1.52E-10), decreased triglycerides in most fractions of LDL particles (16.7-25.5 nm, MatDIET p = 0.003) and impaired glucose tolerance exclusively in SHR offspring. The transcriptome assessment in offspring revealed networks of transcripts reflecting the shifts induced by maternal HSD with major nodes including mir-126, Hsd11b1 in the brown adipose tissue ; Pcsk9, Nr0b2 in liver and Hsd11b1, Slc2a4 in white adipose tissue.

Conclusion: Our results show that Zbtb16 gene substantially modulates the effect of HSD-induced programming on metabolic and transcriptomic profiles of the offspring.

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NFIA controls the brown fat gene programme by co-localising with PPARgamma at cell type-specific enhancers

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Background and aims: Brown fat dissipates energy in the form of heat by means of the uncoupling protein-1 (UCP1) on the mitochondrial inner membrane. In humans, brown fat activity is inversely correlated with body mass index. Therefore, stimulating development and/or function of brown fat would be a novel strategy for the treatment of obesity and its complications. However, global landscape of brown fat development is not entirely understood.

Materials and methods: To profile the tissue-specific accessible chromatin landscape, we performed FAIRE-seq analyses of murine interscapular brown fat and white fat using 8-week-old male C57BL/6J mice. We next performed motif analyses of depot-specific open chromatin regions to seek a novel transcriptional regulator. To examine whether NFIA can induce adipocyte differentiation from myoblasts, we introduced NFIA into C2C12 myoblast cell lines using retroviral vectors. To evaluate the physiological relevance of NFIA in BAT in vivo, we analyzed BAT in wild-type and NFIA-KO mice.

Results: Here, we identified nuclear factor I-A (NFIA) as a novel transcriptional regulator of brown fat. The binding motif for Nuclear factor I (NFI) transcription factor is enriched within brown-fat-specific open chromatin regions. Of the four isoforms of NFI family, NFIA is highly expressed in brown fat compared to white fat or muscle. Introduction of NFIA into myoblasts results in lipid accumulation, activation of the brown-fat-specific gene program and suppression of muscle genes. Conversely, the brown fat of NFIA knockout mice displays impaired expression of the brown-fat-specific genes and reciprocal elevation of muscle genes. Mechanistically, NFIA selectively co-localize with PPARgamma at the brown-fat-specific enhancers, and co-localization of NFIA facilitates the binding of PPARgamma, leading to increased chromatin accessibility and active transcription.

Conclusion: Collectively, these results indicate that NFIA is a novel key transcription factor that co-localizes with PPARgamma and activates the brown-fat-specific gene program.

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The pivotal role of O-GlcNAcylation in cold-induced thermogenesis by brown adipose tissue

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Background and aims: Brown adipose tissue (BAT) is important for non-shivering thermogenesis under cold conditions in rodents and also plays significant roles in glucose and lipid metabolisms. During cold exposure, glucose and fatty acid oxidations increase in order to maintain core temperature by stimulating heat production in BAT.O-GlcNAcylation is the post-translational modification that is characterized by the addition of N-acetylglucosamine to various proteins by O-GlcNAc transferase (Ogt). Since O-GlcNAcylation is considered as a "nutrient sensor" for glucose and fatty acids, it could influence BAT function. However, little is known about the role of O-GlcNAcylation in BAT. Therefore, we investigated the physiological role of O-GlcNAcylation in BAT.

Materials and methods: BAT-specific Ogt knockout (Ogt-BKO) mice were generated using the Cre-LoxP system. We crossbred Ogt-flox ($Ogt^{f/f}$) female mice with uncoupling protein 1 (UCP1) promotor Cre mice. We have confirmed that Ogt protein expressions and subsequent O-GlcNAcylations were decreased only in BAT in Ogt-BKO mice.

Results: Ogt-BKO mice led to hypertrophy and displayed an accumulation of enlarged lipid droplets in BAT cells, although there were no differences in body weights compared to control mice. During cold exposure, Ogt-BKO mice showed severe cold intolerance, and BAT UCP1 protein expression was severely diminished. In addition, a significant reduction in protein expression of peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC-1α), a key regulator of thermogenesis, was observed. Also, mitochondrial related proteins were significantly decreased, accompanied by decreased PGC-1α expression. Blood glucose levels in Ogt-BKO mice during an intraperitoneal glucose tolerance tests under cold condition were lower than those in control mice, although there were no significant differences at room temperature. Moreover, the impaired cold-induced thermogenesis in Ogt-BKO mice was recovered

by following glucose injection or oral intake of a normal diet, but not by following olive-oil injection or oral fat load.
Conclusion: O-GlcNAcylation in BAT is essential for cold-induced thermogenesis and mitochondrial biogenesis by modulating PGC-1α. Ogt deletion in BAT preferentially utilize glucose, and defective fat utilization. Our findings suggest that O-GlcNAcylation plays a crucial role in glucose and lipid homeostasis in cold environments and may act as a "metabolic switch" in BAT.
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Disclosure: **N. Ohashi:** None.

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IL-10/JAK1/STAT3 signalling loss links changes in mitochondria cristae architecture and nonshivering thermogenesis in brown adipose tissue

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Background and aims: Several studies has shown that obesity is linked to chronic low-grade inflammation. The anti-inflammatory cytokine interleukin-10 (IL-10) has a key role as a regulator of obesity-induced inflammation. Since non-shivering thermogenesis in brown adipose tissue (BAT) is important to whole body energy balance, disruption on BAT thermogenesis may lead to obesity. Given that IL-10 activates JAK1/STAT3 pathway and activated STAT3 contributes to regulation of brown adipose tissue differentiation, here we addressed whether IL-10 signaling regulates brown fat function.
Materials and methods: We analyzed protein levels in BAT using immunoblotting. In vivo role of IL-10 signaling in nonshivering thermogenesis was evaluated with ^{18}fluorodeoxyglucose uptake by BAT (measured using PET/CT) during mild cold exposure and interscapular temperature using infrared thermography (FLIR® instruments) in IL-10 deficiency mice. Metabolic activity of BAT was measured using fluorescence lifetime imaging microscopy (FLIM) of NADH and FAD and isolated brown-fat mitochondria respiration using Oxygraph-2k respirometer. Morphological examination of mitochondria was performed with transmission electron microscope (TEM).
Results: IL-10 KO mice upon to high-fat feeding (HF) displayed a higher weight gain compared to wild-type mice. Since no difference on caloric ingestion or activity was detected between IL10d and wild type mice, we observed that IL-10 KO mice consumed less oxygen than control mice. HE staining revealed the presence of larger adipocytes in interscapular BAT depot in IL-10 KO mice. Consistent with this, IL-10 KO developed impaired glucose tolerance in ipGTT. To evaluate in vivo role of IL-10 signaling in adaptive thermogenesis, we challenged the IL-10 KO mice with cold exposure (16°) for 1 hour. After that, the IL-10 KO showed decreased ^{18}fluorodeoxyglucose uptake by BAT using PET/CT and decreased interscapular temperature (using infrared thermography). As assessed by UCP1 expression, IL-10 KO exhibited impaired UCP1 increment after HF feeding in relation to WT mice. Nevertheless, IL-10 KO showed similar oxygen consumption and interscapular temperature change after β3-agonist (CL-316,243) stimulation. In vitro, we measured the oxygen consumption of isolated BAT mitochondria, and the IL-10 KO mitochondria showed a significantly lower consumption when compared to wild type, mainly due a decrease in UCP-1 related consumption. Indeed, UCP-1 protein levels were decreased in IL-10 KO BAT. In addition, in vivo FLIM of NADH and FAD, the mean lifetime of FAD was longer than the mean lifetime of NADH in the IL-10 KO mice in relation to WT mice, as well as the IL-10 KO mice showed increased cellular redox ratio and consequent decreased cellular metabolic activity. Besides, we performed TEM on BAT mitochondria of the two groups and revealed that IL-10 signaling loss is accompanied by decreased cristae density and mitochondria fragmentation. The treatment with TNF-alfa antibody and gain-of-function experiment reverted these structural abnormalities, pointing out that this anti-inflammatory cytokine signaling is involved in the mitochondria disruption.
Conclusion: Thus, in the absence of IL10 there is an impairment of thermogenesis leading to increased body mass gain and mitochondria fragmentation.
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The reduced adipocyte mitochondrial oxidative phosphorylation are linked to impaired beta 3-adrenergic receptor signalling in obese adults

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Background and aims: Obesity is increasing in an epidemic manner in most countries and constitutes a public health problem by enhancing the risk for cardiovascular disease and metabolic disorders such as type 2 diabetes. Early indications suggest that brown adipose tissue (BAT) metabolism is defective in obesity and type 2 diabetes mellitus, which may have important pathological and therapeutic implications. The beta3-adrenergic receptor (β3-AR) pathway is a dominant signaling pathway to activate BAT thermogenesis in humans, selective β3-AR agonist had been proved to stimulated glucose uptake in the BAT depots and increase resting metabolic rate in healthy adult humans, but minimal efficacy on BAT activity was observed in obese subjects after administration of adrenergic agonist. The lack of efficacy of β-AR agonists in obese subjects appears to be due to an impaired β-AR signaling in adipose tissues. The underlying mechanisms for the β-AR resistance remain poorly understood. Our objective was to determine whether the reduced adipocyte mitochondrial oxidative phosphorylation level in obesity is associated with β3-AR expression in non-diabetes adults.
Materials and methods: Omental and/or abdominal subcutaneous adipose samples were collected from 86 age-matched non-diabetic men and women undergoing elective abdominal surgery, mature adipocytes were separated from adipose tissue by collagenase digestion. Mitochondria oxygen consumption rate (OCR), the abundance of mtDNA-encoded NADH dehydrogenase 1 genes (ND1/28S), Citrate synthase (CS) activity, expression levels of β3-AR, HSL and CPT-1 of mature adipocytes were measured using a real-time quantitative PCR system, Clark-type oxygen electrode, spectrophotometric method and western-blot.
Results: The β3-AR mRNA expression of mature adipocyte in obese group was lower (1.8±0.92 vs. 5.6±2.21, P=0.01) compared to non-obese group. Mitochondria OCR (125.01±60.76 vs. 64.52±29.6 pmol/(s*ml)/mg protein, P=0.007) and CS activity levels (1.4 ± 0.18 vs. 0.76 ± 0.06 μmole/min/mg protein, respectively, P = 0.006) were higher in non-obese group than obese ones. A positive correlation between adipocytes mitochondrial OCR and β3-AR mRNA expression level was reported(r=0.6, P<0.05). There were no significant difference between obese and non-obese groups in the abundance of ND1/28S (P>0.05), which showed the different oxidative phosphorylation level between two groups suffered from the difference in the adipocyte mitochondrial capacity, not in the mitochondrial content. The HSL enzyme activity (1.6 folds, P=0.01), CPT-1 enzyme activity (1.8 folds, P=0.008) and CPT-1 mRNA expression levels (2.1 folds, P=0.006) in non-obese group was higher compared to obese group.
Conclusion: Obese individuals suffered from impaired mitochondrial oxidative capacity, followed by lipid metabolism disorders and increased oxidative stress. Promoting BAT recruitment is an important aspect to be considered for obesity who do not possess sufficient amounts of active BAT. The lack of efficacy of cold and β3-AR agonist in recruiting BAT was partly due to impaired β3-AR pathway in obese individuals. The

underlying mechanisms for the β3-AR pathway dysfunction remain poorly understood and should be further investigated.
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Adenosine and A_{2A} receptors in human brown adipose tissue

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Background and aims: Brown adipose tissue (BAT) has emerged as a potential target to combat obesity and diabetes in humans. Novel strategies to activate BAT and improve metabolism are needed. Adenosine is a purine nucleoside released locally in BAT when noradrenaline and ATP are released from sympathetic nerves. Recently it was found that adenosine activates murine and human brown adipocytes, and recruits beiging of white fat via adenosine A_{2A} receptors (A2AR). Furthermore, studies with mice have shown improvements in glucose homeostasis after administration of A2AR agonists. In this study we aimed to investigate the significance of adenosine and A_{2A} receptors in human BAT in vivo. Using PET imaging we measured perfusion and A2AR in supraclavicular BAT and other tissues in healthy men.
Materials and methods: We studied healthy male subjects using PET imaging (n=10, age 25 ± 6 years, BMI 24.5 ± 1.7 kg/m^2). Perfusion of BAT, white adipose tissue (WAT) and muscle were measured with [^{15}O]H_2O PET/CT in basal conditions, during cold exposure and during intravenous infusion of adenosine (0.14 mg/kg/min). To investigate adenosine A_{2A} receptors in BAT, PET imaging with A2AR PET radioligand [^{11}C]TMSX was done once in basal conditions and once during controlled cold exposure (average 16.2 ± 1.6 Celsius degrees, cooling blanket Blanketrol III, Cincinnati Sub-Zero). Before all PET scans the study subjects fasted overnight and avoided caffeine and strenuous exercise for minimum of 24 hours. Distribution volume (DV), i.e. the ratio of the radioligand concentration in tissue to that in plasma at equilibrium, was quantified using Logan plot. BAT and WAT biopsies were obtained from 3 study subjects and are currently being analyzed.
Results: Intravenous adenosine infusion caused a maximal perfusion effect in supraclavicular BAT. As expected, cold exposure doubled the perfusion of BAT compared to basal conditions (basal 8.5 ± 4.5 vs. cold 19.6 ± 8.6, P=0.004) and interestingly adenosine further increased perfusion by nearly 50% (cold 19.6 ± 8.6 vs. adenosine 28.6 ± 7.9, P=0.03). In line with previous studies, i.v. adenosine also increased muscle perfusion 9-fold (P=0.03) and WAT perfusion 5-fold (P=0.004). DV of the A2AR radioligand [^{11}C]TMSX in BAT was significantly lower in cold conditions when BAT was activated compared to baseline conditions (0.89 ± 0.1 vs. 1.36 ± 0.5, P=0.038). DV of [^{11}C]TMSX in muscle was similar as reported in earlier studies and cold exposure caused no significant change.
Conclusion: Exogenous adenosine increased BAT blood flow even more than cold exposure. Adenosine A_{2A} receptor density can be quantified in human BAT using [^{11}C]TMSX PET/CT. Cold exposure decreased the density of A_{2A} receptors available for radioligand binding in human BAT. This may be due to an increase in locally released endogenous adenosine during cold activation of BAT. These results support that adenosine and A2AR are significant in human BAT physiology.
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Disclosure: **M. Lahesmaa:** None.

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Secretin increases glucose uptake in human brown adipose tissue

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Background and aims: GI peptides have a role in energy metabolism and in the development of diabetes and obesity. Obesity and type 2 diabetes are associated with impaired cold-induced brown adipose tissue (BAT) function. Recent preclinical findings suggest that secretin activates BAT in mice. The aim of our study was to investigate whether secretin activates BAT in humans.
Materials and methods: Fifteen healthy, normal-weight males were recruited. Data is presented on the subjects (n=7, mean age 46.6 ± 11.9 years, mean BMI 23.7 ± 2.0) who have completed all studies, while studies for the rest of the subjects will be finalized by August 2017. Supraclavicular BAT glucose uptake (GU) and blood flow were measured with [^{18}F]-FDG- and [^{15}O]-H_2O-PET-CT. The protocol included three separate study days: one during cold exposure and after placebo (saline) or secretin (2 IU/kg of secretinpentahydrochloride) injections. Administration of secretin and placebo were randomized and blinded. Graphical image analysis was blinded. Plasma secretin concentration was measured at baseline and at specific time points during all scans.
Results: Plasma secretin concentration increased after i.v. injection of secretin to a postprandial level for 30 minutes. Compared to saline, secretin administration stimulated an increase in BAT glucose uptake (GU 1.5 ± 0.8 vs GU 0.8 ± 0.4 μmol/100g•min, secretin vs. placebo p=0.04, N=7). Secretin did not increase BAT flow compared to placebo (4.0 ± 2.0 vs 5.6 ± 2.0 ml/100g•min, p=0.12, N=7). In the whole group, cooling tended to increase BAT GU (4.0 ± 4.3 vs. 0.8 ± 0.4 μmol/100g•min, cold vs. placebo, p=0.08, N=7), with three out of seven subjects having metabolically active BAT.
Conclusion: Secretin increases supraclavicular BAT glucose uptake compared to placebo in humans independently of perfusion. These results indicate a potential role of secretin in postprandial thermogenesis and merit further human studies addressing the pharmacological potential of secretin in BAT activation.
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Disclosure: **S. Laurila:** None.

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Association of glycaemic control with the depletion of mucosal-associated invariant T (MAIT) cells in cardiometabolic diseases

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Background and aims: Cardiometabolic diseases (CMDs) represent a considerable burden for health care systems. Systemic low-grade inflammation is an important and common mediator of CMDs. We recently reported that a subset of T cells, circulating mucosal-associated invariant T (MAIT) cells, not only decrease in frequency in obesity and type-2 diabetes (T2D), but are also strongly proinflammatory. It is unknown if MAIT cell reduction and modified phenotype worsens with CMD severity. Here, we assessed circulating MAIT cell characteristics in 439 subjects from the EU-Metarcardis cohort and examined potential mechanisms underlying MAIT cell loss from the bloodstream.
Materials and methods: The frequency of MAIT cells identified as $CD3^{+}CD161^{hi}V\alpha7.2\ TCR^{+}$ cells by flow cytometry of blood samples was examined in 66 healthy subjects and 373 patients with different stages of CMD severity. These patients were stratified in 5 groups of CMDs as follows: metabolic syndrome, obesity, T2D, coronary artery disease with or without heart failure. We examined the relationships between MAIT cell characteristics and CMD clinical phenotypes.
Results: Compared to healthy controls, blood MAIT cell frequency was significantly decreased in CMD groups (median values: 3.5% among controls vs. 0.4% - 1.6% in the different CMD groups, all p from age-adjusted ANCOVA < 0.05). The most substantial decrease was observed in patients with coronary artery disease and heart failure (0.4%). Moreover, absolute MAIT cell numbers were decreased among CMD patients despite an increase of overall circulating leukocytes. MAIT cell abundance was associated with metabolic and inflammation variables such a glycated hemoglobin ($r=-0.28$, $p<0.0001$), hs-CRP ($r=-0.24$, $p<0.0001$) and IP-10 ($r=-0.22$, $p<0.0001$). In vitro exposition of immune cells to increasing doses of glucose promoted MAIT cells apoptosis while conventional T cells were not altered.
Conclusion: A reduced proportion of circulating MAIT cells is a common cellular signature observed in CMD patients, and is related to markers of glycemic control and of chronic inflammation. Thus, MAIT cell loss may in part be related to inflammatory and metabolic stress, notably hyperglycemia, in these patients.
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Disclosure: **K.E. Assmann:** None.

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Perilipin family determines lipid droplet storage in macrophages and alters their inflammatory character

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Background and aims: Macrophages play a role in the growth and vulnerability of atherosclerotic plaques. Macrophage polarity (the balance between pro-inflammatory macrophages (M1) and anti-inflammatory macrophages (M2)) is associated with an increased incidence of stroke. Macrophages store lipids in cytosolic lipid droplet (LD), surrounded mainly by the perilipin (PLIN) family. In adipose tissue, immature small LDs are surrounded by PLIN2 being replaced by PLIN1 to large LDs. PLIN1 coordinates multiple related proteins for the maturation of LDs and the lipolysis stimulated by promoted PKA. However, there have been few reports on the roles of PLIN1 in macrophage development and the inflammatory polarity. The aim of this study was to investigate the relationship between LD proteins and the inflammatory character of macrophages.
Materials and methods: The experiments used tissues from human arteries in 65 patients who had undergone a carotid endarterectomy, and cultured macrophages were generated from healthy human peripheral blood mononuclear cells. Tissue plaques were evaluated using immunostaining, real-time PCR, and western blotting. Monocytes were harvested and differentiated into macrophages. M1 macrophages were differentiated with TNF-α, and M2 macrophages with human IL-4. Macrophages were incubated in the presence of cholesterol (oxidized LDL or VLDL) for 24 hours or 7 days following differentiation to each macrophage type. Inflammatory changes and expression of PLIN were investigated. LD size in the macrophage cytosol and the expression of PLIN were histologically assessed. Western blotting was also used for the evaluation of PLIN expression. SiRNA was used to suppress the expression of PLIN1 in cultured macrophages.
Results: In human arterial plaques, PLIN1 was expressed in both plaques from symptomatic stroke patients (n=31) and non-stroke patients (n=34). PLIN2 mRNA expression in the symptomatic group increased to 3.4-fold relative to the non-stroke group. In cultured human M1 macrophages, CD11c (M1 marker) expression increased depending on cholesterol incubation time. PLIN1 were only expressed in day 7th with cholesterol while PLIN2 were identified from 24 hours and enhanced in day 7th. After 24 hours, there were only small LDs covered with PLIN2, and LDs surrounded by PLIN 1 were formed from day 7th. In M2 macrophages, there was no expression of CD11c in the whole period and the expression of CD163 (M2 marker) decreased slowly. PLIN1 emerged as early as 24 hours. LDs became large size formed by PLIN1 after 24 hours, and further grew after 7 days. Differentiated M2 macrophages in the presence of PLIN1 siRNA had small LDs coated with PLIN2.
Conclusion: PLIN2 was mainly expressed in arterial plaques in symptomatic stroke patients. Anti-inflammatory M2 macrophages rapidly formed large LDs by PLIN1 and stored large amount of lipid internally, and that may alleviate lipotoxicity damage to surrounding vascular tissues. In this process, however, pro-inflammatory character was gradually acquired through excess cholesterol stimulation, and the ability of lipid accumulation might have decreased like the small storage in M1 macrophages, covered with PLIN2. It was suggested that these mechanisms could cause plaque rupture.
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Disclosure: **K. Cho:** None.

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hsCRP can be precisely calculated by a linear-mixed-model from lysophosphatidylethanolamine (LPE) species in the NUtriGenomics Analysis in Twins (NUGAT) study

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Background and aims: Recently, we were able to demonstrate a strong association of several lysophosphatidylethanolamine species (LPE) with hsCRP in the NUGAT-study during a high-fat intervention. An increased hsCRP concentration is a known risk marker for type 2 diabetes mellitus and cardiovascular disease. The aim of the present investigation was to quantify the relation between hsCRP and LPE species and to fit a model

for calculation of hsCRP concentration from LPE species and other relevant parameters.
Materials and methods: In the NUGAT-study 46 healthy mono- and dizygotic twin-pairs first were standardized for their nutritional behavior by a carbohydrate-rich low-fat diet for 6 weeks (LF), immediately followed by an isocaloric high-fat diet for 1 week (HF1) and additional 5 weeks (HF6). At each clinical investigation day (CID) plasma was taken for measurement of metabolic parameters, lipid metabolites, cytokines and hsCRP (ELISA). A linear-mixed-model was fitted to calculate the development of hsCRP concentration from lysophospholipid species levels, and further relevant parameters.
Results: Lysophosphatidylethanolamine species (LPE18:0, 18:1, 18:2, 20:4) and hsCRP concentrations were strongly correlated (ρ=-0.323 to -0.382, p=0.014 to 0.040). While LPE species did not change from LF to HF6 in subjects with hsCRP<1 at HF6, all LPE species decreased significantly in those with hsCRP≥1. In a linear-mixed-model to calculate the hsCRP concentration at HF6, CID (LF, HF1, HF6, as fixed factor) as well as hsCRP at LF, LPE18:0, LPE18:1, LPE18:2, LPE20:4, LPE22:6, chemerin, and leptin (at LF, HF1and HF6) revealed as meaningful parameters. The final model allowed an extremely precise prediction of the hsCRP concentrations at HF6. When we correlated the predicted and measured values, the correlation amounted to ρ=0.989 (p<0.001).
Conclusion: Our data demonstrate that mainly LPE species are strongly associated to the concentration of circulating hsCRP. Reasonably, the concentration of LPE-species, which are thought to behave in an anti-inflammatory manner, decreases during the high fat-diet in those subjects with a high hsCRP at the end of the intervention. The exact mechanisms are not known. Possibly, LPE species are converted into lysophosphatidic acid (LPA) by phospholipase D (PLD), which elicits inflammatory potential by binding to LPA-receptors, or there might be a direct interaction between LPE species and hsCRP.
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Disclosure: **M.A. Osterhoff:** None.

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The saturation of membrane phospholipids would be a trigger for K^+ efflux and NLRP3 inflammasome activation in human monocytes/ macrophages

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Background and aims: Obesity is worldwide epidemic now recognized as a low-grade inflammatory disease, favoring the development of cardiometabolic diseases. IL-1β production mediated by the NLRP3 inflammasome in adipose tissue macrophages and circulating monocytes plays an important role in the development of insulin resistance in obese patients. We previously demonstrated that saturated fatty acids (SFAs) whose levels are increased in adipose tissue and blood of obese patients are able to induce the activation of NLRP3 inflammasome in human monocytes and macrophages, with a concomitant increase in the percentage of saturated membrane phospholipids. Interestingly, unsaturated fatty acids (UFAs) prevent both events by redirecting SFAs towards triglycerides synthesis. However, the molecular link between the phospholipids saturation and NLRP3 inflammasome activation is not yet elucidated.
Materials and methods: PMA-differentiated THP-1 cells have been treated for 8 hours with BSA (control), BSA/C18:0 alone or combined with BSA/C18:1. Cellular [K^+] and IL-1β secretion were quantified by atomic emission spectrometry and ELISA, respectively. Lipidomic analyses were performed on peripheral blood mononuclear cells (PBMCs) of 14 lean patients and 20 normoglycemic (NG), 14 prediabetic and 8 type 2 diabetic (T2D) obese patients classified according to body mass index, fasting glycemia and HbA1c.
Results: Since K^+ efflux is a common step to many NLRP3 inflammasome activation settings, we tested its involvement in C18:0-mediated NLRP3 inflammasome activation in PMA-differentiated THP-1 cells. We demonstrated that increasing extracellular K^+ concentrations is sufficient to inhibit C18:0-induced NLRP3 inflammasome, suggesting the implication of a K^+ efflux. Indeed, a significant decrease in cellular [K^+] is measured in response to C18:0 vs BSA (85,72 % vs 102,80% of cellular [K^+], p< 0,001) and is strongly correlated with IL-1β production (Pearson r = -0,9790 ; p<0,05). The combined treatment C18:0+C18:1 reverses both K^+ efflux (85,72 % vs 112,00% of cellular [K^+] ; p< 0,001) and IL-1β production. Interestingly, in an ongoing lipidomic study performed on PBMCs from lean and obese patients, we observed a significant increase in the percentage of saturated phosphatidylcholine (PC) that is correlated with the glycemic status in the obese population (11.84% vs 12.62% vs 13.47% of total PC in NG, prediabetic and T2D patients respectively; p<0.05 NG vs prediabetic, p<0.001 NG vs T2D, p<0.05 prediabetic vs T2D).
Conclusion: We propose that stearate (C18:0), by inducing an increase in the saturated phospholipids levels, leads to a K^+ efflux and NLRP3 inflammasome activation. An inhibition of the plasma membrane Na,K-ATPase could explain the K^+ efflux and we are currently investigating this hypothesis. The oleate (C18:1), by maintaining low levels of saturated PC, prevents K^+ efflux and NLRP3 inflammasome activation.
Disclosure: **M.A. Gianfrancesco:** None.

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Pro-inflammatory action of omentin through activation of the NFκB, p38 and ERK pathways in primary human adipocytes

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Background and aims: Omentin was described as anti-inflammatory, insulin-sensitizing and atheroprotective adipokine in mice. In humans, data are controversial and the mode of action of omentin is unknown. As obesity and its pro-inflammatory state are important risk factors for type 2 diabetes and its complications, we aimed to investigate whether and by which mechanisms omentin may affect the secretion of inflammatory proteins by human adipocytes.
Materials and methods: We used human preadipocytes isolated from subcutaneous adipose tissue from five non-diabetic donors. Differentiated adipocytes were treated without or with (i) 500 ng/ml omentin, (ii) 2000 ng/ml omentin or (iii) 20 ng/ml tumor necrosis factor alpha (TNFα) as positive control for 24 h. We analyzed the supernatants for 92 inflammation-related biomarkers using the Proseek®Multiplex Inflammation I kit (Olink Bioscience). Subsequently, we identified potential upstream regulators of the omentin- and TNFα-induced secretion profile using Ingenuity Pathway Analysis. To validate these results, we measured the active forms of these potential regulators in cell lysates using Western blotting.
Results: Omentin dose-dependently induced twenty-nine inflammatory mediators. These biomarkers were upregulated 16.3±4.0-fold (mean ±SEM) by 500 ng/ml omentin and 73.7±22.9-fold by 2000 ng/mL omentin (p<0.0001). In addition, 12 of these proteins were also upregulated by TNFα by 56.1±25.8-fold (p=0.0005). The largest groups of co-regulated proteins were chemokines (e.g. IL-8/CXCL8; monocyte chemotactic proteins 2 and 3/CCL2 and 3) and proinflammatory cytokines

(e.g. IL-6, 7 and 1alpha). Omentin also increased the release of the soluble form of the receptor TNFRSF11B. The pathway analysis identified the nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NF-κB), the p38 mitogen-activated protein kinase (MAPK) and the extracellular-signal regulated kinase (ERK1/2 MAPK) as upstream regulators for most of the regulated proteins upon stimulation of both omentin and TNFα. On the cellular level, we found that omentin (at 2000 ng/mL) increased the phosphorylation levels of NF-κB p65 (Ser536) by 2.1±0.3-fold ($p<0.05$). Omentin also enhanced the phosphorylation levels of p38 (Thr180/Tyr182) by 2.6±0.4-fold ($p<0.05$) and of ERK1 (T202/Y204)/ERK2 (T185/Y187) by 1.7±0.2-fold ($p<0.01$). TNFα increased the phosphorylation levels of NF-κB p65 (Ser536) by 5.6±0.9-fold ($p<0.001$) and p38 (Thr180/Tyr182) by 4.4±1.0-fold ($p<0.01$).

Conclusion: Omentin induced the release of pro-inflammatory cytokines and chemokines in primary human adipocytes by activation of the inflammatory NF-κB, p38 and ERK pathways. We hypothesize that omentin might act via TNFRSF11B or other receptors of the TNF superfamily which will be investigated in further studies. This might explain the comparable pro-inflammatory effects of omentin and TNFα and might link both proteins with cardiometabolic disease risk.

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Disclosure: **C. Niersmann:** None.

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Lysozyme as new player in obesity-related adipose tissue inflammation

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Background and aims: Lysozyme (LYZ) is an antimicrobial enzyme that is expressed in white blood cells. The pathophysiology of obesity implies a chronic low-grade inflammation of adipose tissue associated to macrophages infiltration. To the best of our knowledge no previous studies have examined circulating or adipose tissue (AT) LYZ in human or rodent obesity. In the present study, we aimed to investigate human LYZ in adipose tissue and plasma according to obesity status, inflammation and insulin resistance.

Materials and methods: LYZ gene expression and circulating levels were cross-sectionally analysed in subcutaneous (SAT) and visceral (VAT) adipose tissue (real-time quantitative PCR) and in plasma (ELISA) from subjects with a wide range of fatness and insulin resistance. Adipose tissue LYZ gene expression was also analysed after bariatric surgery-induced weight loss (in humans) and after high-fat diet-induced weight gain (in rats). LYZ was also investigated at mRNA and protein level in human primary mature adipocytes and macrophages before and after lipopolysaccharide (LPS) or LPS-conditioned medium from M1 macrophages (MCM).

Results: The expression of LYZ mRNA was comparable to lipogenic genes in AT (FASN, leptin and PPARG). Although LYZ was expressed in adipocytes, its main expression was detected in cells of the stromal vascular fraction, specifically in $CD14^+$ cells. In both SAT and VAT, LYZ mRNA was increased concurrently with BMI ($p<0.0001$), percent fat mass ($p<0.0001$) and obesity-associated metabolic disturbances (fasting serum glucose, fasting triglycerides, HOMA-IR and C-reactive protein). In addition, AT LYZ mRNA was significantly and positively associated with expression of AT inflammation markers, such as tumor necrosis factor alpha (TNFα), leptin or lipopolysaccharide binding protein (LBP), while negatively with markers of adipogenesis (FASN, GLUT4, PPARG, IRS1). Bariatric surgery-induced weight loss resulted in decreased SAT LYZ mRNA (-68.3% decrease, $p<0.0001$) in parallel to reduction of AT inflammation and the improvement of AT adipogenesis. The high-fat diet-induced weight gain led to increased expression (141.5% increase, $p=0.003$). Similar to adipose tissue LYZ mRNA, plasma LYZ was also increased with obesity and weight gain, and significantly correlated with obesity-associated metabolic disturbances. In vitro experiments revealed that inflammatory factors (LPS or MCM) promoted lysozyme biosynthesis and secretion in both adipocytes and macrophages.

Conclusion: Altogether these findings suggest lysozyme as a new player in obesity-associated adipose tissue inflammation and dysfunction.

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Disclosure: **J. Latorre:** None.

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Influence of metabolic endotoxaemia on adipose tissue from obese subjects

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Background and aims: Circulating lipopolysaccharides (LPS) from gut microbiota have been proposed as a triggering factor for the low-grade inflammation in obesity and metabolic diseases. Likewise, impaired adipose tissue (AT) lipid handling and storage is associated with the development of obesity-related metabolic diseases such as insulin resistance or diabetes. Low-grade inflammatory state has been related to AT dysfunction. Animal studies showed that LPS modify the expression of factors involved in AT function. Here, we aimed to analyze the relationship between LPS and the expression of key factors for adipose function and inflammation in human obesity.

Materials and methods: Visceral and subcutaneous AT (VAT and SAT, respectively) samples were obtained during bariatric surgery from morbidly obese patients to analyze gene expression (n=33) as well as to perform in vitro assays (n=5). Patients were classified according to their LPS levels (high LPS levels, H-LPS group and low-LPS levels, L-LPS group). In vitro differentiated adipocytes from healthy obese subjects were stimulated with a range of LPS doses and gene expression and protein secretion analyzed at different time-points.

Results: H-LPS group had significantly lower mRNA levels of SREBP1 ($p<0.05$), FABP4 ($p<0.01$) and LEP ($p<0.05$) in VAT and FABP4 in SAT ($p<0.01$), but higher mRNA levels of the inflammatory genes CSF3 and IL6 in VAT ($p<0.05$); TLR2, CD14 and MCP1 in SAT ($p<0.05$) than L-LPS group. VAT FASN gene expression also tended to be lower in H-LPS group than in L-LPS group ($p<0.1$). Concordantly, LPS stimulus led to a significant decrease in FABP4 and LEP gene expression as well as in Leptin secretion in visceral and subcutaneous differentiated adipocytes from obese subjects ($p<0.05$). This decrease was related to a significant increase in the gene expression and secretion of MCP1, IL6 and CSF3 ($p<0.05$) in both visceral and subcutaneous differentiated adipocytes.

Conclusion: Metabolic endotoxemia can influence AT physiology by enhancing AT inflammation and impairing the expression of key factors for lipid handling and AT function in human obesity.

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Up-regulation of novel proinflammatory adipokine Wnt1 inducible signalling pathway protein 1 (WISP1) in liver fibrosis

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Background and aims: Wnt1 inducible signaling pathway protein 1 (WISP1) is a novel proinflammatory adipokine that was associated with visceral obesity in humans, but not with non-alcoholic fatty liver disease (NAFLD). Animal studies showed that WISP1 is upregulated in liver fibrosis. ADP ribosylation factor related protein 1 (ARFRP1) is a small trans-Golgi-associated GTPase playing critical role in postprandial lipoprotein maturation in liver. Mice lacking the Arfrp1 gene develop fibrosis. We investigated the relationship between WISP1 and fibrosis in liver and adipose tissue in mice and humans.
Materials and methods: Two mouse models with either liver or fat specific Arfrp1 knockout displaying fibrosis in respective tissues were fed standard chow diet for 2 and 12 weeks. Gene expression of WISP1 was measured in liver, muscle and adipose tissue; serum WISP1 levels were determined using ELISA. Moreover, circulating WISP1 levels were detected in 37 patients with type 2 diabetes and NAFLD (age 64 ± 6 years, BMI 30.2 ± 3.6 kg/m², HbA_{1c} 7.0 ± 0.6%, liver fat 15.0 ± 1.8%). Levels of liver enzymes, hyaluronic acid (HA), amino-terminal propeptide of type III procollagen (PIIINP), tissue inhibitor of metalloproteinases 1 (TIMP1), and enhanced liver fibrosis (ELF) score were determined in fasting blood samples. Furthermore, microarrays were performed in adipose tissue biopsies of 15 healthy men (age 45.1 ± 3.8 years, BMI 27.7 ± 1.1 kg/m^2).
Results: In $Arfrp1^{liv-/-}$ mice displaying liver fibrosis, WISP1 gene expression was enhanced in liver ($p < 0.01$), but not in muscle or adipose tissue. In contrast, WISP1 expression was not altered in white subcutaneous and visceral or brown adipose tissues of $Arfrp1^{ad\text{-}ER-/-}$ mice showing fibrotic lesions in white adipose tissue. Additionally, serum levels of WISP1 were increased only in $Arfrp1^{liv-/-}$ mice (3559 ± 144 pg/ml vs. 2397 ± 82 pg/ml in control mice, $p < 0.001$) and not in $Arfrp1^{ad\text{-}ER-/-}$ mice (2598 ± 262 pg/ml vs. 2548 ± 104 pg/ml in control mice). In humans, WISP1 levels were significantly correlated with circulating TIMP1 ($\rho = 0.527$, $p < 0.001$) but not with liver fat content, liver enzymes, HA, PIIINP, and ELF score. Microarray data analysis in adipose tissue samples of healthy humans showed no associations of WISP1 with expression of any markers of fibrosis.
Conclusion: Our data gives further hints for the role of WISP1 in mice with liver fibrosis. In contrast, WISP1 does not seem to play a role in adipose tissue fibrosis in mice. In subjects with NAFLD we observed no association with markers of fatty liver and fibrosis, except TIMP1. Additional research in patients with severe liver disease is needed to confirm these observations.
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Disclosure: **M. Markova:** None.

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Effect of acute hyperinsulinaemia on circulating immune cells in type 2 diabetes offspring

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Background and aims: Diabetes Type 2 (T2DM) and associated complications as cardiovascular disease are mediated among others by increased inflammatory state. Hyperinsulinemia, which precedes impaired glucose levels in T2DM, may contribute to inflammatory state development, especially in subjects with genetic predisposition to T2DM. Thus, the aim of our study was to investigate/compare the effect of acutely elevated insulin levels on activation of immune cells in offspring of T2DM patients and control subjects.
Materials and methods: 17 non-obese male offspring of T2DM patients and 11 control subjects without family history of diabetes matched for age and BMI (36±7 years, BMI = 25.4 ± 0.9 kg/m^2) were investigated. The subjects were healthy, non-smokers, without hypertension, thyroid or other endocrine disease, and without history of obesity. Family history of diabetes for offspring subjects was considered as at least two first-degree relatives (parents, siblings) or one first-degree relative and two second-degree relatives (grandparents, uncle, aunt) were diagnosed with T2DM. All subjects underwent 2hours' hyperinsulinemic-euglycemic clamp, blood samples were taken before and at the end (generally at 120 min) of the clamp for flow cytometric analysis of lymphocyte and monocyte populations. Markers used for monocyte populations were CD45, CD14, CD 163, CCR2, CD36, TLR2, and TLR4; lymphocytes were analysed by CD3, CD4, CD8, CD193, CD196, and CD183; and cell numbers were expressed as percentage of gated events.
Results: In response to acute hyperinsulinemia, a decrease in CD45+/3+/4+ (T_H) lymphocyte content was observed in both groups of subjects (OFF:-12.7 ± 2.9%, $p<0.001$; CON:-10.2 ± 3.0 %, $p=0.006$). A relative content of T_H lymphocytes subpopulation expressing CD194 (T_H2 and Treg cells) was increased by 15.7 ± 4.4% ($p =0.017$) in a control group, the trend to increase was observed in T2DM offspring (13.9 ± 6.2%, $p=0.07$). T_H2 cell population (defined as CD194+/196-/183-) was increased in control subjects (15.1 ± 4.7%, $p =0.014$). No change in a relative content of monocyte population CD45/14+, and its subpopulations expressing CD163, CCR2, CD36, TLR2 and TLR4 was observed, however, increased expression (MFI) of TLR2 and decreased expression of CD36 scavenger receptor was detected on monocytes in both groups of subjects.
Conclusion: Contrary to hypothesis, hyperinsulinemia induced changes in lymphocyte and monocyte populations / activation status, which cannot be considered as strictly pro-inflammatory. The response of analyzed blood immune cells to hyperinsulinemia was not significantly different in T2DM offspring when compared to control subjects, with the exception of T_H2 lymphocytes, which were increased only in control group.
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Disclosure: **M. Šiklová:** None.

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Effect of postpartum weight change on the risk of glucose intolerance three years after delivery

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Background and aims: Both gestational diabetes (GDM) and post-pregnancy weight gain are risk factors for a later development of glucose intolerance (GI). However, little information is known about whether the risk of GI is independently influenced by pre-pregnancy body weight, weight gain during and after pregnancy and the diagnosis of GDM. Our objective was to examine the effect of the weight data of prior GDM and control women (pre-pregnancy weight, weight gain during pregnancy, weight changes after pregnancy) on the development of GI in view of the known risk factors into account,after 3.4±0.7 (mean±SD) years after delivery.
Materials and methods: 94 GDM and 43 control (with normal glucose tolerance during pregnancy) women who gave birth at our hospital in 2005-2006 participated in a nested case-control study, after exclusion of the pregnant women and women with known diabetes at the follow-up. The glycemic status at the time of the follow-up was determined by a standard oral glucose tolerance test (OGTT). The diagnosis of diabetes, impaired fasting glucose and impaired glucose tolerance were defined as GI. The correlation between individual weight parameters, the known risk factors for GI and GI was examined with multiple logistic regression.
Results: During follow-up, GI was diagnosed in n = 37 cases (27%). The weight before pregnancy of current GI women (70.1 ± 14.8 vs. 62.6 ± 10.0 kg, P = 0.007) and their post-pregnancy weight gain (4.0 ± 8.4 vs. -1.9 ± 7.7 kg) was significantly greater than in the case of women with currently normal glucose tolerance (non-GI women). Anamnestic GDM (94 vs. 54%, P <0.0001) and gestational hypertension (22 vs. 3%, P = 0.003) were more common in the GI group. In a multi-variant model, of the three examined weight parameters only pre-pregnancy body weight (OR: 1.04 95% CI: 1.001-1.07) and post-pregnancy weight gain (OR: 1.10 95% CI: 1.04-1.18) were independent predictors. After adjustment of the GDM (OR: 6.56 95% CI: 1.41-30.8) only weight gain after pregnancy remained the independent predictor of GI (OR: 1.09 95% CI: 1.01-1.17) of the weight parameters.
Conclusion: In our study, we confirmed that the important risk factors for the development of glucose intolerance after pregnancy are anamnestic GDM and gestational hypertension, as well as weight gain after pregnancy. The correlation between pre-pregnancy weight and glucose intolerance is explained by the diagnosis of GDM. After pregnancy, to reach and maintain optimum body weight (especially in the case of former GDM) with lifestyle intervention is of primary importance regarding the prevention of glucose intolerance.
Disclosure: **T. Tänczer:** None.

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A family history of diabetes and placenta

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Background and aims: Heritability of diabetes is associated with hyperinsulinemia, impaired endothelial function and inflammatory up-regulation. However, no studies have examined whether a family history of diabetes effects placental vascular circulation.The present study was designed to investigate the impact of first-degree family history of diabetes (FHD) on placental vascular circulation and inflammatory lesions.
Materials and methods: 339 pregnant women were divided into two groups according to presence of FHD: Group 1 included 255 subjects without FHD, and group 2 included 145 subjects with FHD. Placental histology was performed for vascular circulation, as well as inflammatory lesions of maternal and fetal origin. Maternal and neonatal outcome parameters were collected.
Results: Maternal vascular supply (MVS) abnormalities of the placenta were significantly higher in subjects with FHD, compared to subjects without FHD (p<0.005). Fetal vascular supply (FVS) abnormalities, as well as maternal and fetal inflammatory response did not differ significantly between groups. In the GLM analysis, FHD was an independent and significant predictor of MVS abnormalities and more than doubled the risk of this outcome. Gestational diabetes incidence was significantly higher in subjects with FHD (p<0.0001). Significant by-group differences in gestational diabetes persisted even after adjustment for age and BMI. Although incidence of gestational hypertensive disorders was significantly higher in individuals with a family history of diabetes, after adjustment FHD did not significantly predict this outcome.
Conclusion: A first-degree FHD is significantly and independently associated with an increased rate of maternal vascular perfusion abnormalities and risk of gestational diabetes.
Disclosure: **M. Shargorodsky:** None.

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Identification of gestational diabetes and pregnancy outcomes: a population study

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Background and aims: According to recommendations of the Regional Health Service of Tuscany, a region in the central Italy, glucose tolerance should be tested by a 75g-2h-oral glucose tolerance test (OGTT) in all pregnant women at higher risk of gestational diabetes (GDM). From the regional database including all screened pregnancies in year 2015, we estimated compliance to guidelines and an algorithm to identify GDM, including also its outcomes at delivery.
Materials and methods: After excluding all those who were diabetic prior to the pregnancy, target population of this study included all women, aged≥15yr, resident in Tuscany, Italy, who delivered, after 22nd week, in year 2015. Deliveries were identified by the certificates of care at delivery (CEDAP). OGTT performance was ascertained by querying regional fluxes of laboratory data and population was divided by eligible or not eligible to be tested by current guidelines. GDM was identified by an algorithm based on adherence to at least one among following criteria: a) insulin prescription during pregnancy, stopped after delivery, b): at least one documented visit by a diabetologist or inclusion into an educational program for patients with diabetes, c): OGTT performed by six months after delivery. Validation of GDM diagnosis was done in two groups of unselected pregnancies whose GDM status was known, and crossing GDM by algorithm with GDM assessed by hospital ICD-9 code (648.8).
Results: Total cohort was composed by 22,730 women and OGTT was tested in 18,006 cases, in 72.38% of non eligible women and in 82% of eligible ones (p<0.0001). Prevalence of OGTT performers increased with age, pre-pregnancy overweight/obesity, higher education degree while housewives and students had a lower chance of receiving an OGTT as compared with employed women. GDM rates, tested by algorithm ,were 11.11% in total population, 14.37% in eligible and 7.02% in non eligible women. Validation of GDM algorithm was similar after matching results with known populations (positive predictive values: 0.805 and 0.920),

and lower after crossing cases by hospital discharges through ICD9 codes (positive predictive value: 0.252). Women with GDM, compared to those without had higher relative risk (RR) of caesarian section (RR:1.22; 95%CI:1.14-1.30), macrosomic newborns (RR:2.22; 1.10-4.47), preterm deliveries (RR:1.56; 1.36-1.78) and neonates with Apgar score<10 (RR:1.31;1.23-1.39); p<0.05 in all cases.

Conclusion: Overall compliance to guidelines seems to be high in this population (more than 80% in eligible women). GDM prevalence, as estimated by an algorithm, was about 11%. The high prevalence in not eligible women and the non optimal outcomes in GDM women, suggest to reconsider the application of selective screening.

Disclosure: **C. Lencioni:** None.

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Gestational diabetes registers: a study of effectiveness in Australia, the MAGDA study

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Background and aims: The risk of developing Type 2 Diabetes Mellitus (T2DM) within 5-10 years is sevenfold higher in women who have GDM vs no GDM. Australia implemented a National Gestational Diabetes Register (NGDR) in July 2011 as a mechanism to support follow-up of women with GDM history aiming to reduce T2DM progression rates. Registration is not mandated. This study (part of the Mothers After Gestational Diabetes in Australia - MAGDA study) aimed to evaluate the extent to which the NGDR can contribute to diabetes prevention among women diagnosed with GDM.

Materials and methods: This was a retrospective data linkage study in Victoria (VIC) and South Australia (SA) (data available 2008 - 2014) from state birth records, National Diabetes Support Services (NDSS) that runs the NGDR, and principal pathology providers in SA and Victoria. The study utilised GRHANITE™ data acquisition and privacy-preserving record linkage technologies that facilitated the linkage of highly protected databases for the first time. Mothers in Australia are recommended to have a follow-up OGTT test 6 weeks postnatally to detect ongoing diabetes. NGDR posts a screening reminder letter to the GP and mother 8-16 weeks postnatally, 10 months after birth and annually commencing 2 years after birth. Diabetes-related pathology testing (OGTT, GCT, HBA1c, BGLU) were used as indicators of screening follow-up for GDM postnatally. All GDM-diagnosed mothers registered on NGDR who had births between 2010 and 2013 and had a linked, diagnostic laboratory GDM screening test result (i.e. who utilise the labs where we have the data) were followed-up. We compared postnatal diabetes-related testing rates for two years before and after the NGDR inception.

Results: VIC 2010 - 2011 8,076 mothers with GDM diagnosis, screening data available for 37%. Of these 53% had a 6 week OGTT. VIC 2012 - 2013 11,458 with GDM, screening data 52%. 58% had a 6 week OGTT. SA 2010 - 2011 2,484 with GDM, screening data 78%. 43% had 6 week OGTT. SA 2012 - 2013 3,104 with GDM, screening data 82%. 44% had 6 week OGTT. Peak testing occurred in both states at 6 weeks but rates are suboptimal at 58% VIC and 44% SA. The NGDR Register registration rates in the study population 2012-2013 were 90% (VIC), 75% (SA). There was only a 5% increase in screening in VIC after register implementation, 1% in SA. Reminder letters from the register were sent AFTER the peak OGTT testing found at 6 weeks. There was no indication of any screening activity related to the dates NGDR letters were posted at any time.

Conclusion: 6-week postnatal testing rates show negligible improvement regardless of any evolution of patient management processes. The NGDR postnatal reminder letters demonstrate no effect on postnatal diabetes screening at any time in either state. Engaging women who have had GDM in follow-up programs is difficult. Early evidence suggests that managing recall and testing of women who have had GDM at a local level (e.g. GP clinics) is likely to be more effective than letters. NGDR registration rates are high but other strategies must be found to use its information to effect change. Testing mothers before discharge from hospital may be more effective than an under-subscribed 6-week OGTT test.

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Identifying women with recent gestational diabetes at greatest need of early post-partum testing for diabetes and intermediate hyperglycaemia

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Background and aims: Gestational diabetes (GDM) is a strong risk factor for future diabetes, and prevention efforts should target this group of women. Yet, given the overwhelming duties of recent motherhood, adherence to oral glucose tolerance testing (OGTT) is low, particularly early on. Thus, strategies to prioritize efforts to ensure screening at post-partum of those at highest risk are highly needed. The purpose of this study is to assess diabetes risk over the first 20 months post-partum among women with GDM participating in the LINDA-Brasil study so as to find simple means of identifying those at highest need for early testing.

Materials and methods: LINDA-Brasil, an ongoing clinical trial of diabetes prevention at post-partum, is nested within a cohort of women with recent GDM. The cohort currently comprises 2856 women with GDM recruited from high risk pregnancy clinics. We obtained data on clinical characteristics and pregnancy treatments by chart review and interviews, and detected diabetes post-partum by OGTT. We encouraged all women to perform testing, beginning 8 weeks post-partum, and used various approaches to increase adherence, including no cost testing at nearby labs; phone reminders and free clinic visits. Results are expressed as mean (SD). Cox regression was used to estimate relative risks (RR) of different predictors of diabetes in the early post-partum period.

Results: Mean BMI before pregnancy was 30.1 (6.4) kg/m2 and mean age 31.9 (6.2) y; 19.3% used insulin during pregnancy and an additional 16.3%, oral hypoglycemic agents. At post-partum, among those who completed an OGTT, diabetes prevalence was 2.3% among non-antidiabetic medication users, and 15.8% among antidiabetic medication users. By 20 months post-partum, incidence rates were 4% and 27%, respectively. Use of insulin during pregnancy identified those with a six-fold higher risk of having diabetes (RR 6.0; 95%CI 3.9-9.1); use of oral hypoglycemic medication, 1.9 (95%CI 1.3-2.9). Each 5 unit increase in BMI increased risk by 20% (RR 1.2; 95%CI 1.0-1.4). Most incident cases (82%) used insulin (65%) or oral hypoglycemic agents alone (17%) during pregnancy.

Conclusion: Use of insulin or oral hypoglycemic agents during pregnancy identifies most future cases of diabetes developed over 20 months post-partum. Anti-diabetic medication use during pregnancy constitutes a simple means of identifying women most likely to have or develop diabetes soon after giving birth, and thus most likely to benefit from more intensive efforts to ensure early post-partum testing.

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Disclosure: **C.D. Castilhos:** None.

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Routine resting energy expenditure measurement increases effectiveness of dietary intervention in overweight and obese patients

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Background and aims: Primary outcome of this study was to compare weight changes in two groups of overweight or obese individuals: subjects who had a diet prescribed on the base of Resting Energy Expenditure (REE) measured by indirect calorimetry and subjects whose REE was estimated by a predictive equation. In addition, we analyzed differences in weight and metabolic parameters variation in subjects with and without an adequate to predicted REE and in subjects with Respiratory Quotient (RQ) ≤ 0.9 and with RQ > 0.9.
Materials and methods: We retrospectively analyzed data of a 355 overweight and obese patients: 215 on a diet based on REE measured by indirect calorimetry (IC group) and 140 following a diet based on REE estimated by the Harris-Benedict equation (NO-IC group). Anthropometric and metabolic parameters were evaluated for eighteen months since baseline. A multivariable model was used including age, presence of diabetes, endocrine disorders, hypertension or metabolic syndrome. Propensity score adjustment was used to adjust for known differences between the groups being compared. Coarsened Exact Matching (CEM) method was used to match patients according to the baseline covariates used for estimating the propensity score.
Results: A significant greater decrease of body weight was observed in the group that performed indirect calorimetry compared to the group who did not perform it. Eighteen months after the first clinical visit, weight loss rate in the IC group was -9.54 ± 0.07% vs Baseline, whereas in the NO-IC group was -1.95 ± 0.05% vs Baseline, showing a statistically significant difference between the groups ($p<0.001$). No significant differences were observed between patients with not adequate to predicted REE compared to patients with adequate to predicted REE. Particularly, 18 months since the first visit, weight loss in the subjects with adequate to predicted REE was -9.33 ± 0.06% vs Baseline, and with no adequate to predicted REE was -9.72 ± 0.07% vs Baseline, showing no significant difference between groups. The IC group was further divided in subjects with RQ ≤ 0.9 and with RQ > 0.9. There was no significant difference in the variation through time between the two sub-groups (weight: IC RQ ≤ 0.9 vs RQ > 0.9: p=0.908. BMI: IC RQ ≤ 0.9 vs RQ > 0.9: p=0.921). Finally, we analyzed metabolic biomarkers in IC and NO-IC groups considering variations through time: a significant difference between the two groups was found in triglycerides which decreased faster in the IC group (p=0.03).
Conclusion: A weight reduction program based on indirect calorimetry appears more effective than a dietary program based on predictive formulas. This retrospective study suggests the routine use of indirect calorimetry utilization in all weight reduction procedures.
Disclosure: **S. Massarini:** None.

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Food addiction in obese patients

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Background and aims: Food addiction is a controversial concept based on the fact that similar behaviors are found and identical neurobiological pathways (reward circuit and dopaminergic system) are activated in both food intake and psychoactive substance use. This could explain the difficulties of patients presenting symptoms of food addiction to lose control on food consumption, failure in attempts to reduce their food intake and inability to abstain from specific types of food or reduce their consumption. Excessive sugar intake could be a significant component of food addiction due to the activation of the reward circuit in the same way as classic substances (e.g. alcohol, tobacco,cocaine). The prevalence and the role of food addiction in obesity remain unclear and need to be evaluated. The aim of our study was to assess the prevalence of food addiction in obese patients before a weight loss program and to better evaluate the relationship between food addiction and some aspects of eating disorders.
Materials and methods: In a total of 115 participants with obesity, 93 (39 males and 54 females, age 44± 13 yrs, body mass index (BMI) 38.8 ± 8 kg/m2) have been evaluated before starting a weight loss program. The French version of the Yale Food Addiction Scale (YFAS) was used for both the diagnosis and the severity of food addiction. The diagnosis of food addiction was met if participants endorsed three or more criteria as well as at least one of the two clinical significance items (impairment or distress). The severity of food addiction was a simple sum of the seven diagnostic criteria. Different aspects related to eating disorders were evaluated by the French version of the Eating Disorder Inventory-2 (EDI-2) questionnaire.
Results: Ninety-three participants filled up the surveys and 30 of them (32%) presented the diagnosis of food addiction regarding the YFAS score. In a simple regression analysis, we have seen a positive relationship between the severity of food addiction and the severity of bulimia ($p=0.04$, $R^2=0.13$). The same relationship was seen between the severity of food addiction and the lack of confidence in recognizing and accurately identifying emotions and sensations of hunger and satiety, the interoceptive awareness, ($p=0.007$, $R^2=0.23$). Food addiction was not related to body mass index, gender or age.
Conclusion: The prevalence of food addiction in our population of obese patients and the lack of relationship between food addiction and body mass index, gender and age confirm anterior data. The highlighting of two aspects related to eating disorder, the bulimia and the difficulties with interoceptive awareness, can stress out the accuracy of food addiction as a distinct disease compared to other eating disorders. This could give some information for a more adapted care of food addicts focused on both treatment of bulimia and reconnection with hunger and satiety. The food addiction could be of particular importance in patients with diabetes and need to be elucidated.
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Disclosure: **L. Locatelli:** None.

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Serum branched chain amino acids, dietary macronutrients and development of type 2 diabetes in the Finnish Diabetes study

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Background and aims: Branched chain amino acids (BCAA) have been associated with type 2 diabetes (T2D) risk in some studies. Still it is not known what is the relation between BCAA and T2D and what causes the elevation of serum BCAA's content. We hypothesize that BCAAs mediate the effect of energy-yielding nutrients in T2D.
Materials and methods: An exploratory study comprised 128 men and 279 women who participated in the randomized Finnish Diabetes Prevention Study (DPS) and were provided either intensive or general

lifestyle counselling to reduce T2D risk. Participants were overweight (BMI>25), middle-aged (40-65 years) and had impaired glucose (IGT) tolerance at baseline. For median of 9 years, participants were followed-up with repeat annual oral glucose tolerance tests for detecting T2D. In the present study we used the anthropometric measurements, blood samples, and dietary data (3-day food diaries) from baseline and year 1. BCAA were measured from fasting serum by sandwich ELISA as a part of metabolomics analysis. Sex-specific quartiles of baseline BCAA were used to categorize participants and Cox regression was used to analyze diabetes risk among the BCAA categories. Linear regression was used to analyze the association between BCAA and intakes of macronutrients.
Results: Serum BCAA concentration at baseline was associated with the development of T2D (Quartile (Q)4 vs. Q1 HR=1,72 [1,07-2,75]; Q3 vs. Q1 HR=1,69 [1,05-2,70]; Q2 vs. Q1 HR=1,06 [0,63-1,77]; p for trend 0,03) in a model adjusted for sex, age, education, BMI, intervention/control group. In men, the correlation between baseline BCAA with baseline energy intake was inverse (β=-0,23 p=0,01), adjusted for age, education, BMI. In men, carbohydrate intake was inversely correlated (β=-0,38 p=0,01) with BCAA, but association was attenuated (p=0,07) after adjustment for age, education, BMI. In women, baseline fat intake was correlated with BCAA (β=0,26 p=0,04), but correlation was attenuated after adjusting for age, education and BMI (p=0,08). In women, the change in intake of saturated fat was correlated with change in BCAA (β=0,17 p=0,04) in a model adjusted for energy intake, age, education and BMI.
Conclusion: Our results confirm that high serum BCAA concentration is a risk marker of future T2D in people with IGT. Our results suggest inverse association between carbohydrate intake and BCAA in men and positive association between fat intake and BCAA in women. Macronutrient intake may modify BCAA, and this may in part explain the associations between macronutrients and T2D risk.
Clinical Trial Registration Number: NCT00518167
Supported by: AKA, YJS, NNF, FDRD, Juho Vainion Säätiö
Disclosure: **J. Kivelä:** Employment/Consultancy; TETHYS Bioscience Inc. Grants; The Academy of Finland (128315, 129330, 131593), The Juho Vainio Foundation, Novo Nordisk Foundation, Yrjö Jahnsson Foundation, Finnish Diabetes Research Foundation.

PS 045 Preventing and treating diabetes more effectively

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Preventing progression to type 2 diabetes in women who have had gestational diabetes: Back to the drawing board?

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Background and aims: Women who have had GDM are seven times more likely to develop type 2 diabetes (T2DM) than women who with normoglycaemic pregnancies. Lifestyle modification has been shown to reduce the risk of progression. Mothers after Gestational Diabetes in Australia (MAGDA) was a five year systems approach to the prevention of T2DM conducted in South Australia and Victoria from 2011-2015.
Materials and methods: Using a systems approach comprising three studies, we examined the processes for intervening to reduce their risk of T2DM. The National Gestational Diabetes Register (NGDR) sends reminder letters to women for 6-8 week postnatal OGTT and annual follow-up by their family physician. The performance of NGDR was assessed by comparison with two state perinatal data collections and with blood samples from registered women analysed by pathology laboratories. A Collaborative for 12 months involving 17 family medicine practices established practice based registers and recall systems for blood testing and T2DM prevention plans. A clinical trial of a group based lifestyle modification program involving 573 women was run during the first postnatal year.
Results: One quarter of woman were not registered with NGDR. Neither the reminder letter for 6-8 wk postnatal OGTT nor the annual reminder letters made any difference to follow-up blood testing rates. The Collaborative involving family medicine led to doubling the rates of review and testing of women (from 30 to 60% over 12 months). Specific T2DM prevention plans were offered to these women but the uptake was low (10%). Among the women recruited to the postnatal intervention, at baseline 10% had IGT and 2% IFG. The mean changes (ITT analysis) over 12 months were as follows: -0.23 kg body weight in the intervention group compared with +0.72 kg in the usual care group: change -0.95 kg group by treatment interaction P=0.04. There were no other significant anthropometric or biomedical changes. Only 10% of women attended all sessions, 53% attended one individual and at least one group session, and 34% attended no sessions. Loss to follow-up was 27% and 21% for the intervention and control groups respectively.
Conclusion: The NGDR registration rate can be improved by using the state perinatal data collections to identify low reporting providers. Reminder and recall letters for NGDR do not appear to work but NGDR could be used to populate family practice-based registers which seem more successful. Adoption of the IADPSG guidelines will increase the numbers diagnosed with GDM. The method used to evaluate NGDR could be used in a risk stratification study. A 1 kg weight difference may be significant for reducing T2DM risk but the level of engagement during the first postnatal year was very low. Engagement of women at this life stage has been found to be difficult by many studies and at this stage 90% are not at high risk of progression to T2DM. Interventions should only be offered to women with IFG/IGT or HbA1c in the pre diabetes range. Research should be directed at understanding why uptake among women who have had GDM is low and how it could be increased.
Clinical Trial Registration Number: AusNZ Clinical Trials Register 126100003580066
Supported by: National Health and Medical Research Council AppID 533956
Disclosure: **J.A. Dunbar:** None.

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Care of transgender patients with diabetes

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Background and aims: Estimated incidence of transgender persons based on self-report is 871 per 100,000 people. The prevalence of Type 1 diabetes (T1D) is estimated to be 230-340 per 100,000 people. Prevalence of Type 2 diabetes (T2D) is as high as 6,700 per 100,000. Trans patients with diabetes mellitus (DM) seek care for gender dysphoria. Patients with DM are at higher risk for cardiovascular disease (CVD), osteoporosis, obstructive sleep apnea (OSA) and depression. It is unclear if cross hormone therapy increases risk of cardiovascular disease in trans women. Studies suggesting increased risk are confounded by use of ethinyl estradiol, known to increase clotting. Although controversial in hypogonadal natal men, data suggest no CVD risk increase in trans men treated with testosterone. There is no data in trans patients with DM. Hormones used in trans patients are known to have effects on lipid profiles, blood pressure, weight and glucose. Androgen therapy and suppression of endogenous estrogen production in trans men is associated with lower bone mass and OSA. Trans women on estrogen have preserved bone density. The dysphoria and psycho-social issues add to the complexity of managing the DM and co-morbidities. Our institution has a multidisciplinary gender health clinic providing care to 300 transgender patients, 4 with T1D and 5 with T2D. To provide optimal care we follow the American Diabetes Association (ADA) recommended guidelines. We describe our transgender patients with DM focusing on ADA targets and discuss management interventions we identified to improve quality of care.

Materials and methods: Trans patients are cared for by a multidisciplinary team with endocrinology, psychiatry, pharmacology, certified diabetes educator, nutrition, nursing and social services involved. Most recent data was accessed in patients with DM. Means and standard deviation (SD) were calculated when appropriate.

Results: Results are shown in Table 1.

Conclusion: Hormone therapy in our patients is associated with increased modifiable risks associated with DM. These risks need to be optimally managed and discussed with patients. Our results suggest that even with our multidisciplinary team, recommended targets for patients with DM are not always achieved. Triglycerides (TG) tended to be high in trans women most likely due to DM and use of estrogens. Low density lipoprotein cholesterol (LDL-C) was higher and high density lipoprotein (HDL-C) lower in T2 obese patients. Statins were not used in the majority of patients, possibly due to their age. Hypertension was not an issue, maybe due to consistent use of spironolactone as an anti-androgen in trans women. Vitamin D levels were lower than what is considered replete and should be addressed to prevent metabolic bone disease. Trans women with T2D were obese and should be aggressively managed in a weight loss program. A high percentage of patients had a current or past diagnosis of substance abuse and nicotine use requiring interventions and support services. Other behavioral disorders were prevalent in our patients making psychiatry an integral component of our team.

Table 1. Description of transgender patients with Type 1 and Type 2 Diabetes

	T1D Transmen (n=1) Age-21 BMI-26	T1D Transwomen(n=3) Age*-28.3 (10.4) BMI*- 23.7 (7.8)	T2D Transwomen(n=5) Age*-42 (2.8) BMI*-39.0 (9.6)
HbA_{1c}*	8.2	9.6 (1.2)	9.0 (2.4)
LDL-C*	113	129 (44))	88.6 (22.9)
HDL-C*	56	72(10)	41 (2.8)
TG*	37	166(99)	183 (111)
BP systolic/diastolic*	112/70	115 (11.4)/69(8.3)	122 (8.1)/76(7)
Nicotine use **	0/0	1/3	1/2
Substance Use Disorder**	0/0	2/3	0/3
Vitamin D** ng/dl	Not available	22	14 (7)
Statin therapy	0	1	3
Antihypertensive therapy	0	0	1
Behavioral health dx***	0	3	4

*Mean (SD), **Current/Past Use, ***Other than Gender Dysphoria and/or Substance Use Disorder

Disclosure: **P. Kapsner:** None.

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A Randomised trial of a Proactive Inpatient Diabetes Service (RAPIDS) demonstrates decreased adverse glycaemia and hospital-acquired infections

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Background and aims: In hospitalised patients, both hypoglycaemia and significant hyperglycaemia are associated with adverse outcomes. We hypothesised a proactive inpatient diabetes service (IDS), which electronically identifies inpatients with diabetes and provides immediate management, will decrease the incidence of adverse glycaemia & hospital complications.

Materials and methods: RAPIDS (ACTRN12616000265471) was a cluster-randomised trial on 8 wards of a tertiary hospital. Consecutive inpatients with diabetes or new-onset hyperglycaemia (random blood glucose [BG] ≥11.1 mmol/L without known diabetes) were recruited. Networked glucose meters were used to record capillary BG measures from admission until discharge, or day 14 for long-stayers. There was a 10-week baseline observational phase followed by a 12-week active phase during which the wards were cluster-randomised into 4 intervention and 4 control wards. Intervention wards received proactive IDS (endocrinologist or nurse practitioner who aimed to see patients within 24h of admission), while control wards continued usual care (a referral-based consultation service). Primary outcome (incidence of adverse glycaemic days [AGD]: patient-day with any BG <4.0 or >15.0 mmol/L) and secondary outcomes (patient-day mean glucose, hospital-acquired infections and length of stay) were compared between baseline and active phases within each group and subjected to multivariable analysis, adjusting for patient clinical features.

Results: We investigated 1002 patients (87% type 2 diabetes; 29% insulin-treated; HbA1c: 7.5±1.7%) totalling 5447 patient-days & 19062 BG measures. Incidence of AGD decreased significantly in the intervention wards (243 vs. 186 per 1000 patient-days [23% decrease], $p < 0.001$), but there was no significant change in the control wards (291 vs. 261 per 1000 patient-days, $p= 0.08$). On multivariable analysis, proactive IDS was independently associated with 24% decrease in the incidence of AGD ($p=0.005$). Proactive IDS also decreased the patient-day mean glucose (mean [SD]: 9.0 [2.7] vs. 9.5 [3.2] mmol/L, $p<0.001$), and the incidence of hospital-acquired infections (crude incidence: 8% vs. 3%, $p=0.02$; adjusted odds ratio: 0.28, 95% CI: 0.11-0.74, $p = 0.01$). There was no difference in hospital length of stay.

Conclusion: This large randomised trial of a proactive inpatient diabetes service decreased the incidence of adverse glycaemia and hospital-acquired infections. This proactive treatment paradigm may change the approach to inpatient diabetes care.

Clinical Trial Registration Number: ACTRN12616000265471
Supported by: Australian Diabetes Society
Disclosure: **M. Kyi:** None.

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A randomised clinical trial of electronic secure messages to prompt patients with poorly controlled diabetes to prepare for their primary care visits

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Background and aims: Despite robust evidence to guide clinical care, most patients with diabetes do not meet all goals of risk factor control. Improved patient-provider communication during time-limited primary care visits may represent one strategy for improving diabetes care.
Materials and methods: We conducted a pragmatic, physician-randomized, multi-site intervention to test the hypothesis that prompting patients with poorly controlled diabetes (A1c > 8.0%) to prepare for their visit would lead to more effective primary care interactions. Patients of physicians in the intervention arm received pre-visit emails asking them to pick and briefly describe their top 1 or 2 visit priorities while patients in the control arm continued with usual care. The study was conducted from 3/1/2015 - 10/21/2016 within a large integrated care delivery system in Northern California. Outcomes included validated measures of visit interactions and changes in A1c.
Results: We enrolled 146 primary care physicians (PCPs, 90.1% consent rate) from 30 primary care practices. Over the course of the study, 609 patients in the treatment arm and 547 patients in the control arm had visits with their PCPs. Among 408 randomly-selected patients completing post-visit surveys (58% response rate), intervention patients were more likely to have prepared a list of questions for their doctor (72.2% vs. 63.3%, p = 0.04), been given choices about diabetes treatment to think about (81.1% vs. 72.9%, p = 0.038), and asked about any medication problems (79.7% vs. 72.3%, p = 0.07). There was no significant difference between study arms in changes in A1c.
Conclusion: A simple pre-visit preparation tool improved primary care visit interactions but not A1c relative to usual care. Translating better primary care visits into better glycemic control may require additional, non-visit based interventions.
Clinical Trial Registration Number: NCT02375932
Supported by: NIDDK
Disclosure: **R.W. Grant:** None.

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Results of a cluster randomised controlled trial to improve primary care management of diabetic peripheral neuropathy symptoms: the Diabetes Telephone study

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Background and aims: Diabetic peripheral neuropathy is common among patients with diabetes and difficult to treat given the limited efficacy and tolerability of available medications. We tested the hypothesis that an automated system for eliciting and communicating symptom changes and medication side effects could improve symptom management among newly treated patients.
Materials and methods: Using a cluster randomized controlled trial design, we randomized 1,096 primary care physicians to treatment (3 brief automated calls over six months to monitoring patient symptoms and treatment side effects) or control condition (3 brief non-interactive automated calls over 6 months containing diabetes educational information) and recruited 1,270 of their patients (response rate 83%) newly treated for symptoms to the study. We assessed change in quality of life, patient-provider communication and patient-reported symptoms including pain interference, sleep disruption, and lower extremity function at 8 months and the likelihood of ever receiving a clinically meaningful dose of treatment during the 12-month post intervention period.
Results: Mean patient age was 67 (s.d.11.7) years and 53% were female; 65% had a haemoglobin A1c greater than 7.0%. Demographic and clinical characteristics, as well as baseline quality of life scores, were similar in both arms. Among the 1252 patients with complete data, we found no statistically or clinically significant effects of the intervention on change in quality of life [-0.002 (-0.01, 0.01), p=0.61] or symptoms [pain interference: 0.57 (-0.41, 1.54), p=0.25; sleep disruption: 0.26 (-0.19, 0.85), p=0.21; lower extremity functioning: 0.055 (-0.96, 1.07), p=0.92] or in clinical care [minimum effective dose: 0.04 (-0.20, 0.28); p=0.76]. More than 70% of intervention patient responses to the first call prompted a physician alert, primarily related to medication side effects (51%). Twenty-two percent of intervention patients discontinued treatment by the end of 2 months; the most commonly cited reasons for discontinuation included side effects, lack of symptom relief and concerns about taking too many medications. The majority of patients who continued treatment reported taking medication on a daily basis (>90%) and more than 20% reported self-titrating medications.
Conclusion: This pragmatic trial to monitor and communicate diabetic peripheral neuropathy symptoms as well as treatment associated adverse effects did not significantly improve patient quality of life measurements. The limited exposure of physicians to the intervention and the lack of a training component may have contributed to the absence of an observed effect. We found high rates of medication related adverse effects and low rates of symptom relief despite persistent use of prescribed medications. Given the high prevalence of diabetic peripheral neuropathy and the limited ability of medications to improve symptoms without acceptable adverse events, more research is required to identify effective interventions for improving patient quality of life.
Clinical Trial Registration Number: CE-1304-7250
Supported by: Patient-Centered Outcomes Research Institute [CE-1304-7250; SC14-1403-11992
Disclosure: **A.S. Adams:** Grants; Patient-Centered Outcomes Research Institute Assessment of Prevention, Diagnosis, and Treatment Options Program Award [CE-1304-7250], Division of Diabetes Translation, Centers for Disease Control and Prevention [U58 DP002641], National Institute for Diabetes, Digestive and Kidney Disorders [R01DK099108], National Health, Lung, Blood Institute [R01 HL117939].

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Organisation of diabetes care is associated with systolic blood pressure level: a cross-sectional study of 230,958 people with type 2 diabetes

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Background and aims: Sweden is one of top-performers in Europe in providing diabetes care. People with Type 2 diabetes mellitus (T2DM) is mainly treated within the primary health care system. Several quality improvement strategies has been identified as successful in the management of people with T2DM. However, current research is limited by not adjusting for several important confounders at the primary health care center (PHCC) and individual levels (e.g., clinical, socioeconomic and comorbidity data). The aim of this study aim was to investigate the association between the organization of diabetes care (i.e., personnel resources and organizational features) in primary health care and the level of systolic blood (SBP) among people with T2DM.
Materials and methods: People with T2DM (n = 230 958) attending 846 PHCCs were included in this cross-sectional study. The PHCC level data were based on managers answering the Swedish National Survey of the Quality and Organisation of Diabetes Care in Primary Healthcare (SwedQOP) questionnaire addressing PHCCs' personnel resources and

organizational features. People with T2DM and clinical data were obtained from the Swedish National Diabetes Register and linked to registers containing individual level data on socioeconomic status and comorbidities. Data were analyzed using a generalized estimating equations (GEE) linear model. The final GEE model comprised valid values for 787 PHCCs and 180 928 people with T2DM.

Results: After adjusting for PHCC and individual level confounders, personnel resources at the PHCCs being significantly associated with decreasing SBP levels were number of whole time equivalent (WTE) registered nurse (RN)/500 people with T2DM (-1.02 mmHg; $P < 0.001$) and RNs' number of European Credit Transfer and Accumulation System credits in diabetes-specific education (-0.02 mmHg; $P < 0.001$). PHCCs' organizational features associated with a decreasing SBP were PHCCs providing group education (-0.32 mmHg; $P < 0.001$) and having a system for checking that patients participated at their annual review at general practitioners (GPs) (-0.31 mmHg; $P = 0.027$). The opposite effect (i.e. increasing SBP) was found regarding duration of regular visits to GPs (0.47 mmHg per additional 15 minutes duration; $P < 0.001$) and RNs (0.28 mmHg per additional 15 minutes duration; $P < 0.001$).

Conclusion: The present large sample size cross-sectional study found that personnel resources and organizational features of PHCCs were associated with individual SBP level for persons with T2DM. The findings adds important knowledge, which may stimulate decision-makers to prioritize these factors for achieving a successful diabetes care management.

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Disclosure: **R. Husdal:** None.

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Patterns of glucose-lowering medication use in patients with diabetes and heart failure: insights from the Diabetes Collaborative Registry (DCR)

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Background and aims: Although heart failure (HF) and type 2 diabetes (T2D) often coexist, the optimal glucose management strategies in these patients has been challenging to determine, particularly as novel medication classes emerge. Some medications may have safety concerns in patients with HF (TZDs, certain DPP-4i's, sulfonylureas, insulin) while others could be potentially beneficial (metformin, SGLT2i) but may need to be used with caution in particular sub-groups (e.g., advanced kidney disease). We used DCR to evaluate current patterns of glucose-lowering medication use in adults with T2D with and without HF.

Materials and methods: DCR is a US-based outpatient registry of adults with diabetes seen in primary care, cardiology, and endocrinology practices and includes 5114 providers from 374 practices. We compared use of glucose-lowering medications in adults with T2D with vs. without HF. We used hierarchical, modified Poisson regression models to examine whether HF was associated with preferential use of each medication class, adjusting for factors that could impact selection of one medication class over another: age, chronic kidney disease (CKD), coronary artery disease (CAD), number of glucose-lowering medications, and insurance. Site was included as a random effect to account for clustering of patients within sites.

Results: Among 669,308 adults with T2D on at least 1 glucose-lowering medication, 179,133 (27%) had a diagnosis of HF (29% HF with reduced ejection fraction [HFrEF], 71% HF with preserved ejection fraction [HFpEF]). Patients with T2D and HF (compared with no HF) were more likely to be older, men, and to have CAD, atrial fibrillation, and CKD. In unadjusted comparisons, those with HF were more likely to be treated with insulin and sulfonylureas and less likely to be treated with metformin, TZD, DPP-4i, GLP-1 RA, and SGLT2i (Table). These associations remained significant after adjustment for patient factors and site, with the most prominent associations of HF with greater use of insulin and less use of metformin, TZD, and SGLT2i (Table).

Conclusion: In a large US-based outpatient registry, we found that a quarter of adults with T2D had a diagnosis of HF, which was predominantly HFpEF. Although some patterns of T2D medication use in patients with vs. without HF seemed consistent with existing evidence (less use of TZD and, potentially, DPP-4i), others appeared contrary to evidence (less use of metformin and SGLT2i).

Table. Use of Glucose-Lowering Medications in Patients With vs. Without HF

	Unadjusted Use		Adjusted Relative Rate of Use with HF[1]	
	HF n=179,133	No HF n=490,175	Relative Rate[2] (95% CI)	p-value
Insulin	39.6%	25.9%	1.38 (1.35-1.41)	<0.001
Metformin	59.1%	74.3%	0.85 (0.83-0.86)	<0.001
Sulfonylurea	35.3%	29.9%	1.04 (1.03-1.05)	<0.001
Thiazolidinedione	7.2%	8.4%	0.79 (0.75-0.82)	<0.001
DPP-4 inhibitor	14.5%	14.7%	0.94 (0.92-0.96)	<0.001
GLP-1 RA	4.5%	5.3%	0.94 (0.91-0.97)	<0.001
SGLT2 inhibitor	2.6%	3.8%	0.83 (0.79-0.88)	<0.001

[1] Each medication class was modeled separately (i.e., 7 Poisson regression models)

[2] Adjusted for site (random effect), age, CKD, CAD, number of glucose-lowering medications, and insurance

Disclosure: **S.V. Arnold:** None.

PS 046 New approaches reaching glucose target

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Predicting post one-year durability of glucose-lowering monotherapies in patients with new-onset type 2 diabetes

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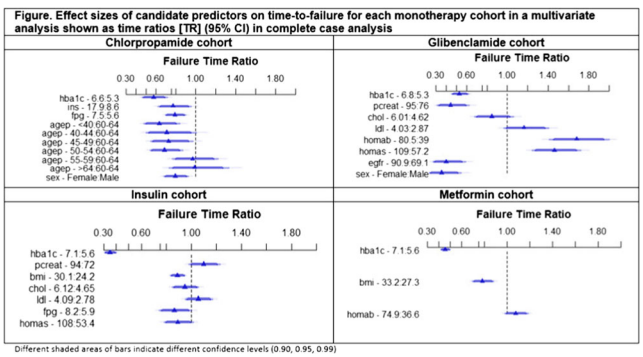
Background and aims: Current NICE guidelines for type 2 diabetes (T2D) recommend identifying the most appropriate glucose-lowering therapy for a given individual. This decision depends not only on the initial glycaemic response, but also the durability of its glucose-lowering effect over time. We investigated the latter by examining possible predictors of post one-year durability for glucose-lowering monotherapies in people with new-onset T2D.

Materials and methods: We used data from 2,339 UKPDS participants who remaining on their randomized glucose-lowering monotherapy at one year and had an HbA1c <7.5% (46% of 5,102 enrolled). Of these, 453 (19%) were treated with chlorpropamide, 391 (17%) glibenclamide, 1,047 (45%) basal insulin, and 448 (19%) overweight participants with metformin. Monotherapy glycaemic failure was defined as either an HbA1c ≥7.5%, or the protocol-driven requirement for glycaemic rescue with the addition of a second glucose-lowering therapy. We calculated the post one-year time-to-failure for each glucose-lowering monotherapy and developed prognostic models to investigate the phenotypic and biomarker characteristics that best predicted this time point. Potential variables were identified firstly by univariate accelerated failure time (AFT) models, and then we performed multivariate AFT regression models to identify those covariates that remained independently and significantly associated with time-to-failure.

Results: Monotherapy failure occurred in 1,712 (73%) of participants, comprising 272 (12%), 267 (11%), 824 (35%) and 349 (15%) respectively for chlorpropamide, glibenclamide, basal insulin and metformin. Median time-to-failure was 4.9 years (IQR 2.0, 10.1) overall, but differed by monotherapy being 7.2 (4.6, 9.6), 5.3 (3.3, 8.7), 5.2 (2.1, 7.1), and, 4.0 (2.4, 6.0) years respectively. The strongest independent predictor of time-to-failure was the one-year HbA1c, with higher values associated with reduced durability for all monotherapies. Additional covariates that contributed independently were: age, sex, fasting plasma glucose (FPG) & insulin for chlorpropamide; total cholesterol, LDL-cholesterol, creatinine, eGFR, HOMA-%B & HOMA-%S for glibenclamide; body mass index (BMI), total cholesterol, FPG, LDL-cholesterol, creatinine & HOMA-%S for basal insulin; BMI & HOMA-%B for metformin. The magnitude and direction of the effect sizes, expressed as time ratios [TR] and 95% CIs are shown in the Figure.

Conclusion: Median post one-year time-to-failure differed between the first-line glucose-lowering monotherapies studied. The one-year HbA1c value was the major time-to-failure determinant for all therapies, with additional factors that differed by monotherapy. Information such as this could be used to develop a time-to-failure calculator that might help guide the selection of glucose-lowering therapies for people with T2D.



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Second line treatment after metformin monotherapy of type 2 diabetes in a real life setting 2006 to 2015: nationwide data comparison between Denmark, Norway and Sweden

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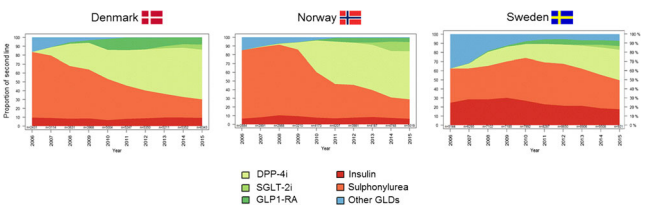
Background and aims: International type 2 diabetes (T2D) guidelines advocate metformin as the first step in pharmacological treatment, followed by a choice of different glucose-lowering drugs (GLD) for 2nd line e.g. sulphonylurea, insulin, dipeptidyl peptidase-4 inhibitors (DPP-4i), sodium glucose cotransporter-2 inhibitors (SGLT-2i), and glucagon-like peptide-1 receptor agonists (GLP1-RA). Although similar health care systems, GLD guidelines differ and it is not known whether treatment patterns vary between countries. The aim was to compare T2D 2nd line GLD treatment patterns after metformin monotherapy fail, using nationwide registries in three Nordic countries with similar demography and public health care systems.

Materials and methods: T2D patients treated with GLD between 2006-2015 were identified in the Registries of Medicinal Product Statistics (Denmark) or Prescribed Drug Register (Norway, Sweden), and linked with National Patient- and/or Cause of Death Registries. Second line treatment was defined as ≥6 months metformin monotherapy, followed by a filled prescription of a new GLD class. Date of 2nd line drug start was defined as index date.

Results: In total, 124,250 patients initiating 2nd line treatment were identified. In 2015, Swedish patients were older at index date (65.0 years) vs Norway (61.7 years) and Denmark (62.0 years), a trend which was stable during the whole observation time. Proportions of female T2D patients receiving 2nd line treatment was similar across all countries (c:a 40%). Antihypertensives [ACEi and ARBs] and statins was more extensively used in Denmark (90%) and Sweden (89%) vs Norway (80%). GLD use show a rapid change in the 2nd line treatment in Denmark and Norway whereas the uptake of newer GLDs is slower in Sweden (Figure). In 2015, newer GLDs (DPP-4i, SGLT-2i and GLP1-RA) were more extensively used in Denmark (70%) and Norway (71%) vs Sweden (44%). Conversely, almost twice as high use of older GLDs (insulin, sulphonylureas and other GLDs [except metformin]) were used in Sweden (56%) vs Denmark (30%) and Norway (29%) when initiating 2nd line.

Conclusion: During the last decade, a rapid increase in the use of newer GLDs was observed in Denmark and Norway but slower in Sweden. The use of older GLDs (such as insulin and SU) was almost twice as high in Sweden vs Denmark and Norway in 2015. Despite neighbouring countries with similar demography and health care systems we found large differences in GLD use highlighting the need for continuous attention to updated treatment guidelines.



Supported by: AstraZeneca
Disclosure: **F. Persson:** Employment/Consultancy; AstraZeneca. Grants; AstraZeneca. Honorarium; AstraZeneca. Lecture/other fees; AstraZeneca.

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Changes in HbA_{1c} and weight after initiation of second-line therapy in patients with type 2 diabetes in clinical practice

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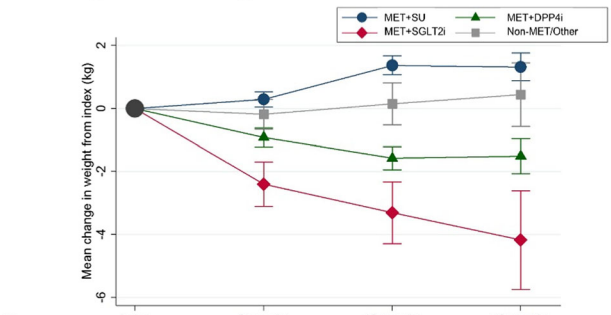
Background and aims: Intensification of first-line glucose-lowering therapy is often required in patients with T2DM. This study examined the use of second-line therapies in people with T2DM in UK clinical practice, and the association between second-line therapy and change in HbA_{1c} and weight over 18 months.
Materials and methods: This was a comparison of patients receiving different second-line glucose-lowering therapies in the UK Clinical Practice Research Datalink (CPRD). Patients with T2DM aged ≥18 years who initiated a second-line oral therapy between 1 August 2013 and 30 April 2015 were included.
Results: Patients identified initiating a second-line therapy (N=11,979) had a mean age of 62 years, 58.0% were male, mean (SD) HbA_{1c} 9.0 (1.8) % and mean (SD) body weight 92.8 (22.2) kg. Median time to initiation of second-line therapy was 4.5 years after diagnosis of T2DM, and 2.0 years after initiation of first-line therapy. The first-line therapy for the majority of patients (75.9%) was metformin monotherapy. When considering only those with metformin monotherapy as first-line therapy; second-line therapies were sulphonylureas (SU; 51.2%), dipeptidyl peptidase-4 inhibitors (DPP-4i; 31.9%), and sodium-glucose cotransporter 2 inhibitors (SGLT2i; 4.8%), with 12.1% on non-metformin therapy or other combinations. In those who initiated second-line therapy and remained on this therapy at each time point, mean baseline-adjusted changes in HbA_{1c} at 6 months were: metformin+SU -1.33%, metformin+DPP-4i -1.11%, metformin+SGLT2i -1.26% and non-metformin therapy/other combinations -1.03%. Decreases from baseline values seen at 18 months were: metformin+SU -1.21%, metformin+DPP-4i -1.23%, metformin+SGLT2i -1.46% and non-metformin therapy/other combinations -1.18%. Changes in body weight over 18 months for each second-line therapy are shown in the Figure. The low number of patients included in this analysis at later time points should be taken into consideration when interpreting the data.
Conclusion: In UK clinical practice, intensification of treatment with a second-line therapy occurred when patients had a high HbA_{1c}, and ~2 years after the initiation of first-line therapy. All choices of second-line therapy after metformin monotherapy were associated with reductions in HbA_{1c} over 18 months, while changes in body weight varied by second-line therapy, with sustained reductions seen over 18 months with addition of either SGLT2i or DPP4i to metformin.

Figure. Baseline-adjusted mean (SE) change in body weight from index in those who initiated, and remained on, second-line therapy after metformin monotherapy



N	Index	6 months	12 months	18 months
Metformin+SU	4606	1550	1092	567
Metformin+DPP4i	2880	993	714	359
Metformin+SGLT2i	440	185	103	46
Non-metformin/ other combination	1091	403	220	109

BMI, body mass index; DPP4i, dipeptidyl peptidase-4 inhibitors; MET, metformin; SGLT2i, sodium-glucose cotransporter 2 inhibitors; SU, sulfonylurea

Supported by: AZ
Disclosure: **J.P.H. Wilding:** None.

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Treatment patterns and associated factors in 13,379 patients with type 2 diabetes initiating a second-line therapy: the DISCOVER study

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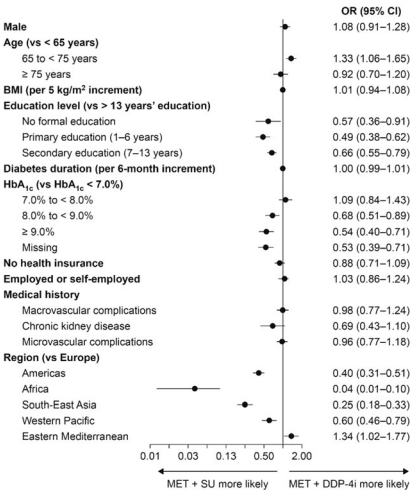
Background and aims: Data on treatment patterns in patients with type 2 diabetes are scarce in many countries. DISCOVER is a global, prospective, observational study of patients with type 2 diabetes initiating second-line therapy.
Materials and methods: Data were collected using a standardized case report form. First- and second-line treatments were assessed in 13 379 patients from 32 countries. Multinomial logistic regression models were used to assess factors associated with second-line treatment options among patients initially prescribed metformin (MET) monotherapy.
Results: Over half of patients (59.7%) received MET monotherapy as first-line treatment. Of these, 22.0% received a sulfonylurea (SU) + MET as second-line therapy, and 23.7% received a dipeptidyl peptidase-4 inhibitor (DPP-4i) + MET. A total of 37.2% of patients discontinued MET. Associations between baseline factors and the likelihood of receiving either SU + MET or DPP-4i + MET as second-line therapy are shown in the Figure. Patients with HbA_{1c} > 8.0% (vs. HbA_{1c} < 7.0%) and those in Africa (vs. Europe) were more likely to receive SU + MET than DPP-4i + MET. Close to 50% of the variation in choice of second-line therapy was explained by country.
Conclusion: Substantial variation exists in choice of second-line therapy worldwide. Despite the advent of new drugs, SUs remain widely used as second-line therapy in combination with MET.

Figure. Likelihood of receiving a sulfonylurea (SU) or a dipeptidyl peptidase-4 inhibitor (DPP-4i) as add-on to metformin (MET) at second-line, after receiving first-line MET monotherapy. CI, confidence interval; OR, odds ratio.



Clinical Trial Registration Number: NCT02322762
Supported by: AZ
Disclosure: **A. Nicolucci:** Grants; Novo Nordisk, Sanofi-Aventis, Artsana, Dexcom. Honorarium; Novo Nordisk, Medtronic, AstraZeneca, Eli Lilly.

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Distance from glycaemic goal at the time of treatment intensification in patients with type 2 diabetes failing metformin monotherapy in the UK

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Background and aims: Previous clinical trials have shown that intensive blood glucose control can substantially lower the risk of chronic micro- and macrovascular diabetic complications. While there are data to suggest that many patients with diabetes do not adequately achieve glycemic control, it is not well known how distant these uncontrolled patients are from typical glycemic targets. The objective of the study was to quantify the distance from a HbA1c target (<7%) among patients failing metformin monotherapy at the time of treatment intensification.
Materials and methods: Data was extracted from UK Clinical Practice Research Datalink (CPRD) with patients receiving metformin between Jan 2012- Dec 2014 (study period) and at least 12 months of continuous enrollment prior to the first fill of metformin (index date). The study population included patients who failed metformin monotherapy after at least 3 months of therapy (HbA1c ≥7%) and received treatment intensification with an add-on drug. Patients with Type 1 diabetes mellitus, pregnancy/gestational diabetes or other secondary diabetes were excluded. Distance from goal was calculated as the median difference of HbA1c at the time of treatment intensification and HbA1c goal (<7%).
Results: We identified 19,804 T2DM patients meeting study criteria. At the index date, before intensification, patients had a mean age of 64.6 years (Standard Deviation= 12.1 years); 27.4% of patients had a history of microvascular complications; 29.0% had a history of macrovascular complications; 1.3% had a history of severe/recurrent hypoglycemia in the one year prior. Median difference from HbA1c goal was 1.5%, with 25% of patients between 1-2% (n=4884) and 40% greater than 2% (n=7895) from goal. This median difference was 1.6% after exclusion of elderly patients (aged ≥ 65 years), those with microvascular and macrovascular complications and those with a history of severe/recurrent hypoglycemia (total n=6175), for whom a modified goal of 8% may be appropriate rather than 7%.
Conclusion: Among patients failing metformin monotherapy, majority of patients were greater than 1% away from the HbA1c goal of 7%.
Supported by: Merck Co. & Inc
Disclosure: **G. Fernandes:** Employment/Consultancy; Merck & Co., Inc., Kenilworth, NJ USA.

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Efficacy of self-monitoring blood glucose in the context of a chronic care model for type 2 diabetes patients not treated with insulin

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[1]UOS Integrazione Cure Primarie e Specialistiche, Azienda Ospedaliera ICP, Milan, [2]CORESEARCH - Center for Outcomes Research and Clinical Epidemiology, Pescara, Italy.

Background and aims: Patient-centered chronic care models are widely recognized as the most effective approach for the management of chronic diseases, but their implementation in the real world is a challenge. In Italy, the SINERGIA model was developed in one diabetes outpatient clinic and it improved metabolic control and major cardiovascular risk factors in an observational cohort of over 1000 patients. In the SINERGIA model self-monitoring blood glucose (SMBG) represents the core of education. Starting from these findings, the Self-Care study was designed to compare the efficacy of the SINERGIA model vs. usual care in the context of a randomized clinical trial.
Materials and methods: Multicenter, randomized (1:1), controlled clinical trial of 12 month follow-up plus a 12 month observational phase. Eligible patients were: age ≥ 45 years, first referral to diabetes clinic, HbA1c 7.0% - 9.0%, no SMBG and no insulin treatment. Patients allocated to SINERGIA group received the experimental education based on the use of SMBG as key tool to enhance patient empowerment in the context of a chronic care model; all subjects randomized to this group received the same glucose meter, i.e. BGStar®. Patients allocated to usual care group received the standard diabetes education provided by the center; in these patients SMBG was prescribed only if deemed appropriate, based on physician's judgment and patient needs; any glucose meter could be prescribed. Changes after 12 and 24 months from baseline in HbA1c levels, weight, blood pressure, lipid profile and health-related quality of life (HRQoL) scores (i.e. PDM, PHCO, SF-12, DTSQ, ADDQOL, and VAS) were assessed.
Results: Overall, 21 centers randomized 121 subjects to SINERGIA and 120 to control (Drop-out: 9.9% and 11.7%, respectively). Patients had mean age 61.8±8.8 years and diabetes duration 5.8±6.3 years. SMBG was prescribed to 70.8% of individuals allocated to usual care group. HbA1c levels were reduced by -0.47% (95%CI -0.66; -0.29) in SINERGIA vs. -0.32% (95%CI -0.51;-0.13) in usual care (p=0.27) after 12 months (primary end-point) and by -0.39% (95%CI -0.59;-0.19) vs. -0.18% (95%CI -0.39;-0.02) in usual care (p=0.17) after 24 months. Weight and lipid profile improved in both groups. Trends of additional benefits on blood pressure were found with SINERGIA. Psychological well-being (expressed as Mental Health Score - SF12) was unchanged after 12 and 24 months in SINERGIA and worsened in usual care; PDM score, i.e. the level of patient involvement in the decision making process, increased in SINERGIA group only.
Conclusion: The study was unable to demonstrate the superiority of SINERGIA vs. usual care, but suggests that SINERGIA *per se* was effective in improving and maintaining HbA1c levels and cardiovascular risk factors, without worsening psychological well-being at SMBG initiation. The study documents that SMBG training accompanied with education targeted to patient autonomy adds value to current care.
Clinical Trial Registration Number: NCT02082028
Supported by: Sanofi SpA, Milan, Italy
Disclosure: **I. Ciullo:** None.

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The SMBG study: a randomised controlled trial of self-monitoring of blood glucose in non-insulin treated type 2 diabetes

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Diabetes Research Unit Cymru, Swansea University, Swansea, UK.

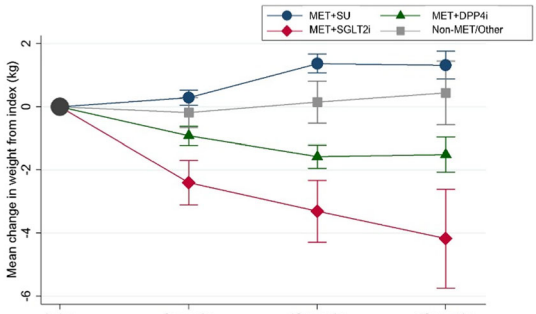
Background and aims: The benefit of Self-Monitoring of Blood Glucose (SMBG) in people with non-insulin treated type 2 diabetes (T2DM) continues under debate, with inconsistent evidence from observational studies and randomised controlled trials. However, there is growing consensus that structured SMBG can improve glycaemic control and overall well-being. The aim of the SMBG Study was to evaluate structured SMBG (2 days of paired blood glucose daily profiling, weekly) with standardised intervention (trained nurses using standardised treatment algorithms) with and without monthly telecare vs no SMBG in sub-optimally controlled, non-insulin treated people with T2DM.
Materials and methods: A 12 month, multi-centre, randomised controlled trial was performed in people with established (>1 year) T2DM, not on insulin therapy, with poor glycaemic control (HbA1c ≥7.5% ≤ 13%). A total of 666 participants were recruited from 16 primary and secondary care sites across Wales and England with 446 people randomised into one of three groups. Group 1 (G1), a control group that

received usual diabetes care with no SMBG; Group 2 (G2), undertook structured SMBG with clinical review every 3 months; Group 3 (G3), undertook structured SMBG with additional monthly telecare support. In Groups 2 & 3, participants and healthcare professionals were blinded to HbA1c results and glycaemic management was based on SMBG results alone.

Results: The demographic profile and baseline characteristics are detailed in the table. There were no significant differences between the three groups at randomisation. 323 participants attended the final visit at 12 months (G1 n=116; G2 n=99; G3 n=108) with no significant difference in participant withdrawal/loss to follow up. The mean (SD) HbA1c at 12 months was significantly lower for all groups compared to baseline (8.3(1.31)%, 7.4(1.22)% and 7.3(0.89)% for Groups 1, 2 and 3 respectively) with a significant reduction of 0.3% in G1 ($p<0.01$), 1.1% in G2 ($p<0.001$) and 1.3% in G3 ($p<0.001$) at 12 months. The percentage of those achieving HbA1c target of 7% or below was significantly higher ($p<0.001$) in the two SMBG groups compared to the control group (G1=17.2% vs G2=46.5% and G3=43.5%). Those reaching HbA1c of 7.5% or below was also significantly higher in the SMBG groups (G1= 33.6%, G2=63.6%, G3=66.6%, $p<0.001$). There was no statistical difference between groups 2 and 3. There was also no difference in the number of participants prescribed insulin: G1 (3), G2 (4) and G3 (4).

Conclusion: This study demonstrates that the use of structured SMBG with a standardised management algorithm provides significant benefits in terms of glycaemic control with a mean reduction of 0.9% (95% CI -1.18 to -0.62, $p<0.001$) between the combined SMBG groups compared to the control group.

Figure. Baseline-adjusted mean (SE) change in body weight from index in those who initiated, and remained on, second-line therapy after metformin monotherapy



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Metformin+SU	4606	1550	1092	567
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BMI, body mass index; DPP4i, dipeptidyl peptidase-4 inhibitors; MET, metformin; SGLT2i, sodium-glucose cotransporter 2 inhibitors; SU, sulfonylurea

Clinical Trial Registration Number: ISRCTN21390608
Supported by: EFSD/Lifescan. Roche Diabetes Care GmbH equipment & unrestricted grant
Disclosure: **S.N. Parsons:** None.

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Automated frequent insulin dosage titrations to enhance therapy effectiveness; lessons from the d-Nav® insulin guidance

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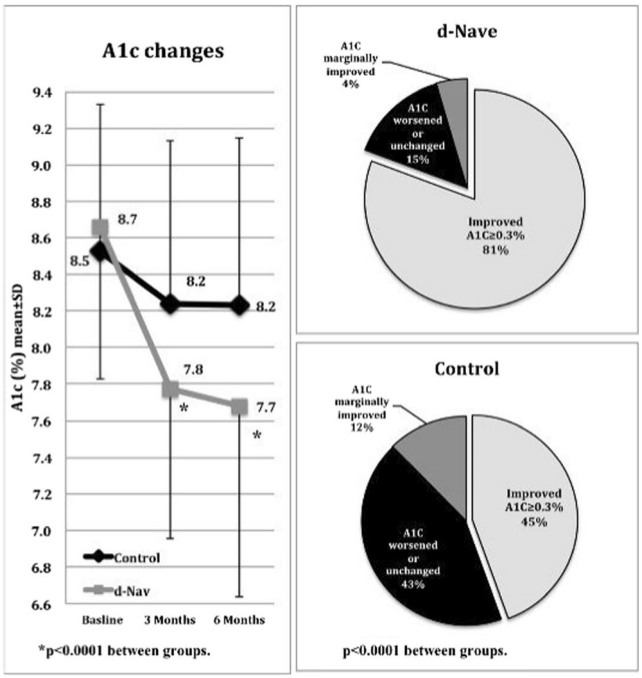
[1]University of Michigan, Ann Arbor, [2]International Diabetes Center, Minneapolis, [3]Hygieia Inc., Livonia, [4]Henry Ford Medical Center Endocrinology, Detroit, [5]Iowa Diabetes and Endocrinology Research Center, Des Moins, USA.

Background and aims: Insulin therapy is used by a quarter of type 2 diabetes patients, yet most do not achieve glycemic goals. Unless dosage is frequently adjusted, variations in insulin requirements hamper its effectiveness. In reality, dosage adjustments are done sporadically due to providers' workload in caring for a large number of patients. d-Nav® has been developed to overcome this barrier by bridging the titration gap. d-Nav is a handheld device that automatically analyzes stored glucose patterns and titrates insulin at least weekly, based on individual needs. Previous experience has underscored the role of care specialists' in supporting effective use of d-Nav. We equipped a diabetes specialty team with d-Nav to assess its impact on insulin management of patients with type 2 diabetes compared to close patient support without automatic dosage titration.

Materials and methods: We conducted a 6-month, multicenter, randomized-controlled trial with 181 sub-optimally controlled patients with type 2 diabetes. Standard care, which was delivered by a diabetes specialist team who contacted patients 7 times (control group), was compared to the same with the addition of d-Nav (d-Nav group).

Results: In the d-Nav group, A1c decreased by 1% from 8.7±0.8% (mean±standard deviation or SD) to 7.7±1.0%, while in the control group A1c decreased by 0.3% from 8.5±0.8% to 8.2±0.9% ($p<0.0001$)(See Figure). In the d-Nav group, clinically significant improvement in A1c (≥0.3%) was seen in 80.7% of subjects and 52.3% achieved A1c≤7.5%. Conversely, in the control group 44.4% improved A1c≥0.3% and 21.0% achieved A1c≤7.5%. In nearly half of the control patients (43.2%), A1c worsened or was unchanged during the study compared to 14.8% in the d-Nav group (see Figure).

Conclusion: When equipped with automated insulin titration capabilities, a diabetes specialist team can deliver an effective insulin therapy to the majority of patients. Automated insulin titration along with provider support can significantly advance the standard of care in insulin users with type 2 diabetes.



Clinical Trial Registration Number: NCT02424500
Supported by: National Institutes of Health, NIDDK (award number 2R42DK085974-02A1)
Disclosure: **I. Hodish:** Stock/Shareholding; I am a co-founder of Hygieia INC.

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Real-world basal insulin intensification patterns in the United States

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Background and aims: Basal insulin is an important component of the intensification treatment strategy in patients with type 2 diabetes mellitus (T2DM). Nevertheless, many patients remain uncontrolled (A1C≥7%) after initiating basal insulin. This study examined the prevalence of patients uncontrolled on basal insulin in a real-world setting and treatment intensification patterns and outcomes.
Materials and methods: Adults diagnosed with T2DM initiating a basal insulin analog (insulin glargine or detemir) with no GLP-1 or bolus insulin use from Jan-2010-Sep-2014 were identified in the QuintilesIMS PharMetrics Plus Health Plan Claims Database linked to ambulatory Electronic Medical Records. Patients were persistent on basal insulin for ≥6 months, and had ≥12 months of continuous health plan enrollment. Intensification patterns (increase in basal dose [of ≥10%], addition of bolus insulin, GLP-1 or a new oral antidiabetic drug) and outcomes were assessed among patients who were uncontrolled (A1C ≥7%) 6-months post-initiation. Kaplan-Meier (KM) analysis evaluated time to treatment intensification. Sensitivity analyses examined the definitions of uncontrolled (A1C≥8% and A1C>9%) and increase in basal dose (10 Unit (U) increase).
Results: Of 427 eligible patients with A1C available at 6 months, 59% were male, mean age was 53.9 years, mean A1C before basal insulin was 9.5%, mean follow-up was 29.4 months and mean basal insulin dose at initiation was 29.6U (median 24U). Six months after initiating basal insulin, 81% of patients (n=346) were uncontrolled (52% A1C≥8%, 27% A1C>9%). Mean 6 month dose was 31.0U (median 25U). Among patients uncontrolled 6 months after basal insulin initiation, 88% (n=306) subsequently intensified treatment over the follow-up (69% [n=238] by 12-months post-initiation). In KM analysis, patients first intensified treatment in a median of 58 days (60 days using A1C≥8%, 50.5 days using A1C>9). Most (67%; n=231) first intensified treatment by increasing the basal insulin dose, and mean basal insulin dose increased to 61.7U (median increased to 38U) at time of intensification. Among patients that first intensified by increasing the basal insulin dose, 92% (n=140 out of 152) with A1C values in the subsequent 6 months remained uncontrolled (76% A1C≥8%, 72% A1C>9%). In KM analysis, only 33.8% added another antidiabetic agent by 1-year post-intensification. When the definition of increased basal dose was changed to a 10U increase, only 47% (n=164) of uncontrolled patients intensified by 12-months post-initiation, with 28% (n=97) intensifying first by increasing the basal insulin dose.
Conclusion: The vast majority of patients remained uncontrolled 6 months after basal insulin initiation. Subsequently, most patients failed to achieve glycemic targets despite gradual uptitration of basal insulin. This suggests that a substantial unmet need exists for more timely insulin uptitration and intensification with other agents among T2DM patients not achieving glycemic control with basal insulin. Further research is needed to uncover potential areas for intervention to improve outcomes for patients uncontrolled on basal insulin.
Supported by: Novo Nordisk
Disclosure: **B. Thorsted:** Employment/Consultancy; Novo Nordisk. Stock/Shareholding; Novo Nordisk.

PS 047 Ultralong acting insulin analogues: new studies

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Effect of insulin degludec versus insulin glargine on glycaemic control and daily fasting blood glucose variability in insulin-naive Japanese patients with type 2 diabetes

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Background and aims: Insulin degludec (IDeg) is an ultra-long-acting insulin that has a smooth time/action profile over more than 42 hours.A pharmacodynamic study demonstrated that day-to-day glucose variability was four-fold smaller in patients with type 1 diabetes receiving IDeg than in those using insulin glargine (IGlar).
Materials and methods: Eligible patients were randomly allocated at a 3:1 ratio to receive once-daily IDeg (n=31) or IGlar (n=12). Both basal insulins were administered before breakfast and titrated to achieve a target FBG < 110 mg/dl. The primary endpoints were the change in HbA1c from baseline to 24 weeks of treatment, as well as the standard deviation (SD) and coefficient of variation (CV) of FBG from 8 to 12 weeks and from 20 to 24 weeks. Secondary endpoints included the QOL evaluated by the Diabetes Therapy-Related QOL questionnaire.
Results: After 24 weeks, HbA1c was decreased by 1.6% in the IDeg group and 1.7% in the IGlar at the same insulin dosage. At 24 weeks, FBG was significantly lower in the IDeg group than in the IGlar group and the CV of FBG was significantly smaller in the IDeg group. The frequency of total and severe hypoglycemic episodes did not differ between the groups. In the IDeg group, QOL showed significant improvement regarding anxiety and dissatisfaction with treatment. At 24 weeks, FBG (measured before breakfast) was significantly lower in the IDeg group than in the IGlar group, P=0.0122).
Conclusion: Treatment with IDeg or IGlar achieved similar improvement in glycemic control in insulin-naïve patients with type 2 diabetes. The day-to-day variation of FBG was smaller in patients receiving IDeg than in those with IGlar.
Clinical Trial Registration Number: UMIN000011827
Disclosure: **Y. Aso:** None.

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Effect of insulin degludec on HbA_{1c}, weight and hypoglycaemia: data from the Association of British Clinical Diabetologists (ABCD) nationwide degludec audit

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Background and aims: The Association of British Clinical Diabetologists (ABCD) nationwide degludec audit was set up to study the effect of the use of insulin degludec in routine clinical practice in the UK. The aim of this analysis was to look at the effect of switching to insulin degludec from another basal insulin on HbA1c, weight and hypoglycaemia.
Materials and methods: The ABCD nationwide degludec audit currently includes 433 people of whom 253 have type 1 diabetes (T1D) and 173 have type 2 diabetes (T2D). Of those with T1D 154 (61%) were switched to insulin degludec due to hypoglycaemia, and 99 (39%) for other reasons. Of those with T2D 57 (33%) were switched to insulin degludec due to hypoglycaemia, and 116 (67%) for other reasons. Baseline HbA1c and weight were compared with the latest results with 2-sided p-values using a paired t-test. Weight and HbA1c comparisons were grouped according to type of diabetes and whether degludec was started due to hypoglycaemia or for other reasons. Hypoglycaemia was recorded as having decreased, stayed the same or

increased after switching to insulin degludec, and data were compared for those with T1D and T2D where degludec was started due to reasons of hypoglycaemia. For a statistical comparison, it was assumed that where a similar rate of hypoglycaemia was reported using insulin degludec and the other basal insulin there was in fact a lower rate of hypoglycaemia with insulin degludec than the other basal insulin with probability 0.5. 2-sided p-values were calculated testing the hypothesis that a similar proportion of people experience a lower rate of hypoglycaemia on insulin degludec compared with the other basal insulin using the normal approximation to the binomial with a continuity correction. .

Results: Changes in HbA1c and weight are summarised in the attached table. For those switched to insulin degludec for hypoglycaemia in T1D there was a significant reduction in minor, severe and nocturnal hypoglycaemia ($P < 0.0001$ for all). For those with T2D switched to insulin degludec due to hypoglycaemia there was only a significant reduction in minor hypoglycaemia ($p < 0.05$), although it should be noted that the numbers in all groups were small.

Conclusion: For people with T1D, there was a significant reduction in HbA1c if insulin degludec was started for reasons other than hypoglycaemia, and a significant reduction in minor, severe and nocturnal hypoglycaemia if hypoglycaemia was the reasons for switching to insulin degludec. In either case there was no significant change in weight. For people with T2D, there was a significant reduction in HbA1c where insulin degludec was started for reasons other than hypoglycaemia, with no significant change in weight. For those with T2D switched to insulin degludec for hypoglycaemia, there was significant weight loss and a significant reduction in minor hypoglycaemia.

Type of diabetes	T1D		T2D	
Reason for starting degludec	Hypoglycaemia	Other	Hypoglycaemia	Other
Change in HbA1c after degludec (mean +/- SE)	+0.9+/- 1.4 mmol/mol (ns) n=99	-7.9 +/- 1.9 mmol/mol (p=0.0002) n=69	-2.4+/-1.8 mmol/mol (ns) n=40	-11.7+/-2.4 mmol/mol P<0.0001) n=99
Change in weight after degludec (mean +/- SE)	-0.20 +/- 0.60 kg (ns) n=83	+1.00 +/- 0.54 kg (ns) n=52	-2.37 +/- 0.91kg (P=0.01) n=37	-0.61 +/- 0.70 kg (ns) n=73

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Disclosure: **A.N. Lumb:** Grants; The audit is supported by an unrestricted grant from Novo Nordisk to the Association of British Clinical Diabetologists.

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Insulin degludec (IDeg) shows consistent risk reductions across hypoglycaemia definitions vs insulin glargine U100 (IGlar U100) in the SWITCH 1 and 2 trials

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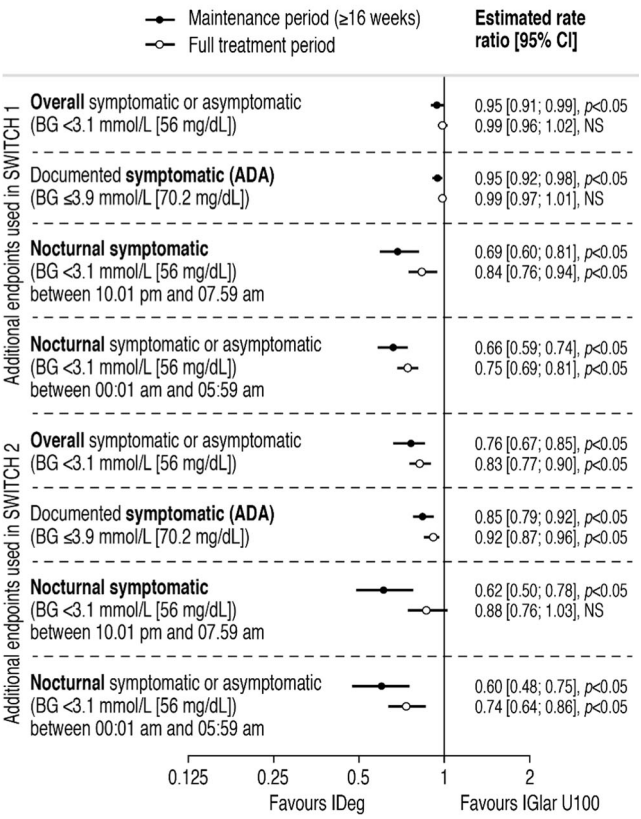
Background and aims: Insulin degludec (IDeg) is a basal insulin with a long duration of action and a flat glucose-lowering profile. The risk of hypoglycaemia with IDeg vs. insulin glargine U100 (IGlar U100) was compared in two trials in patients with type 1 (SWITCH 1) or type 2 diabetes (SWITCH 2) using a range of clinically relevant definitions of hypoglycaemia.

Materials and methods: Two 2x32-week, double-blind, treat-to-target crossover trials compared the risk of overall (severe [requiring third-party assistance and external adjudication] or blood glucose [<3.1 mmol/L (56 mg/dL)] confirmed) symptomatic (accompanied by typical symptoms of hypoglycaemia) hypoglycaemia, nocturnal (severe or blood glucose-confirmed; between 00:01 and 05:59 am) symptomatic hypoglycaemia and severe hypoglycaemia with IDeg once daily (OD) vs. IGlar U100 OD. *Post hoc* analyses investigated the risk of asymptomatic hypoglycaemic episodes, hypoglycaemia during sleep, and the American Diabetes Association (ADA) definition of symptomatic confirmed (blood glucose ≤3.9 mmol/L [70.2 mg/dL]) hypoglycaemia between treatments (Figure). All endpoints were analysed in the 16-week maintenance period and the full treatment period (32 weeks).

Results: HbA_{1c} non-inferiority was confirmed in both trials. SWITCH 1 and SWITCH 2 had significant reductions in overall symptomatic hypoglycaemia and nocturnal symptomatic hypoglycaemia in both the maintenance and the full treatment periods with IDeg vs. IGlar U100. SWITCH 1 had a significant reduction in severe hypoglycaemia with IDeg in the maintenance and the full treatment periods; in SWITCH 2 the rate ratio of severe hypoglycaemia was significantly lower with IDeg in the full treatment period. Significant risk reductions with IDeg vs. IGlar U100 were found in the maintenance period for overall and nocturnal symptomatic or asymptomatic hypoglycaemia. When the definition of the nocturnal period was expanded to 10:01 pm-07:59 am, rates of nocturnal symptomatic hypoglycaemia during sleep were again significantly lower with IDeg vs. IGlar U100 in the maintenance period. The finding of significantly lower rates of hypoglycaemia in the maintenance period with IDeg was retained when the ADA definition of symptomatic hypoglycaemia was used. For both SWITCH 1 and SWITCH 2, results were pointing in the same direction in the full treatment period for all definitions.

Conclusion: Hypoglycaemia reductions were consistent across hypoglycaemia definitions, especially during the nocturnal period, in SWITCH 1 and SWITCH 2.

Figure. Rate ratios of hypoglycaemia across different definitions



BG, blood glucose; CI, confidence interval; IDeg, insulin degludec; IGlar U100, insulin glargine U100; Nocturnal, severe or BG confirmed hypoglycaemia occurring at nighttime during sleep; NS, not significant; Overall, severe or BG confirmed hypoglycaemia; Severe, an episode requiring third-party assistance and external adjudication.

Clinical Trial Registration Number: SWITCH 1 (NCT02034513); SWITCH 2 (NCT02030600)

Supported by: Novo Nordisk A/S
Disclosure: **C.H. Wysham:** Employment/Consultancy; Astra Zeneca, Boehringer Ingelheim, Eli Lilly, Janssen, Novo Nordisk, sanofi. Lecture/other fees; Astra Zeneca, Boehringer Ingelheim, Insulet, Eli Lilly, Janssen, Novo Nordisk, sanofi.

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Converting to IDegLira is efficacious regardless of pre-trial insulin dose in patients with type 2 diabetes uncontrolled on insulin glargine U100

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Background and aims: This post hoc analysis of the DUAL V study investigated the safety and efficacy of initiating the fixed-ratio combination insulin degludec/liraglutide (IDegLira) once daily at 16 dose steps/units (U) (16 U IDeg; 0.58 mg liraglutide) in adults with type 2 diabetes (T2D) uncontrolled on 20-50 U of insulin glargine U100 (IGlar U100), vs continued IGlar U100 up-titration, across pre-trial daily insulin dose groups (20-<30; ≥30-<40; ≥40-≤50 U). This analysis aimed to confirm that there is no loss of glycaemic control when switching to 16 U IDegLira from a higher pre-trial basal insulin dose.
Materials and methods: DUAL V was a 26 week open-label, treat-to-target trial that randomised patients (n=557) with T2D uncontrolled (HbA_{1C} 7-10%) on IGlar U100 (20-50 U) plus metformin to either IDegLira or continued IGlar U100 titration**.**
Results: HbA_{1c} reductions from baseline to end of trial (EOT) were significantly greater with IDegLira vs IGlar U100 for all dose groups (Table). For all dose groups, compared with IGlar U100, IDegLira was insulin sparing, resulted in body weight loss vs body weight gain, and was associated with lower rates of hypoglycaemia (p<0.05, all treatment contrasts). There were no clinically relevant increases in self-measured plasma glucose levels when converting from any dose group to 16 U IDegLira, and no withdrawals due to hyperglycaemia with IDegLira in first 8 weeks. Fasting plasma glucose reductions were similar between treatment arms for all dose groups. For all endpoints except EOT insulin dose, treatment effect was consistent across dose groups.
Conclusion: In conclusion, regardless of pre-trial insulin dose group, IDegLira resulted in significantly greater HbA_{1c} and body weight reductions and lower hypoglycaemia rates vs IGlar U100 at a lower EOT insulin dose. Importantly, there was no loss of glycaemic control when converting from any dose between 20-50 U of IGlar U100 to the starting dose of 16 U IDegLira. This maintenance of glycaemic control at a lower insulin dose is likely attributable to the liraglutide component.

Table Efficacy and safety in DUAL V (NCT01952145) by pre-trial insulin dose category

	20–<30 U/day			≥30–<40 U/day			≥40–≤50 U/day		
	IDegLira (N=142)	IGlar U100 (N=127)	Treatment contrast [95% CI]	IDegLira (N=60)	IGlar U100 (N=68)	Treatment contrast [95% CI]	IDegLira (N=76)	IGlar U100 (N=83)	Treatment contrast [95% CI]
Baseline HbA_{1c}, %	8.3	8.1	–	8.5	8.2	–	8.3	8.4	–
Δ HbA_{1c}, %	−1.9	−1.3	ETD: −0.54 [−0.74; −0.33]**	−1.8	−1.0	ETD: −0.60 [−0.90; −0.30]**	−1.6	−1.1	ETD: −0.63 [−0.90; −0.36]**
Δ FPG, mmol/L	−3.1	−2.8	ETD: 0.06 [−0.43; 0.54]	−2.4	−2.8	ETD: 0.24 [−0.47; 0.94]	−2.7	−2.6	ETD: −0.26 [−0.89; 0.38]
Δ Body weight, kg	−1.3	1.3	ETD: −2.67 [−3.48; −1.86]**	−0.8	2.3	ETD: −3.40 [−4.58; −2.22]**	−1.9	2.1	ETD: −3.93 [−4.99; −2.86]**
EOT daily insulin dose[‡], U	38.1	53.3	ETD: −16.12 [−20.92; −11.33]**	40.6	68.8	ETD: −30.01 [−36.98; −23.04]**	46.3	84.2	ETD: −37.72 [−43.98; −31.46]**
Hypo events/PYE	2.93	4.71	ERR: 0.58 [0.35; 0.93]*	2.11	5.02	ERR: 0.33 [0.15; 0.68]*	1.05	5.61	ERR: 0.23 [0.12; 0.47]**

Data are mean unless otherwise stated. *p<0.05; **p≤0.0001; [‡]Maximum 50 U IDegLira; EOT, end of trial; ETD, estimated treatment difference; ERR, estimated rate ratio; FPG, fasting plasma glucose; IDegLira, insulin degludec/liraglutide combination; IGlar U100, insulin glargine 100 units/mL; hypo, unable to self-treat and/or blood glucose <3.1 mmol/L; LOCF, last observation carried forward; PYE, patient-year of exposure; U, units. Change in HbA_{1c}, FPG and body weight from baseline to EOT as well as insulin dose at EOT were analysed using an ANCOVA model where missing data were imputed using LOCF. Hypoglycaemic episodes were analysed using a negative binomial regression model with a log link and the logarithm of the time an event was considered treatment emergent as offset.

Clinical Trial Registration Number: NCT01952145
Supported by: Novo Nordisk A/S
Disclosure: **L.F. Meneghini:** Employment/Consultancy; Novo Nordisk, Sanofi Aventis.

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Real-world use of IDegLira is effective at moderate doses in patients with type 2 diabetes across all baseline treatment regimens and reduces concomitant antidiabetic therapy

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Background and aims: IDegLira is a once-daily, fixed-ratio combination of insulin degludec and liraglutide, the glucagon-like peptide-1 receptor agonist (GLP-1RA), for which the safety and efficacy has been demonstrated in the DUAL clinical trial programme. Results from the observational study, EXTRA, have demonstrated the effectiveness of IDegLira in a real-world setting. This analysis examined the titration of IDegLira and concomitant changes to treatment regimen associated with these glycaemic improvements, in patients grouped according to their baseline regimen.
Materials and methods: This European, multicentre, retrospective chart review, included 611 patients with type 2 diabetes, aged ≥18 years, who started IDegLira at least 6 months prior to data collection. Patients were grouped according to baseline regimen: non-injectable therapies, GLP-1RA, insulin + GLP-1RA (free combination), basal insulin, and multi-dose insulin, all subgroups were ± oral antidiabetic drugs (OADs). Clinical characteristics were assessed at baseline (BL, defined as the most recent recording during the 6-month period prior to first prescription of IDegLira) and follow-up (F/U; 6 months ± 45 days for each time point) after commencing IDegLira.
Results: After 6 months, IDegLira was titrated to a moderate dose in all subgroups (mean 30.2 dose steps/U; Table). The association between IDegLira dose at BL (and F/U) and change in HbA_{1c} was not significant (p=0.7610 [BL] and p=0.2447 [F/U]). Across all F/U IDegLira doses (<10; ≥10-<20; ≥20-<30; ≥30-<40; ≥40-<50; ≥50 dose steps), HbA_{1c} was reduced (-2.0; -0.9; -0.8; -1.0; -1.1; -0.8%). GLP-1RA dose was significantly reduced following IDegLira initiation in GLP-1RA and free combination subgroups. Total daily insulin dose was significantly reduced in patients receiving multi-dose insulin at BL; this was largely due to 60.5% (78/129) of patients discontinuing prandial insulin following IDegLira initiation. Of the 481 patients across all subgroups using ≥1 OAD at BL, 154 (32%) discontinued ≥1 OAD following IDegLira initiation. At F/U 11.0% (62/566) reached the max dose of 50 dose steps, and the majority came from the free combination (31) or multi-dose insulin group (15).
Conclusion: The clinical benefit of initiating once-daily IDegLira in a real-world setting is observed irrespective of F/U IDegLira dose at 6 months, regardless of previous therapy, and associated with a reduction in concomitant antidiabetic therapy, both total daily insulin dose and non-insulin agents.

Change in IDegLira dose, insulin dose and OAD use by baseline regimen (all ± OADs)

	Non-injectable therapy (n=112)	GLP-1RA (n=57)	Basal insulin (n=109)	Insulin + GLP-1RA (free combination) (n=135)	Multi-dose insulin (n=153)	Overall (n=566)
IDegLira initiation dose, dose steps	17.2 (10.6)	16.8 (8.5)	20.0 (9.4)	30.9 (14.0)	21.2 (9.9)	22.0 (12.1)
ΔIDegLira dose, dose steps	8.5 [6.6; 10.4]*	10.6 [7.7; 13.6]*	8.5 [6.5; 10.6]*	6.0 [4.4; 7.7]*	8.5 [6.8; 10.3]*	8.1 [7.3; 9.0]*
F/U IDegLira dose, dose steps	25.7 (12.4)	27.4 (11.4)	28.5 (11.1)	36.9 (13.7)	29.7 (11.9)	30.2 (12.9)
BL total insulin dose, U	–	–	31.1 (21.2)	44.3 (33.9)	65.7 (40.6)	48.8 (36.6)
F/U total insulin^ dose, U	–	–	29.4 (13.2)	41.8 (23.8)	45.0 (33.1)*	39.6 (26.5)*
BL GLP-1RA dose, DDD	–	1.2 (0.3)	–	1.3 (0.7)	–	1.3 (0.6)
F/U GLP-1RA^ dose, DDD	–	0.7 (0.3)*	–	0.9 (0.3)*	–	0.8 (0.3)*
No. of pts + OAD at BL; F/U	98; 44	54; 15	103; 54	116; 20	110; 21	481; 154
ΔHbA_{1c}, %	-1.6 [-1.9; -1.2]*	-1.0 [-1.3; -0.7]*	-0.9 [-1.2; -0.7]*	-0.6 [-0.8; -0.4]*	-0.7 [-0.9; -0.5]*	-0.9 [-1.0; -0.8]*

*p<0.0001, significance assessed using a two-tailed t-test. Data are based on effectiveness analysis set and presented as mean (SD) or [95% CI]; ^Includes IDegLira dose.
Δ, change from baseline to F/U; BL, baseline; DDD, defined daily dose based on World Health Organisation daily dose; GLP-1RA, glucagon-like peptide-1 receptor agonist; F/U, follow-up (6 months); OAD, oral antidiabetic drug.

Clinical Trial Registration Number: NCT02754817
Supported by: Novo Nordisk A/S

Disclosure: **B. Schultes:** Honorarium; Novo Nordisk, AstraZeneca, Sanofi Aventis. Lecture/other fees; Novo Nordisk.

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More consistent antilipolitic and antiketogenetic action of insulin Glargine U300 vs U100 in type 1 diabetes

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Background and aims: To compare PK/PD of insulin (I) glargine U300 (Gla300) and U100 (Gla100) during euglycemic clamp.
Materials and methods: We studied 18 T1DM subjects (8M, age 40±12 yrs, DM duration 27±12 yrs, BMI 23.3±2 kg/m^2, A1C 7.06±0.4 %) after 3 month treatment with Gla100 or Gla300 titrated to fasting euglycemia (randomized, crossover). In the clamp, subjects received the dose of Gla100 (0.30±0.04 U/kg) or Gla300 (0.37±0.07 U/kg) they had daily at 8 PM.
Results: N=10 subjects. Both Gla100 and Gla300 maintained euglycemia for 24 h. Plasma I was initially lower (0-12h, 8.7±3.5 vs 9.2 ±4.7 μU/ml) (p=0.068), but later greater on Gla300 vs Gla100 (12-24h, 7.6±2.4 vs 5.5±1.7 μU/ml) (p=0.022), whereas plasma glucagon was similar (51±9.2 and 50±12 pg/ml, p>0.2). Glucose infusion rate (GIR) was lower in initial 0-12 h but greater later at 18-24 h with Gla300 vs Gla100 (p<0.05), although 24h GIR was no different. Serum FFA concentration was lower for 24 h (319±70 vs 459±120 μmol/L), as 3-B-OH-butyrate (412±98 vs 641±138 μmol/ml) with Gla300 vs Gla100 (p<0.05). Serum alanine and lactate were not different.
Conclusion: As compared to Gla100, Gla300 (~23% higher dose, but equivalent glucose effect) modulates 24h glucose metabolism more physiologically, and is also more effective in suppressing lipolysis and ketogenesis for 24 h without affecting glucagon concentration, likely the result of more physiological plasma 24 h plasma I replacement with Gla300.
Clinical Trial Registration Number: EudraCT: 2015-002135-17
Supported by: Investigator initiated trial supported by Sanofi
Disclosure: **P. Lucidi:** Other; Travel grants to meetings from Sanofi and Theras.

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Device-supported vs routine titration of insulin glargine 300 U/ml (Gla-300) in type 2 diabetes: efficacy and safety

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Background and aims: Insulin self-titration may help people with T2DM reach glycaemic targets. MyStar DoseCoach™, a combined titration device/blood glucose meter, aids self-titration by providing automated insulin glargine dosing suggestions.
Materials and methods: AUTOMATIX, a randomised, parallel-group, multicentre treat-to-target trial, evaluated the efficacy and safety of device-supported vs routine (investigator recommended) Gla-300 titration regimens. Primary endpoint: percentage of participants reaching a fasting SMPG (FSMPG) target of 5.0-7.2 mmol/l at week 16 without severe hypoglycaemia. Secondary endpoints included: percentage of participants reaching target FSMPG at week 16 without confirmed or severe hypoglycaemia; change in mean FSMPG, HbA_{1c} and daily insulin dose (baseline to week 16). Number of participants experiencing hypoglycaemia and adverse events were also reported.
Results: In total 151 participants with T2DM (insulin naïve/insulin pre-treated) were randomised 1:1 to device-supported or routine titration with Gla-300. Although not significant, a higher proportion of participants using device-supported vs routine titration achieved the primary endpoint (**Table**). Between titration arms, comparable numbers of participants experienced hypoglycaemia and TEAEs/SAEs (**Table**).
Conclusion: The results show that device-supported titration with Gla-300 has a good efficacy/safety profile and may aid glycaemic target achievement.

Table. Outcomes from the AUTOMATIX study

	Device-supported titration (on-treatment period) (n=75)	Routine titration (on-treatment period) (n=76)
Participants[a] reaching mean FSMPG target range (5.0–7.2 mmol/) at week 16, estimated[b] %		
Without severe hypoglycaemia	45.9	36.8
Estimated weighted difference (95% CI)[c]	9.04 (−6.748 to 24.829)[NS]	
Without confirmed (≤3.9 mmol/l)) or severe hypoglycaemia	34.3	14.5
Estimated weighted difference (95% CI)[c]	19.75 (6.284 to 33.207)[ND]	
Change in mean FSMPG from baseline to week 16, mmol/l[a]	−2.51 (2.34)	−2.27 (2.29)
Change in mean HbA_{1c} from baseline to week 16, %[a]	−1.16 (0.84)	−1.01 (0.88)
Change in average daily Gla-300 dose from baseline to week 16, U/kg[d]	0.213 (0.185)	0.157 (0.153)
Hypoglycaemia, number of participants (%)[d]		
Any time of day (24 h)		
Any event	26 (34.7)	29 (38.2)
Confirmed (≤3.9 mmol/l) or severe hypoglycaemia	22 (29.3)	27 (35.5)
Nocturnal (00:00–05:59 h)		
Any event	8 (10.7)	11 (14.5)
Confirmed (≤3.9 mmol/l) or severe hypoglycaemia	7 (9.3)	10 (13.2)
TEAEs, n (%)[d]	34 (45.3)	29 (38.2)
SAEs, n (%)[d]	2 (2.7)	3 (3.9)

[a]mITT population.
[b]Estimated proportion of participants obtained by averaging all the imputed proportions of participants reaching the endpoint (a multiple imputation method was used to address missing values in the mITT population).
[c]Estimated difference of proportions obtained by combining the difference in percentage, weighted by the randomisation stratum of previous use of insulin (insulin naïve, insulin pre-treated), between titration groups of all different imputed data sets, using Rubin's formula.
[d]Safety population.
[NS]Not statistically significant (superiority testing).
[ND]Superiority not determined.
Values are expressed as mean (SD) unless otherwise stated.
CI, confidence interval; SAE, serious adverse event; SD, standard deviation; FSMPG, fasting self-monitored plasma glucose; mITT, modified intention-to-treat; TEAE, treatment-emergent adverse event

Clinical Trial Registration Number: NCT02585674
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Disclosure: **F. Flacke:** Employment/Consultancy; Sanofi. Stock/Shareholding; Sanofi.

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More physiological circulating insulin and modulation of hepatic glucose production with insulin Glargine U300 vs U100 in type 1 diabetes

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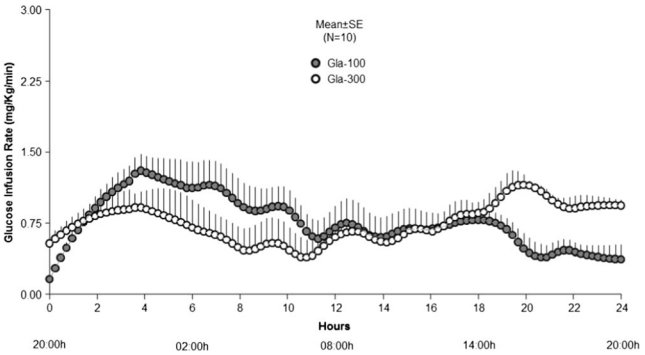
Background and aims: To compare PK/PD of insulin (I) glargine U300 (Gla300) vs U100 (Gla100).
Materials and methods: We studied 18 Type 1 DM subjects (8M, age 40 ±12 yrs, DM duration 7±12 yrs, BMI 23.3±2 kg/m^2, A1C 7.06±0.4 %) after 3 month treatment with Gla100 or Gla300 titrated to fasting plasma glucose (PG) 100 mg/dl (random crossover). In the euglycemic clamp, the subjects' individual daily doses (Gla100 0.30±0.04 U/kg, Gla300 0.37±0.07 U/kg) were given at 8 PM.
Results: Plasma I was initially lower (0-12h, 8.7±3.5 vs 9.2±4.7 μU/ml) (p=0.07), but later greater on Gla300 vs Gla100 (12-24h, 7.6±2.4 vs 5.5 ±1.7 μU/ml) (p=0.02). Both Gla100 and Gla300 maintained PG for 24 h (100±2 and 100±1 mg/dl, p=0.44). With Gla300 vs Gla100 G infusion rate (GIR) was initially lower [AUC ratio T/R (95% CI)], $GIR_{0\text{-}12h}$, 0.61 (0.41 to 0.91, p=0.02)] and later greater [$GIR_{18\text{-}24h}$, 2.2 (1.2 to 4.0, p=0.02), although $GIR_{0\text{-}24h}$ was not different [0.94 (0.66 to 1.3, p=0.66)]. Hepatic G production (HGP) was less suppressed on Gla300 for initial 12 h (4.3±2.6 vs 3.5±2.1 μmol/Kg/min), but more so on Gla300 over last 6 h (1.8±1.3 vs 4.9±2.6 μmol/Kg/min) (both p<0.05).
Conclusion: Gla300 (~23% higher dose, equivalent G efficacy) results in more physiological plasma I and HGP vs Gla100. The higher nocturnal

HGP may reduce risk for nocturnal/morning hypoglycemia, the lower afternoon HGP may favor better predinner G control with Gla300 vs Gla100.



Clinical Trial Registration Number: EudraCT: 2015-002135-17
Supported by: Investigator initiated trial, supported by Sanofi
Disclosure: **F. Porcellati:** Employment/Consultancy; board member: Sanofi, Consultant: Sanofi, Eli Lilly and Company.

PS 048 Insulin therapy: new approaches in type 2 diabetes

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Interim results from BEYOND II: evaluation of an intervention to optimise basal insulin use for type 2 diabetes in China

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Background and aims: This study aims to evaluate a physician-targeted educational intervention for improving basal insulin (BI) use in China.
Materials and methods: BEYOND II is a prospective cohort study conducted at 73 endocrinology departments at hospitals in China. Participating physicians undergo a 6-month intervention comprising face-to-face training based on a guideline-recommended BI treatment pathway, reinforced by self-audit and peer-to-peer feedback on clinical practice. Pre- and post-intervention, ~100 adults (≥18 years) with type 2 diabetes mellitus (T2DM) receiving BI for ≥3 months are enrolled at each study centre to assess glycaemic control and BI use. The primary endpoint is the proportion of centres meeting individual improvement goals set by the Study Committee. This is a planned interim analysis.
Results: Patient profiles are summarised in the Table. Of 37 centres completing the study as of December 2016, 24 (64.9%, 95% CI; 47.5 to 79.8%) achieved post-intervention improvement targets. The post-intervention cohort had a higher proportion achieving HbA1c <7% (25.2 vs. 38.5%) and FPG <6.1 mmol/L (14.7 vs. 19.8%), a lower mean HbA1c and FPG, and a lower mean daily insulin dose than the baseline cohort (Table). Hypoglycaemia occurred in <5% of patients in both cohorts. The proportion of physicians (n=353) self-rating as 'confident in most cases' about insulin initiation (77.3 vs. 87.3%; p<0.001), titration (81.0 vs. 85.0%; p=0.022) hypoglycaemia management (77.6 vs. 90.1%; p<0.001) increased post-intervention.
Conclusion: The BEYOND II intervention led to improvements in glycaemic control at 64.9% of study centres by encouraging adoption of guideline recommendations for BI use in clinical practice.

Variable[a]	Baseline cohort (N=3537)	Post-intervention cohort (N=3524)	P-value[b]
Age, years	59.7 (11.6)	59.2 (11.5)	0.086
Males, n (%)	1877 (53.1)	1890 (53.6)	0.634
BMI, kg/m^2	25.1 (3.3)	24.8 (3.1)	<0.001
Disease course, years	10.7 (7.1)	10.5 (7.1)	0.362
Basal insulin dose, IU/kg/day	0.24 (0.10)	0.23 (0.10)	0.033
HbA1c, %	8.16 (1.70)	7.63 (1.49)	<0.001
HbA1c <7%, n (%)	893 (25.2)	1355 (38.5)	<0.001
FPG, mmol/L	9.15 (3.50)	8.30 (3.00)	<0.001
FPG <6.1mmol/L, n (%)	521 (14.7)	699 (19.8)	<0.001
Incidence of hypoglycaemia, n (%)	165 (4.7)	154 (4.4)	0.551

[a]Values are mean (standard deviation) unless otherwise specified; [b]Chi-squared test for categorical variables and Student's t-test for continuous variables. BMI, body mass index; FPG, fasting plasma glucose; HbA1c, glycated haemoglobin.

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Disclosure: **J. Weng:** None.

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Impact of treatment persistence on glycated haemoglobin A_{1c} (HbA_{1c}) trends among patients with type 2 diabetes newly initiated on basal insulin

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Background and aims: Previous studies have suggested that greater treatment persistence is associated with improved clinical outcomes in patients with T2D; however, the relationship between persistence with basal insulin therapy and glycaemic control over time has yet to be investigated.
Materials and methods: This retrospective observational study assessed the impact of treatment persistence on HbA_{1c} levels over time in US insulin-naive patients with T2D newly initiating insulin glargine 100 U/mL or insulin detemir between January 1, 2011 and December 31, 2013. Patients were identified from the Clinformatics database, and HbA_{1c} values were extracted quarterly for each patient. Data were assessed 12 months prior to (baseline) and 18 months after insulin initiation. Non-persistence was defined as a basal insulin refill gap of > 90 days during 18 months follow-up. A generalized estimating equations regression model accounting for the correlation of repeated HbA_{1c} measurements and missing data for each patient during the follow-up period was used to compare HbA_{1c} trends between persistent and non-persistent patients.
Results: In this analysis 3,993 patients with ≥1 HbA_{1c} measure at baseline and ≥1 HbA_{1c} measure at follow-up (mean 2.71 HbA_{1c} measures per patient) were identified. Patients were further categorized as persistent (n = 1,715) or non-persistent (n = 2,278). Approximately 73% of non-persistent patients discontinued basal insulin within 6 months of initiation. Persistent patients had significantly greater HbA_{1c} reduction during the 18-month follow-up period. After controlling for potential confounders in the generalized estimating equations model, persistence was associated with an additional 0.05% decrease in HbA_{1c} per quarter, with an accumulated additional 0.3% decrease in HbA_{1c} over 18 months, compared with non-persistent patients ($P = 0.0004$).
Conclusion: These data suggest that for patients with T2D newly initiated on basal insulin, being persistent with treatment is associated with a consistent decrease in HbA_{1c} over time in real-world clinical settings.
Supported by: Study funding and editorial support provided by Sanofi US, Inc.
Disclosure: **L. Xie:** Employment/Consultancy; Employee of STATinMED Research, under contract with Sanofi U.S., Inc.

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Cost analysis of initiating basal insulin for 6 months in type 2 diabetes patients in China: an observational registry study

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Background and aims: The short-term cost changes after adding-on basal insulins (BIs) therapy in routine clinical practice is still unknown. This study aims to determine the economic impact of converting patients with T2DM from an oral antidiabetic drug (OAD)-only regimen to a BI+ OAD regimen.
Materials and methods: Based on a multicenter, 6-month prospective registry study design, 18,995 patients with type 2 diabetes inadequately controlled (HbA1C ≥7%) by OADs and willing to initiate BI therapy were enrolled from 209 hospitals of eight geographic regions of China from December 2011 to June 2013. Interviews were conducted at baseline (0 month—V1), month 3 (V 2) and month 6 (V 3). Type and dose of BI were at the physician's discretion and patients' willingness. The treatment cost included cost of OAD medication, insulin therapy, and self-monitoring of blood glucose (SMBG). Daily treatment cost per person before and after adding BIs was evaluated. Clinical outcomes such as HbA1c and hypoglycemia were also recorded.
Results: After adding on BIs, the mean ± SD daily treatment cost for insulin-naïve T2DM patients increased from $1.24 ± $2.04 (baseline) to $2.67 ± $1.76 at 3 months and $2.57 ± $1.81 at 6 months, a median (Q1, Q3) increase of 1.51 (0.39, 4.07) times over 6 months. The cost and clinical outcomes in different subgroups are shown in Table 1. The times of cost increased proportionally with the baseline HbA1c level (from 1.33times to 2.08times) and declined as diabetes duration prolonged (from 1.64 times to 1.30 times). The times of costs increase were higher in the insulin glargine 1.59(0.48,4.14) and detemir 1.80(0.58,4.88) groups compared with the NPH group 0.94(0.07,3.17). However, NPH subgroup had the highest increase in percentage of hypoglycemia (7.2%) compared with subgroup of Glargin (1.3%) and Detemir (6.5%). Insulin cost accounted for the highest proportion (48.3%) of costs, with OAD costs ranking the second (36.7%), followed by needles (7.9%) and SMBG (6.9%) costs.
Conclusion: Adding-on BI therapy may increase the daily treatment cost by 1.5 times at 6 months, followed by significant improvement in blood glucose control.

Table 1. Daily treatment cost ($) change and clinical outcomes after initiating basal insulin for 6 months in different subgroups

Group variable	Absolute cost increase (v3-v0)	Times of cost increase at 6 months,	HbA1c reduction (%)	Increase in hypoglycemia (%)	Weight gain (kg)
Total	1.35 ± 2.19	1.51(0.39, 4.07)	2.15±2.07	2.9	0.10±2.94
Baseline HbA1c (%)					
[7, 8)	1.27 ± 1.85	1.33(0.34,3.37)	0.91±1.14	1.4	-0.14±2.80
[9, 11)	1.41 ± 2.52	1.62(0.44,4.33)	2.28±1.42	3.8	0.07±2.97
[11, 13)	1.42 ± 2.50	1.89(0.47,5.33)	3.83±1.74	4.3	0.53±3.08
≥13	1.39 ± 1.87	2.08(0.37,5.59)	6.13±2.25	6.1	0.91±3.17
Diabetes duration (years)					
<5	1.26 ± 2.20	1.64(0.39,4.58)	2.47±2.19	3	-0.03±3.07
[5, 10)	1.36 ± 2.23	1.46(0.39,3.74)	1.88±1.89	3.2	0.15±2.87
[10, 15)	1.47 ± 2.06	1.37(0.39,3.64)	1.91±1.91	2.3	0.30±2.77
≥15	1.54 ± 2.26	1.30(0.33,3.71)	1.75±1.97	2.6	0.31±2.72
BMI (kg/m^2) at baseline					
<24	1.26 ± 2.26	1.44(0.37,3.90)	2.38±2.21	2.8	0.93±2.67
[24, 28)	1.39 ± 2.16	1.58(0.41,4.17)	2.00±1.97	3.3	-0.21±2.79
≥28	1.47 ± 2.07	1.56(0.38,4.32)	1.90±1.84	2.2	-1.33±3.32
BI type at v1					
Glargine	1.49 ± 2.09	1.59(0.48,4.14)	2.09±2.01	1.3	0.06±2.89
Detemir	1.66 ± 2.54	1.80(0.58,4.88)	2.17±2.01	6.5	-0.20±2.88
NPH	0.56 ± 2.14	0.94(0.07,3.17)	2.35±2.29	7.2	0.49±3.13

Data are n, mean ± SD or Median (Q1, Q3). BI, basal insulin; BMI, body mass index; HbA1c, glycated hemoglobin; NPH, neutral protamine Hagedorn; OAD, oral antidiabetic drug; SD, standard deviation.

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Supported by: Sanofi-Aventis (Shanghai, China)
Disclosure: **P. Zhang:** None.

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Clinical benefit of simultaneous initiation of basal insulin and GLP-1 in patients with type 2 diabetes and elevated HbA_{1c} as supported by real-world evidence

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Background and aims: Many guidelines tend to recommend a stepwise treatment approach that may be associated with uncontrolled HbA1c between steps. However, timely and durable glycemic control is a key goal of T2DM treatment. This study compared longitudinal HbA1c in patients with uncontrolled T2DM receiving combination treatment with GLP-1 and basal insulin (BI) initiated either sequentially or simultaneously.
Materials and methods: Patients with T2DM from 1/1/2007-12/31/2014 were identified in the GE Centricity database. Patients receiving combination GLP-1/BI were selected (initiation of combination GLP-1/BI termed index date). Patients were required to have 6 months pre- and 1 year post-index date physician history and pre-index date HbA1c > 7%. Patients were classified as having simultaneous GLP-1/BI initiation if they started both medications within 14 days of each other, otherwise patients were considered sequential initiators. In addition, patients were required to receive combination treatment (i.e., overlapping prescription orders for both medications) for a minimum of 30 of the first 60 days of the post-index date period. HbA1c was compared from 6 months pre- to 1 year post-index date.
Results: A total of 5,453 patients initiated BI first, 3,930 patients initiated GLP-1 first, and 1,823 patients initiated GLP-1/BI simultaneously. Patients were of similar age across all study cohorts (mean [SD] age 56.7 [11.8] years among BI first, 57.0 [10.7] among GLP-1 first, and 55.9 [11.8] years among simultaneous initiators). Baseline HbA1c was similar across all study cohorts (mean [SD] HbA1c 9.1 [1.6] among BI first, 9.1 [1.6] among GLP-1 first, and 9.0 (1.7) among simultaneous initiators), and the change in HbA1c during 1 year follow-up was similar across cohorts (0.7 for both simultaneous initiators and BI first, 0.8 for GLP-1 first). Among the subgroup of patients with baseline HbA1c > 10.0 (N=1,172 BI first, 882 GLP-1 first, and 378 simultaneous initiators), baseline HbA1c was similar (9.6 [2.2] among BI first, 9.3 [2.0] among GLP-1 first, and 9.1 [1.9] among simultaneous initiators), and simultaneous initiators had the largest decrease in HbA1c during follow-up (mean [SD] change in HbA1c was 2.3 [2.1] for simultaneous initiators, 1.8 [1.7] for GLP-1 first, and 2.0 [2.0] for BI first).
Conclusion: Patients with very high HbA1c (i.e., HbA1c > 10) were found to have the largest HbA1c decrease if they simultaneously initiated GLP-1 and BI, indicating that patients may benefit from simultaneous over stepwise treatment initiation.
Supported by: Sanofi
Disclosure: **B. Guerci:** Other; Sanofi US; Eli Lilly and Company; Novo Nordisk Inc.; Novartis Pharmaceuticals Inc.; AstraZeneca; Abbott; Medtronic; Roche Diagnostic; VitalAire; Bristol-Myers Squibb Company; Janssen Pharmaceuticals.

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Use of regular insulin U-500 in type 2 diabetes treatment with pump therapy

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Background and aims: Insulin therapy intensification by insulin pump is helpful in insulin resistant type 2 diabetes. The use of concentrated insulin may help improving glycemic control but few studies are available. We retrospectively report on the utilization of regular insulin 500 U/ml (Eli Lilly, France) in a pump device over a 5-yr period.
Materials and methods: Patients on MDI (basal-bolus) or pump therapy, both using U-100 rapid acting insulin analog, were recruited in two circumstances : i) HbA1c>8% and/or insulin dose >100 UI/d. After U-500 therapy initiation, follow-up was performed at 1 week, 3-6, 12, 18-24, 30-36, 42-48 and 54-60 months for HbA1c, total daily dose (TDD), body weight, incidence of hypoglycemia, treatment drop off.
Results: 52 patients were included, M/F ratio 28/24, age 62,9±8,8, diabetes duration 19±8 yrs, TDD 199±58 UI/d, BMI 39±6 kg/m², HbA1c 8.9 ±1.3%. Mean follow up on U-500 was 20±15 months with 10 drop off. HbA1c decreased by - 0,96% (p=0,001), - 0.95% (p<0,001), - 0,99 % (p= 0,001), - 1.38% (p=0,001), - 1% (NS) and - 2.15% (NS) at 3-6, 12, 18-24, 30-36, 42-48 and 54-60 months. Body weight remained stable and TDD increased by 11-25% among the study period. Non severe hypoglycemia occured in 21% subjects on U-100 and 55% subjects on U500 at 6 months (p=.0.01). 0ne severe hypoglycemia occured during the study period.
Conclusion: Pump therapy delivering U-500 regular insulin durably improves glycemic control in insulin refractory patients with type 2 diabetes who remain uncontrolled with U-100 intensified insulin regimens. Hypoglycemia may be prevented by careful education of U-500 users.
Disclosure: **E. Deberles:** None.

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Associations between insulin dose titration over one year and hypoglycaemia: a descriptive study using 4-T data

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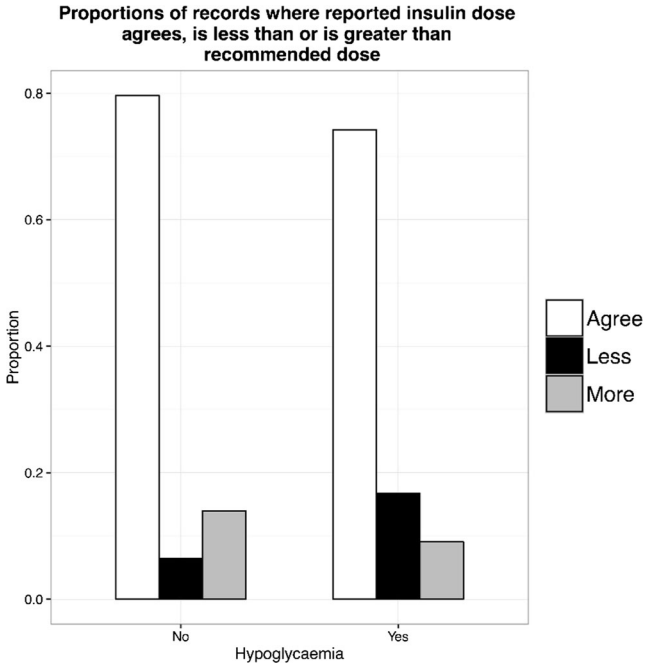
Background and aims: Insulin titration algorithms for type 2 diabetes are well established, but their implementation and the insulin doses used are poorly described. In the 4-T study, participants were randomly allocated to three different insulin regimens, one of which was a basal insulin with daily injections of insulin detemir adjusted with an algorithm based recommendation. Comparing reports of the actual insulin dose used with that recommended provide a means of examining adherence to recommendations for insulin dose.
Materials and methods: In a cohort of people with T2D starting treatment with insulin detemir in the 4-T study, participants received standardized recommendations for dose adjustment from a computerised algorithm. Insulin dose was increased if more than one-third of blood glucose readings in the preceding two weeks were above the target range of 4.0 to 5.5mmol/l and reduced where hypoglycaemia or blood glucose levels of <3.9mmol/l were experienced. Data on recommended insulin doses and subsequent reported actual doses, and reports of hypoglycaemia were recorded. Sequential random intercept logit models, based on whether the reported dose was the same as the recommended dose were used to explore associations with gender, insulin dose taken, pre-breakfast glucose and occurrence of hypoglycaemia.
Results: Insulin dose data were available on 233 (99.6%) of 234 participants in the 4-T trial allocated to detemir insulin. 143 (61.1%) were men. Mean (SD) age was 61.9 (10.0) years and HbA_{1c} 8.4 (0.8)%. Over one year 4,976 paired records of insulin doses recommended and taken were compared. For 3,934 (79.1%) paired records, the insulin dose reported as being taken at the next study contact was the same as the prior recommendation. 24 (10.3%) participants reported taking the recommended

insulin dose for all study visits. In 1042 (20.9%) records the dose reported did not match the recommended dose. Of these, 667 (64.0%) were for a higher dose of insulin and 375 (36.0%) were for a lower dose of insulin than that recommended. For those experiencing hypoglycaemia following the dose recommendation, the proportion of people taking less insulin than recommended was higher than among those not experiencing hypoglycaemia [Figure]. The odds ratio (95% CI) for associations between taking less (relative to taking more) insulin than recommended and the following factors were: more hypoglycaemia (3.65, 2.35-5.65); higher glucose level (0.86, 0.79-0.93); trend over time (1.06, 1.04-1.09); male *vs.* female (0.59, 0.41-0.86) and insulin dose taken 0.98 (0.98-0.99).
Conclusion: These data confirm that around 20% of insulin dose recommendations to people over one year in a clinical trial were not followed, but where the dose was discrepant, a greater rather than a smaller dose of insulin than recommended was more likely to be used.



Supported by: The 4-T trial was funded by Novo Nordisk.
Disclosure: **A.J. Farmer:** Grants; The 4-T trial was funded by Novo Nordisk. (This analysis was supported by NIHR funding for Mei-Man).

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Patterns and trends in insulin intensification among patients with type 2 diabetes in Western Pacific region

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Background and aims: Verifying Insulin Strategy and Initial Health Outcome Analysis (VISION) was an 18-month observational study that explored treatment approaches in patients with type 2 diabetes mellitus (T2DM) initiating insulin in the Western Pacific (WP) region. We summarise demographic and clinical characteristics, patterns of insulin initiation and intensification, health care professional profile and patient-reported outcomes (PROs).
Materials and methods: Patients aged ≥18 years with T2DM initiating insulin therapy in normal clinical course after oral drugs failure were enrolled from 5 WP countries. A total of 1065 patients were enrolled from Hong Kong (n=143), Malaysia (n=237), Philippines (n=188), Taiwan (n=163) and Thailand (n=334). Participant data were recorded by the treating physicians and PROs were assessed using questionnaires.
Results: Mean age of patients was 57.2 years with mean glycosylated haemoglobin (HbA1c) of 9.9%. About 59% of patients had HbA1c >9% at insulin initiation despite 81% being on 2 or more oral anti-diabetic (OAD) agents. Hypertension and dyslipidaemia were the most frequent comorbidities in 70% and 62.9% patients, respectively. Sixty-four percent were being followed by endocrinologist and the rest by non-endocrinologists. More than 70% were using metformin and sulfonylureas (SU) and 26.3% were on DPP4 inhibitors (DPP4i). Basal insulin was initiated in 72% and premixed insulin in 27%. OADs were continued by most of the patients, initiated on basal insulin; whereas, SU use decreased from 51% at baseline to 14.4% in patients initiated on premixed insulin. Intensification of insulin therapy (switching from basal to premixed insulin, adding mealtime bolus doses and/or increasing the dosing frequency) was observed in 66.4% patients on initial basal insulin and 53.1% of patients on initial premixed insulin. By the end of study, proportion of patients with HbA1c ≤9% increased from 33.4% to 54.8% but only 24.7% achieved HbA1c of ≤7.5% after intensification of insulin treatment by their physicians. The proportion of patients completely satisfied with their insulin treatment increased from 19.0% to 28.6% in the first 6 months and remained stable till the end of the study. The quality of life (QoL), EQ-5D-3L Visual Analogue Scale mean scores increased from baseline by the end of the study.
Conclusion: VISION study highlights clinical inertia and importance of early insulin initiation and intensification. More than half of patients had an HbA1c >9% at the time of insulin initiation and only a quarter managed to achieve HbA1c of ≤7.5% by the end of 18 months. About 15% patients were continued on SU despite being on premixed insulin which is not recommended in most guidelines. Insulin regimen change was more frequently needed in the basal group compared to the premixed group. Improvement in treatment satisfaction score and other PROs suggests that in most patients, insulin treatment does improve QoL.
Supported by: Eli Lilly and Company
Disclosure: **A. Jabbar:** Other; Employee of Eli Lilly and Company.

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Impact of human versus analog insulins on occurrence of myocardial infarction

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Background and aims: Prior studies have shown mixed results on the cardiovascular safety of insulin analogs compared to human insulins. This observational study uses rigorous causal inference methods of Comparative Effectiveness Research to emulate a trial evaluating the cardiovascular safety of analog versus human insulins.
Materials and methods: Study subjects were 127,600 adults with type 2 diabetes receiving care between 1/12005 and 12/31/2013 at 4 U.S. health care delivery systems, classified on date of insulin initiation (index date) as using (a) analog insulins with or without human insulin, versus (b) human insulins only. Subjects were followed from index date to first

occurrence of: myocardial infarction (MI), death, health plan disenrollment, or 12/31/2013. In order to estimate the differences between the two groups had patients remained on their insulin regimen (a per-protocol analysis), we used Inverse Probability Weights (IPW) and marginal structural modeling (MSM) analyses in which patients' data were right-censored if they crossed-over to the other exposure group or interrupted insulin therapy during follow-up. Effect estimates were obtained based on two logistic MSM parameterizations for counterfactual hazards. To address concerns over potential residual confounding, we replicated the same analytic approach using three nested sets of covariates assumed to potentially affect (a) both the outcome and the exposure/censoring events, (b) the outcome, and (c) either the outcome or exposure/censoring events.

Results: The mean follow-up time was 21 months. Hazard ratios and cumulative risk differences comparing the two groups were not statistically significant. The estimates of the hazards ratio and its 95% confidence interval were 1.2214 [0.9877;1.4551]; 1.08 [0.8642;1.2959]; and 1.0779 [0.7692;1.3866] for each covariate adjustment set, respectively. The estimates of the cumulative risk difference at 2 years and its 95% confidence interval were 0.0037 [-8e-04;0.0082]; 0.0011 [-0.0034;0.0057]; and -6e-04 [-0.0055;0.0044] for each covariate adjustment set, respectively.

Conclusion: In this large comparative effectiveness analysis using marginal structural modeling with three covariate adjustment sets, we were not able to identify statistically significant excess occurrence of myocardial infarction in those using analog, compared to human insulins.

Supported by: US National Heart & Lung & Blood Institute

Disclosure: **P.J. O'Connor:** None.

PS 049 Insulin analogues: new insulins

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1,5-anhydroglucitol correlates with postprandial glucose in subjects with type 1 diabetes irrespective of HbA_{1c} responder status

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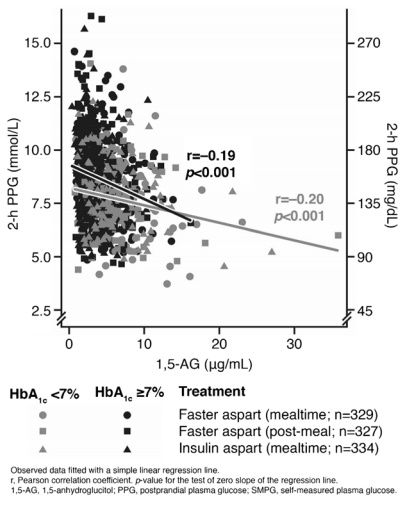
Background and aims: Serum 1,5-anhydroglucitol (1,5-AG) levels decrease (<12 μg/mL) during periods of hyperglycaemia (>180 mg/dL) and reflect plasma glucose control over the previous 1-2 weeks. 1,5-AG is considered particularly useful in evaluating postprandial glucose (PPG) excursions in well- or moderately controlled patients (defined by HbA_{1c}), as 1,5-AG levels can reflect both fasting and postprandial hyperglycaemia in poorly controlled patients. The aim of this *post hoc* analysis was to assess if the extent to which 1,5-AG levels reflect PPG control depends on HbA_{1c} responder status.

Materials and methods: Post hoc analysis of onset 1, a 26-week, phase 3 trial in which subjects with type 1 diabetes (mean HbA_{1c} 7.6%) were randomised to double-blind mealtime fast-acting insulin aspart (faster aspart), insulin aspart (IAsp) or open-label post-meal faster aspart (20 min after the start of the meal), all in combination with insulin detemir. In this analysis, subjects from the three treatment arms were pooled to examine the correlation between absolute values of 1,5-AG and 2-h PPG from 7-9-7 self-measured plasma glucose profiles at week 26 according to HbA_{1c} status (<7% or ≥7%) at week 26.

Results: In onset 1, HbA_{1c} was reduced in all treatment arms; primary endpoint was HbA_{1c} change from baseline at 26 weeks (est. treatment difference [ETD] [95% CI]: mealtime faster aspart vs. IAsp −0.15% [−0.23;−0.07]; post-meal faster aspart vs. IAsp 0.04% [−0.04;0.12]). Glycaemic differences were reflected in 1,5-AG change from baseline (ETD [95% CI]: mealtime faster aspart vs. IAsp 0.50 μg/mL [0.24;0.76]; post-meal faster aspart vs. IAsp -0.16 μg/mL [-0.42;0.10]). 1,5-AG correlated with 2-h PPG for subjects with HbA_{1c} <7% (r=-0.20; $p<0.001$) and HbA_{1c} ≥7% (r=-0.19, $p<0.001$) at week 26. In both HbA_{1c} responder groups, the correlation between 1,5-AG and 2-h PPG was similar within each treatment arm.

Conclusion: These results are consistent with the usefulness of 1,5-AG as a marker of short-term glycaemic control, irrespective of HbA_{1c} responder status.

Figure. Correlation between absolute values of 1,5-AG and 2-h PPG (SMPG) at week 26 by HbA_{1c} responder status



Observed data fitted with a simple linear regression line.
r, Pearson correlation coefficient. *p*-value for the test of zero slope of the regression line.
1,5-AG, 1,5-anhydroglucitol; PPG, postprandial plasma glucose; SMPG, self-measured plasma glucose.

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Supported by: Novo Nordisk A/S
Disclosure: **S. Heller:** Other; Advisory panel: Eli Lilly & Co; Novo Nordisk A/S; Takeda, Sanofi-Aventis, Merck, Boehringher Ingelheim, Speakers' bureau: Novo Nordisk; Eli Lilly & Co; Merck Sharp & Dohme, Takeda, Sanofi-Aventis, AstraZeneca, Boehringer Ingelheim.

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Treprostinil causes local vasodilation, is well tolerated, and results in faster absorption of insulin lispro

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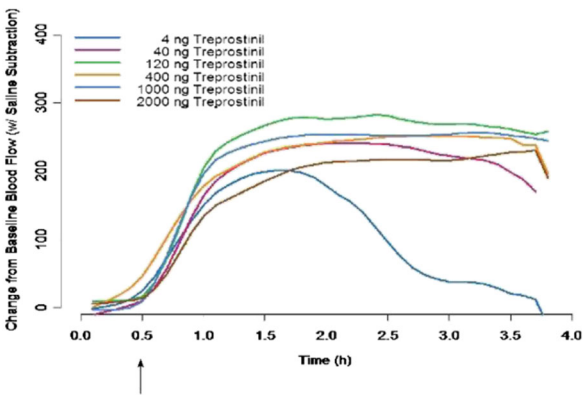
Background and aims: The purpose of this study was to test if an increase in local blood flow induced by s. c. administration of a known vasodilator (treprostinil) at a tolerated dose would speed absorption of insulin lispro.
Materials and methods: This was a phase 1, randomised, placebo-controlled, 2-part exploratory study in healthy subjects. Part A was a dose-escalation study (4 to 2000 ng) of s. c. treprostinil (n=11), a prostacyclin analogue approved for the treatment of symptomatic pulmonary arterial hypertension, versus placebo, to assess safety, tolerability, and local vasodilation. Laser Doppler flowmetry was used to assess local blood flow. Part B was a 4-period, complete crossover, euglycaemic clamp (N=16) to assess the pharmacokinetics and glucodynamics (GD) of insulin lispro (15 U) when administered with 3 concentrations of treprostinil or placebo.
Results: Treprostinil was rapidly absorbed and eliminated with detectable exposure only at the highest dose levels. Local blood flow increased from baseline and had similar magnitude for the 4-hour duration for all treprostinil doses (Figure 1), except the 4 ng dose level in which blood flow returned to baseline at ~3 hours postdose. There was no obvious difference in adverse events (AEs) across treatments and, importantly, no clinically significant increase of those AEs was associated with systemic absorption of treprostinil. There was no suggestion of treatment-related changes in laboratory values, vital signs, or ECG parameters with treprostinil. When insulin lispro was combined with treprostinil, the insulin lispro area under the concentration curve versus time from 0 to 30 min increased up to 92% ($p<0.0001$) and the time to onset of insulin action was reduced up to 29% ($p<0.0001$) compared with when insulin lispro was used alone; however, the total glucose infused was similar.
Conclusion: Coadministration of microdoses of treprostinil increased local blood flow and accelerated the absorption and GD action of insulin lispro without compromising safety, and may represent a way to achieve an ultra rapid prandial insulin.

Figure 1. Mean change from baseline in local blood flow, as measured by Laser Doppler Flowmetry, versus time by treprostinil dose level.



Supported by: Eli Lilly and Company
Disclosure: **J. Leohr:** Employment/Consultancy; Eli Lilly and Company. Stock/Shareholding; Eli Lilly and Company.

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Ultra-rapid BioChaperone Lispro improves post-prandial blood glucose excursions versus insulin lispro in a 14-day treatment study in subjects with type 1 diabetes

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Background and aims: We investigated safety and efficacy of BioChaperone Lispro (BCLIS), an ultra-rapid formulation of insulin lispro (LIS), in a double blind randomised, cross-over study in 36 subjects with type 1 diabetes treated with multiple daily insulin injections (mean ±SD age: 45±12 yrs; BMI: 24.3±2.6 kg/m²; HbA1c: 7.2±0.7%).
Materials and methods: Post-prandial glucose (PPG) excursions were assessed with individualised solid mixed meal tests (MMT) (50% carbs, 30% fat, 20% proteins) with insulin administrations immediately (T0), 15 minutes before (T-15) and 15 minutes after (T+15) meal start on days 1, 2 and 3 and with T0 administration on day 14. Pharmacokinetics (PK) were assessed only for T0 MMTs. Subjects also used individualised BCLIS or LIS doses immediately before meals during two 14-day outpatient periods with unchanged basal insulin regimen.
Results: BCLIS showed similar safety and tolerability to LIS with 12% less hypoglycaemic episodes in the outpatient period. No injection site reactions were observed. PK showed identical total exposure for both formulations, but a higher early exposure (table) and a faster insulin lispro absorption with BCLIS than LIS (early $T_{0.5max}$ Day 1: 0.259 vs. 0.365 h, Day 14: 0.261 vs 0.345 h, $p<0.0001$). In MMTs with T0 administrations, significant reductions in 1-2h PPG excursions by 30-50% were observed with BCLIS vs LIS (table). Both accelerated absorption and better PPG control were sustained over 14 days. In addition, no differences in PPG control were seen between BCLIS injected at T+15 and LIS at T0.
Conclusion: BCLIS was well-tolerated and safe over 14 days of MDI in subjects with type 1 diabetes, significantly improved post-prandial glucose excursions vs LIS at mealtime and could additionally offer the flexibility of post-meal administration.

Table:

	Parameter	Treatment	LS Means T0	LS Means T+15	LS Means T0 (day 14)	p-value T0 BCLIS vs T0 LIS	p-value T+15 BCLIS vs T0 LIS
Blood glucose (BG) parameter	$\Delta AUC_{BG\ 0\text{-}1h}$ (mmol*h/L)	BCLIS	1.11	2.17	0.71	**0.0059**	0.1470
		LIS	1.83	2.72	1.44		
	$\Delta AUC_{BG\ 0\text{-}2h}$ (mmol*h/L)	BCLIS	3.44	5.83	2.00*	**0.0237**	0.2251
		LIS	5.00	7.33	3.50*		
	ΔBG_{1h} (mmol/L)	BCLIS	2.28	4.00	1.33*	**0.0250**	0.0814
		LIS	3.22	5.22	2.39*		
PK parameter	$AUC_{LIS\ 0\text{-}30min}$ (pg*h/mL)	BCLIS	348	NA	362	**<0.0001**	**NA (No PK at T+15)**
		LIS	200		245*		
	$AUC_{LIS\ 0\text{-}1h}$ (pg*h/mL)	BCLIS	1007		1074	**0.0181**	
		LIS	830		936		

* indicates a significant difference within treatment between T0 day 1-3 and T0 Day 14
ΔAUC_{BG} : incremental area under the blood glucose curve
ΔBG_{1h} : blood glucose excursion 1h post meal
AUC_{LIS} : area under the serum lispro concentration

Clinical Trial Registration Number: NCT02528396
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Disclosure: **G. Meiffren:** Employment/Consultancy; Adocia. Stock/Shareholding; Adocia.

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Exploration of the mechanism of accelerated absorption for a novel insulin lispro formulation

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Background and aims: Potential benefits of a prandial insulin with a faster onset and offset compared to currently available insulin analogues include better prandial glycaemic control, less hypoglycaemia and weight gain, and better overall utility in closed-loop artificial pancreas systems. Modelling and simulation suggested that increasing absorption rate would have more influence on the insulin pharmacokinetics (PK) than modulating insulin clearance. To achieve this goal, the excipients citrate and treprostinil were included in LY900014 (LY)—a novel formulation of insulin lispro—to accelerate insulin lispro absorption for improvement of postprandial glucose. Herein we describe the physiological mechanisms that contribute to accelerated absorption and provide pre-clinical evidence of faster insulin lispro absorption.

Materials and methods: An *in vitro* assay was used as a model of transport across the endothelial monolayer, where human dermal microvascular endothelial cells were grown in Transwell® plates and quantitation of the transport of FITC-insulin lispro across the cell monolayer was performed. The Miles assay was used to test vascular permeability in rats following an intravenous dose of Evans Blue dye, which binds tightly to albumin. Test excipients were administered by subcutaneous (sc) injection, and vascular permeability was assessed by measurement of the dye in skin biopsies. Laser Doppler imaging was used to measure vasodilation in the skin of rats following sc dosing of test excipients. To determine the impact of the excipients on glucodynamics and insulin lispro PK, LY was administered by sc injection to diabetic miniature swine. A mixed effects ANOVA was performed with Dunnett's pairwise comparisons of LY to the marketed U100 formulation of insulin lispro.

Results: In the vascular endothelial cell-based permeability assay, citrate induced a dose-dependent increase in permeability of FITC-insulin lispro. In the Miles assay, injection of citrate into the sc tissue of rats resulted in a localised increase in vascular permeability. The vasodilator treprostinil, a prostacyclin analogue approved for clinical use, had little effect in either of these assays. On the other hand, sc injection of very low doses of treprostinil induced localised vasodilation in rats without causing systemic vasodilation. Citrate had minimal effects on vasodilation. When administered by sc injection to diabetic miniature swine, LY resulted in significantly faster glucose lowering at time points up to 30 min post dose, and key PK parameters (t_{max}, early 50% t_{max}, late 50% t_{max}) were significantly faster as compared to the marketed U100 formulation of insulin lispro.

Conclusion: Taken together these results demonstrate that insulin lispro absorption can be accelerated through modulation of injection site blood flow and vascular permeability and provide a mechanistic understanding for the ultra rapid insulin lispro profile that LY has demonstrated in clinical trials.

Supported by: Eli Lilly and Company

Disclosure: **J.S. Moyers:** Employment/Consultancy; Eli Lilly and Company. Stock/Shareholding; Eli Lilly and Company.

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Efficacy and safety of fast-acting insulin aspart are maintained over 52 weeks: comparison with insulin aspart in onset 1

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Background and aims: onset 1 was a phase 3a trial evaluating fast-acting insulin aspart (faster aspart) in adults with type 1 diabetes (T1D) over 52 weeks vs. insulin aspart, in two 26-week treatment periods. The aim of the additional 26-week period was to assess long-term safety and efficacy of faster aspart.

Materials and methods: In the initial 26-week treatment period of onset 1, subjects were randomised to either double-blind mealtime faster aspart, insulin aspart (IAsp) or open-label post-meal faster aspart, each with once- or twice-daily insulin detemir. Subjects on mealtime faster aspart (n=381) and mealtime IAsp (n=380) then continued to the additional 26-week treatment period.

Results: After 52 weeks, mean HbA_{1c} change from baseline was −0.08% with faster aspart and +0.01% with IAsp, with significant est. treatment difference (ETD) favouring faster aspart (ETD: −0.10% [95% CI: −0.19;−0.00]). Following a standardised meal test, change from baseline in 1-h postprandial plasma glucose (PPG) increment significantly favoured faster aspart (−1.05 mmol/L) compared with IAsp (−0.14 mmol/L) (Table). A similar trend toward better efficacy with faster aspart vs. IAsp was seen in change from baseline in 2-h PPG increment after the meal test (Table). The improvements in PPG control were also reflected in 7-9-7 self-measured plasma glucose profiles, with significant difference between treatment arms in favour of faster aspart for mean overall plasma glucose (PG) change from baseline (ETD [95% CI]: −0.23 mmol/L [−0.46;−0.00]), driven by 2-h PPG increments after breakfast and dinner. No statistically significant differences were observed across all meals (Table). The significant increase from baseline to 52 weeks in 1,5-anhydroglucitol with faster aspart compared with IAsp (Table) also suggested fewer hyperglycaemic excursions with faster aspart. Median total insulin dose was 61.3 U/0.77 U/kg with faster aspart and 68.5 U/0.83 U/kg with IAsp. No difference was observed for body weight change (+1.18 kg [faster aspart] vs. +1.05 kg [IAsp]; ETD: 0.13 kg [95% CI: −0.38;0.65]). After 52 weeks, adverse events were similar between faster aspart and IAsp, and as expected for IAsp. Overall severe or blood glucose-confirmed hypoglycaemia rates (PG <3.1 mmol/L) were similar (faster aspart: 53.29 events/patient-year; IAsp 53.19 events/patient-year) (estimated ratio: 1.01 [95% CI: 0.88;1.15]).

Conclusion: No long-term safety issues were identified with faster aspart. Glycaemic control was significantly improved after 52 weeks with faster aspart vs. IAsp. Approaching a profile closer to physiology with faster aspart achieves lower PPG and HbA_{1c} in T1D vs. IAsp.

Table. Estimated treatment differences between faster aspart vs. insulin aspart in change from baseline to 52 weeks in onset 1

	Estimated treatment difference in change from baseline (mealtime faster aspart – mealtime insulin aspart) [95% CI]
Mean HbA_{1c}	−0.10% [−0.19;−0.00]*
Standardised meal test	
1-h PPG increment	−0.91 mmol/L [−1.40;−0.43]* −16.48 mg/dL [−25.17;−7.80]*
2-h PPG increment	−0.42 mmol/L [−1.11;0.27] −7.60 mg/dL [−19.98;4.78]
7-9-7 point SMPG	
Overall PG	−0.23 mmol/L [−0.46;−0.00]* −4.14 mg/dL [−8.23;−0.06]*
2-h PPG increment breakfast	−0.44 mmol/L [−0.87;−0.01]* −7.87 mg/dL [−15.62;−0.13]*
2-h PPG increment lunch	0.14 mmol/L [−0.28; 0.57] 2.57 [-5.12; 10.26]
2-h PPG increment dinner	−0.47 mmol/L [−0.89;−0.05]* −8.49 mg/dL [−16.01;−0.97]*
2-h PPG increment overall	−0.25 mmol/L [−0.52;0.01] -4.55 [−9.30; 0.20]
1,5-anhydroglucitol	0.35 µg/mL (0.05;0.65)*

*Statistically significantly in favour of faster aspart compared with IAsp.
CI, confidence interval; faster aspart, fast-acting insulin aspart; IAsp, insulin aspart; PG, plasma glucose; PPG, postprandial glucose; SMPG, self-measured plasma glucose.

Clinical Trial Registration Number: NCT01831765
Supported by: Novo Nordisk A/S

Disclosure: **C. Mathieu:** Other; Advisory panel: Novo Nordisk, Sanofi-Aventis, Merck Sharp & Dohme Ltd., Eli Lilly and Company, Novartis, AstraZeneca LP, Jansen Pharmaceuticals, Hanmi Pharmaceuticals, Intrexon, Boehringer Ingelheim, Research support: Novo Nordisk, Sanofi-Aventis, Merck Sharp & Dohme Ltd., Boehringer Ingelheim, Speakers' bureau: Novo Nordisk, Sanofi-Aventis, Merck Sharp & Dohme, Eli Lilly and Company, Novartis, AstraZeneca.

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Improved glycaemic control with carbohydrate counting for adjustment of fast-acting insulin aspart versus insulin aspart in subjects with type 1 diabetes

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Background and aims: Insulin delivery based on carbohydrate counting is the gold standard for improving glycaemic control in type 1 diabetes (T1D). The aim of this *post hoc* analysis was to assess the impact of dose adjustment methodology on the efficacy and safety of mealtime fast-acting insulin aspart (faster aspart) and insulin aspart (IAsp) in subjects with T1D.
Materials and methods: *Post hoc* analysis of onset 1, a 26-week, phase 3 trial in which subjects were randomised to double-blind mealtime (0-2 min before the meal) faster aspart or IAsp, both with once- or twice-daily insulin detemir. In onset 1, subjects with previous experience continued carbohydrate counting (baseline HbA_{1c}, faster aspart and IAsp 7.6%) and remaining subjects used a simple bolus algorithm (baseline $HbA_{1c,}$ faster aspart 7.5%, IAsp 7.6%). In this *post hoc* analysis, subjects were grouped by dose-adjustment method.
Results: Faster aspart showed a statistically significant greater reduction in HbA_{1c} vs. IAsp, and non-inferiority was confirmed (Table). With carbohydrate counting, HbA_{1c} reduction was statistically significantly greater for faster aspart vs. IAsp (est. treatment difference: −0.19% [95% CI: −0.30;−0.09]), but was similar for both treatments with the simple bolus algorithm. Rates of hypoglycaemic episodes and bolus insulin dose were similar between treatments across adjustment methods. No significant differences in total insulin dose or weight gain were observed between treatments with either adjustment method.
Conclusion: Faster aspart was effective in glycaemic control regardless of adjustment method. For patients with T1D capable of dosing based on carbohydrate counting, faster aspart may offer improved glycaemic control vs. IAsp, with similar weight gain and insulin dose, and without an increased risk of hypoglycaemia.

Table. Outcome measures after 26 weeks of mealtime faster aspart vs. IAsp for all subjects and by dose adjustment methodology.

		Faster aspart	IAsp	Estimated treatment difference (faster aspart – IAsp), [95% CI]
Change from baseline HbA_{1c} (%)*	All subjects	−0.32	−0.17	−0.15 [−0.23;−0.07]
	Carbohydrate counting	−0.27	−0.08	−0.19 [−0.23;−0.09]
	Bolus algorithm	−0.38	−0.30	−0.08 [−0.21;0.04]
Change from baseline body weight (kg)	All subjects	0.67	0.55	0.12 [−0.30;0.55]
	Carbohydrate counting	0.43	0.48	−0.05 [−0.61;0.50]
	Bolus algorithm	1.03	0.65	0.38 [−0.28;1.04]
		Faster aspart	**IAsp**	**Estimated treatment ratio (faster aspart/IAsp), [95% CI]**
Total daily insulin dose (U/kg)†	All subjects	0.83	0.85	0.98 [0.92;1.04]
	Carbohydrate counting	0.82	0.83	0.99 [0.91;1.06]
	Bolus algorithm	0.84	0.87	0.96 [0.88;1.06]
Daily insulin bolus dose (U/kg)†	All subjects	0.39	0.39	1.00 [0.93;1.07]
	Carbohydrate counting	0.37	0.36	1.02 [0.93;1.12]
	Bolus algorithm	0.43	0.44	0.97 [0.86;1.08]
Severe or BG-confirmed hypoglycaemic episodes/PYE‡	All subjects	58.3	57.9	1.01 [0.88;1.15]
	Carbohydrate counting	53.5	53.3	1.00 [0.84;1.19]
	Bolus algorithm	65.5	65.2	1.00 [0.82;1.23]

*Change from baseline is analysed using a mixed effects model for repeated measures.
†Dose ratios are analysed using a mixed effects model for repeated measures for the log-transformed dose.
‡Hypoglycaemia rates are analysed using a negative binomial model for hypoglycaemic episode count with a log link function, and the logarithm of the exposure time as offset.
For all outcome measures, estimates by treatment are LS mean values derived from the respective models.
BG-confirmed: plasma glucose value <3.1 mmol/L (56 mg/dL)
BG, blood glucose; CI, confidence interval; faster aspart, fast-acting insulin aspart; IAsp, insulin aspart; LS, least squares; PYE, patient-year of exposure.

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Supported by: Novo Nordisk A/S
Disclosure: **A. Philis-Tsimikas:** Stock/Shareholding; Novo Nordisk, Ionis, Gilead. Other; Advisory panel: AstraZeneca, DexCom, Lilly, Merck, Novo Nordisk, Sanofi, DexCom, Janssen, Lilly, Mylan, Novo Nordisk, Sanofi.

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Greater early postprandial suppression of endogenous glucose production is achieved with fast-acting insulin aspart compared to insulin aspart

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Background and aims: Fast-acting insulin aspart (faster aspart) is insulin aspart (IAsp) in a new formulation containing two well-known additional excipients, L-arginine and niacinamide, which result in a stable formulation with accelerated initial absorption after subcutaneous administration. Previous clinical trials have shown that faster aspart provides greater early absorption leading to improved postprandial glucose control compared with IAsp. The aim of the present trial was to investigate the mechanisms behind the lower postprandial glucose achieved with faster aspart versus IAsp.
Materials and methods: In a randomised, double-blind, crossover trial design, subjects with type 1 diabetes (N=40; 21 women/19 men; mean±SD age 42±12 years, BMI 24.1±2.2 kg/m^2, HbA_{1c} 7.3±0.7%) received identical subcutaneous single doses of faster aspart and IAsp (individualised by subject; 0.06-0.28 U/kg) at the start of a standardised mixed meal (containing 75 g carbohydrate labelled with [1-^{13}C] glucose). Postprandial glucose turnover was assessed by the triple-tracer meal method using continuous, variable [6-^{3}H] glucose and [6,6-$^{2}H_2$] glucose infusion.
Results: Early insulin exposure ($AUC_{IAsp,0\text{-}30min}$ and $AUC_{IAsp,0\text{-}1h}$) was greater for faster aspart versus IAsp, leading to smaller postprandial glucose increment at 1 hour (ΔPG_{1h}) (Table). The smaller ΔPG_{1h} with faster aspart was due to greater suppression of endogenous glucose production (EGP) and higher glucose disappearance (ΔAUC_{Rd}) with faster aspart versus IAsp during the first hour post-dose. The greater early suppression of EGP with faster aspart accounted for 78% of the smaller ΔPG_{1h} with faster aspart versus IAsp. Suppression of free fatty acid levels ($AOC_{FFA,0\text{-}1h}$) was 36% greater for faster aspart versus IAsp.
Conclusion: Faster aspart provides improved postprandial glucose control compared with IAsp partly through earlier and greater suppression of endogenous glucose production.

Pharmacokinetic and pharmacodynamic results for faster aspart versus IAsp

Endpoint	Treatment difference Faster aspart-IAsp	Treatment ratio Faster aspart/IAsp	95% CI	P-value
Pharmacokinetics				
$AUC_{IAsp,0\text{-}30min}$		1.93	[1.59;2.34]	<0.001
$AUC_{IAsp,0\text{-}1h}$		1.32	[1.18;1.48]	<0.001
t_{max} (min)	-18.8		[-28.5;-9.0]	<0.001
Plasma glucose				
ΔPG_{1h} (mmol/L)	-0.59		[-1.19;0.01]	0.055
Suppression of endogenous glucose production				
Suppression of $EGP_{0\text{-}30min}$		1.96	[1.13;4.43]	0.017
Suppression of $EGP_{0\text{-}1h}$		1.12	[1.01;1.25]	0.040
Glucose disappearance				
$\Delta AUC_{Rd,0\text{-}1h}$		1.23	[1.05;1.45]	0.012
Free fatty acids				
$AOC_{FFA,0\text{-}1h}$		1.36	[1.01;1.88]	0.042

Suppression of EGP: Calculated as the pre-dose adjusted area over the EGP curve divided by time period (to obtain mean pre-dose adjusted EGP) and then divided by pre-dose EGP.
$AOC_{FFA,0\text{-}1h}$, area over the serum free fatty acid concentration-time curve from 0 to 1 hour; $AUC_{IAsp,}$ area under the serum insulin aspart concentration-time curve; $\Delta AUC_{Rd,0\text{-}1h}$, pre-dose adjusted area under the rate of glucose disappearance-time curve from 0 to 1 hour; ΔPG_{1h}, postprandial plasma glucose increment at 1 hour; EGP, endogenous glucose production; Faster aspart, fast-acting insulin aspart; IAsp, insulin aspart; t_{max}, time to maximum observed concentration.

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Liver-preferential effect of oral basal insulin 320 shows benefit under "overdose" conditions

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Background and aims: Insulin 320 (I320) is an acylated basal insulin analog designed for oral administration. In assessing the safety and mechanism of action of this analog, we examined the response of dogs to an overdose (ie, simulated over-absorption) of I320.

Materials and methods: In order to eliminate variations in bioavailability of I320, it was infused at 40 pmol·kg^{-1}·min^{-1} (basal dose) i.v. for 45 min/day for 3 days before study in all dogs to achieve steady state basal levels. On day 4, ^{3}H-glucose was infused i.v.; after ^{3}H equilibration and basal sampling, dogs were randomly assigned to 1 of 5 groups. Four groups (n=5/group) received a 45-min hepatic portal vein infusion (simulating oral absorption) of I320 at the basal rate or "overabsorption" rates of 80, 120, or 160 pmol·kg^{-1}·min^{-1}. The 5th group (n=3) received regular human insulin (HI) at 24 pmol·kg^{-1}·min^{-1}, a lower dose than the basal I320 dose due to differences in tissue distribution. Sampling continued for 315 min after the insulin infusion ended.

Results: During daily treatments, venous plasma glucose (mM) fell from 5.9±0.1 to 5.4±0.1 (day1), 5.7±0.1 to 5.2±0.1 (day2), and 5.7±0.1 to 5.1 ±0.1 (day3). On day4, basal arterial glucose (6.2±0.1 mM) and endogenous glucose R_a (EndoR_a; 15.0±0.6 µmol·kg^{-1}·min^{-1}) were the same among groups; see Table for glucose parameters during and after insulin infusion. All HI dogs required glucose infusion to maintain arterial glucose ≥2.5 mM, while none of the I320 dogs did. Notably, none of the I320 doses stimulated GlcR_d, while HI increased it >2-fold. Arterial glucagon increased ≤1.8-fold in the I320 groups, while it increased 2.7 fold with HI (P<0.05 vs 40 and 160 I320 doses). Peak arterial cortisol was greater (P<0.05) in HI than in any other group (28±10, 83±16, 157±37, 116±14, and 194±6 nmol/L for 40, 80, 120, and 180 I320 infusion rates and HI, respectively), while peak arterial epinephrine was 752±222, 2943±1800,* 4622±543,* 5251±1646,* and 13280±7472* pmol/L for 40, 80, 120, and 180 I320 infusions and HI, respectively (*P<0.05 vs 40 dose of I320). Arterial glycerol concentrations (reflecting lipolysis) increased dose-dependently in the I320 groups, but HI had the greatest effect (Δ vs. basal: 9±12, 24±18, 51±22, 75±12,* and 112±30* µmol/L with 40, 80, 120, and 180 I320 infusion rates and HI, respectively; *P<0.05 vs 40 dose of I320).

Conclusion: Even the highest dose of I320 did not result in severe hypoglycemia, while all HI-treated dogs required glucose rescue. Over-absorption of I320 during chronic treatment appears to be well tolerated, since the analog stimulates GlcR_d less than HI, allowing more effective counterregulation. Due to the high retention of I320 in the central (blood) compartment, its primary effect appears to be at the liver, which is well equipped to respond to over-insulinization.

Table: 4th day results (mean±SEM) during and after insulin infusion

Insulin infusion (pmol·kg^{-1}·min^{-1})	Arterial glucose nadir (mM)	EndoR_a (µmol·kg^{-1}·min^{-1})		Glucose R_d (peak; µmol·kg^{-1}·min^{-1})	Glucose clearance (peak; ml·kg^{-1}·min^{-1})
		nadir	peak		
40 (I320)	5.7±0.2	12.3±1.3	15.7±1.0	15.5±1.2	2.7±0.1
80 (I320)	4.0±0.5*	10.5±0.9	15.3±0.7	15.8±0.5	3.9±0.4*
120 (I320)	2.9±0.2*	7.1±1.0*	13.6±1.2	15.0±0.9	4.7±0.5*
160 (I320)	2.9±0.2*	7.0±1.2*	14.0±1.3	15.3±1.2	4.8±0.7*
24 (HI)	2.5±0.1*‡	9.6±0.6*	24.2±4.5†	33.3±4.2†	8.8±1.1†

*P<0.05 vs 40 (I320); †P<0.05 vs all other groups; ‡required exogenous glucose for 12-24 min to maintain glycemia ≥2.5 mM

Supported by: Novo Nordisk A/S
Disclosure: **M.C. Moore:** None.

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The effects of local subcutaneous inflammation on insulin pharmacokinetics

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Background and aims: Continuous subcutaneous insulin infusion (CSII) therapy has clinical benefits for diabetes management yet is impacted by an abbreviated 3 day infusion set usage life. As set wear times increase, patients experience altered pharmacokinetic (PK) and pharmacodynamic (PD) profiles, adverse tissue reactions and loss of glycemic control. Previous research has demonstrated that insulin phenolic excipients, m-cresol and phenol, induce acute inflammatory cytokine release *in vitro*. Coupled with inflammation from the indwelling catheter itself, these data build a compelling case for investigating the potential deleterious effect of local inflammation on insulin delivery. This study shows for the first time that local subcutaneous inflammation negatively impacts insulin PK, thereby providing a basis for studying inflammation as a cause of limited CSII set lifetime.
Materials and methods: Female Sinclair swine (n≥5, full crossover design) were given one of two treatments: (1) a 100 μL subcutaneous injection of lipopolysaccharide (LPS) at 10 EU/kg or (2) a 100 μL subcutaneous saline injection (negative control). As a known initiator of the inflammatory cascade, LPS was used to mimic local CSII-induced tissue effects. Twenty-four hours following dose treatment, an insulin PK study was performed by subcutaneously injecting 4 U of U100 insulin lispro into the treated site. Glucose levels were maintained via catheter fluid therapy of 5% dextrose at a rate of 300 mL/hr. Blood samples were taken periodically for six hours following injection, serum separated and analyzed for lispro content via a lispro-specific radioimmunoassay. At the end of the study, biopsies were taken and analyzed via histopathology for inflammatory cell content. PK metrics (C_{max}, t_{max}, AUC and AUC_{60}) and histology data were statistically compared across treatments using either a paired t-test for normally distributed data or a Wilcoxon signed-rank test for non-parametric data ($p \leq 0.05$).
Results: Figure 1 demonstrates the effects of LPS-induced inflammation on subcutaneous insulin PK. C_{max} and AUC_{60} were decreased, while time to reach maximal concentration, t_{max}, was increased in the LPS case ($p<0.05$ for all). These findings suggest that inflammation not only decreases the amount of insulin absorbed, but also slows the rate of insulin absorption. PK changes were accompanied by an increase in nuclei density for the LPS case, confirming inflammatory effect of the treatment. Mechanisms for limited absorption include decreased diffusion in the inflamed tissue as well as degradation of insulin by inflammatory cells.
Conclusion: Here we present for the first time an *in vivo* study demonstrating the effects of inflammation on subcutaneous insulin PK. Results establish that local inflammation induces adverse changes to insulin PK metrics. As shifts in PK/PD and loss of glycemic control are correlated to limited set lifetime, these findings suggest that local inflammation should be further studied as a factor affecting clinical device performance.

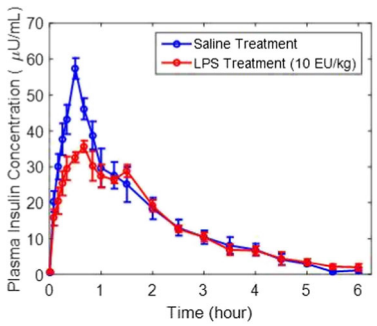

Figure 1: Subcutaneous insulin PK (mean±SEM, n≥5) following site pre-treatment with either 10 EU/kg LPS (red) or saline (blue).

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A meta-analysis comparing glycaemic outcomes with insulin glargine in treat-to-target trials with clinical practice

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Background and aims: The "treat-to-target" approach is a key paradigm in clinical development of novel insulins. Typically, an investigational product and a comparator insulin are titrated aggressively to a predefined uniform glycaemic target over a period of 6 to 12 months, during which safety and efficacy parameters are assessed. However, the glycaemic target used in a treat-to-target trial is almost invariably lower than that commonly used in daily clinical practice, and titration of insulin to reach said target is more aggressive. This meta-analysis was conducted in order to generate a better understanding of potential differences between treat-to-target trials and clinical practice in terms of glycaemic outcomes and insulin dosing.
Materials and methods: A Medline search was conducted focusing on identifying observational studies or real world evidence collected on glargine users. Glycaemic outcomes (HbA1c and fasting plasma glucose (FPG)) as well as insulin doses were recorded and a trial-level meta-analysis was conducted. Similarly, phase III treat-to-target trials of novel insulins (degludec, glargine U300 and LY2605541) were meta-analysed separately. Only trials and English language publications including insulin-naïve patients starting treatment with insulin glargine with a follow-up period of 3 to 12 months were included.
Results: A total of 207 publications were identified for analysis. After exclusion of irrelevant publications and publications lacking data on the key outcomes, 24 publications encompassing a total of 34,648 patients were included in the meta-analysis. The average HbA1c and FPG achieved at the end of the treat-to-target trials in which patients were treated with glargine was 7.12% (54.3 mmol/mol) and 6.4 mmol/l, and the average insulin dose was 0.49 U/kg/day. These outcomes were significantly different from those achieved in the publications based on real world clinical practice, where HbA1c and FPG at end of follow-up was 7.45% (57.9 mmol/mol) and 7.2 mmol/l and the average daily insulin dose was 0.21 U/kg/day.
Conclusion: There are significant and important differences between treat-to-target trials and everyday clinical practice. Patients in treat-to-target trials typically achieve better glycaemic outcomes, but this meta-analysis indicates that the difference may be less pronounced than might have been expected. Furthermore, the improvement in glycaemia should be viewed in the context of a significantly greater amount of basal insulin. The safety and efficacy characteristics of basal insulins in everyday clinical practice may not be readily extrapolated from treat-to-target trials because of differences in insulin dosing and glycaemic outcomes. In order to better understand insulin characteristics in everyday clinical practice, we suggest that drug developers and regulators work together to promote the use of pragmatic randomized insulin trials (PRINT). Combined with evidence from clamp studies and treat-to-target trials, PRINTs would potentially contribute important information to facilitate clinical decision making.
Disclosure: **M. Haglund:** Other; Former employee of Novo Nordisk and Eli Lilly. No current financial relations with either company. This study conducted without financial support from any third party.

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Which C-peptide predicts better the insulin necessity?

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Background and aims: C-Peptide is co-secreted with insulin and is used to evaluate insulin dependency in Diabetes Mellitus (DM). It can be measured in serum (SCP) or urine as concentration (UCP) or total amount in 24h urine (24-UCP). The aim of this study is to evaluate which method better correlates with the need of insulin for treatment in DM.
Materials and methods: We evaluated the measurements of SCP, UCP and 24-UCP performed at our Hospital in 2007-2016. The presence and type of DM were recorded as well as the patients under non-insulin treatments, named insulin independent (II-DM), and patients treated with insulin, named insulin dependent (ID-DM). The total daily units in ID-DM patients was also recorded. Values of $p \leq 0.01$ were considered statistically significant.
Results: A total of 692 C-peptide assays (354 SCP, 166 UCP and 172 24-UCP) were analyzed in 233 patients. Of these, 73 had no diagnosis of DM, 29 were classified as DM-1 and 131 as DM-2. The results of the various methods used are shown in the table I. Mann-Whitney test showed statistically significant differences in ID-DM compared to II-DM patients using the 3 methods ($p \leq 0.01$). The Spearman correlation found a statistically significant correlation between the 3 tests and the total daily insulin units ($p \leq 0.01$; $r = -0.34$; -0.402; -0.316 for SCP, UCP and 24-UCP respectively). The ROC curves were all statistically significant and the areas under curve were 0.72, 0.69 and 0.63 for SCP, UCP and 24-UCP respectively. All patients with SCP, UCP and 24-UCP under 0.4 ng/mL, 12.6 ng/mL and 12.9 μg/24h respectively were treated with insulin.
Conclusion: The value of C-peptide is different in ID-DM patients and correlates with the dose of insulin. SCP seems to be the best predictor of ID-DM and the UCP is the one that best correlates with the dose of insulin administered. The cutoff for the necessity of insulin treatment, at least at our laboratory, seems to be 0.4 ng/mL, 12.6 ng/mL and 12.9 μg/24h for SCP, UCP and 24-UCP respectively.

		SCP (ng/mL)	UCP (ng/mL)	24-UCP (μg/24h)
II-DM	Mean	3,4	54,1	95,9
	Min./Max.	0,4 / 34	12,6 / 181	12,9 / 312,6
ID-DM	Mean	1,8	26,3	57,2
	Min./Max.	0,1 / 16,3	0,1 / 140	0,2 / 442,8
No DM	Mean	1,8	64,9	81,8
	Min./Max.	0,4 / 56	15,2 / 181	12,9 / 235,3
Total	Mean	2.7	37,1	69,2
	Min./Max.	0,1 / 56	0,1 / 140	0,2 / 442,8

Table I – C-peptide values in the different groups studied

Disclosure: **R. Capitão:** None.

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Insulin treated patients titrated to HbA_{1c} target require insulin dose titration every 39 days on average to maintain glucose control

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Background and aims: Effective management of insulin dosing has traditionally proven to be complex and highly individualized. Therapeutic inertia by both providers and patients results in prolonged treatment with non-insulin therapies and presentation of beta cell inadequacy before initiating or intensifying insulin. Standard methods for manual titration of insulin typically follow a one-size fits all approach, with a weight-based or fixed unit starting dose, that increases a fixed amount at set time intervals. It is also unclear as to how frequently patients need dose titrations to sustain improvements in HbA1c or whether conditions, such as the resolution of hyperglycemia-induced oxidative stress, impact a patient's insulin dose after glucose control is achieved. The typical practice is to see a patient back for follow-up every 3-6 months once A1c is controlled. Glucommander is a FDA-cleared and CE marked, integrated, and personalized diabetes therapy management software intended to assist healthcare providers with subcutaneous insulin dose titration by analyzing blood glucose data and calculating patient-specific insulin recommendations for basal, mealtime, and correction doses. Prior studies have demonstrated that Glucommander can assist providers in helping patients safely and effectively achieve their target HbA1c. In one study, 42 patients were titrated to their glucose target range in an average of 11 days and sustained a 2.6% HbA1c improvement over 6 months. Another study of 41 patients showed a 2.7% HbA1c improvement at 3 months. A literature review identified limited evidence to help guide providers in regards to how frequently to review glucoses and titrate insulin after a patient has achieved glucose control. Once patients achieve their target glucose range, Glucommander recommends an increased time interval between interventions with continued remote monitoring with the use of integrated cellular and Bluetooth enabled blood glucose meter partners. The intention of our study was to fill a perceived research gap in regard to insulin titration intervals once a patient has achieved their target HbA1c.
Materials and methods: This retrospective study evaluated 71 patients with both type 1 and type 2 diabetes mellitus, who had their insulin doses adjusted by a healthcare professional who used Glucommander Outpatient to analyze blood glucose data and calculate each dose titration. Patients were on either basal insulin only, or basal and nutritional insulin. We then calculated the average number of days between dose titrations in those patients who reached their A1c target and were followed on Glucommander for greater than 12 weeks.
Results: Patients were managed with Glucommander guided insulin titration for an average of 39 weeks. The average time to achieve target glucose was 12 days; average HbA1c improvement at 12 weeks was 2.6%. After the first three months of treatment, the basal insulin dose was adjusted an average of every 39 days and a nutritional bolus adjustment was made an average of every 38 days.
Conclusion: The current practice of 3-6 month follow-up of a patient after achieving glucose control is insufficient to maintain that control and may put a patient at risk for hypo or hyperglycemia. Our study would suggest that remote glucose monitoring accompanied by insulin titration every 4-6 weeks is ideal in order to maintain adequate glucose control.
Supported by: Glytec
Disclosure: **A.S. Rhinehart:** Employment/Consultancy; Glytec. Stock/Shareholding; Gytec.

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Insulin dependency with and without glucagon: marked difference in insulin requirement between patients with total pancreatectomy and type 1 diabetes

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Background and aims: Patients with total pancreatectomy are completely deprived of not only insulin, but also glucagon. Type 1 diabetes mellitus is also completely deprived of insulin, but glucagon is preserved. Patients with total pancreatectomy and type 1 diabetes are thus similar in absolute insulin deficiency, but different in glucagon, providing unique opportunity to study the contribution of glucagon on glucose metabolism in insulin-dependent state. To investigate the contribution of glucagon on energy metabolism in complete absence of insulin in vivo, insulin requirement and metabolic profiles in patients with total pancreatectomy was studied in comparison with those in type 1 diabetes.
Materials and methods: Subjects studied were 28 individuals with type 1 diabetes and 10 individuals with total pancreatectomy. All individuals were completely deficient in endogenous insulin (fasting CPR < 0.0066 nmol/L) and were hospitalized to optimize their glycemic control with insulin pump. Total, basal and bolus insulin requirements as well as time-

to-time pattern of basal insulin infusion rate were compared between total pancreatectomy and type 1 diabetes.
Results: Insulin requirement in patients with total pancreatectomy were markedly smaller in total daily dose (21.9 vs 35.9 units/day, P=0.003), basal insulin (3.7 vs. 11.4 units/day, P=0.00001) and proportion of basal insulin to total daily dose (16% vs. 33%, P=0.00003) than in type 1 diabetes. In contrast, bolus insulin requirement was comparable between the two groups. Dawn phenomenon as assessed by the difference between the maximum and the minimum insulin infusion rate during night was less remarkable in total pancreatectomy than in type 1 diabetes (P=0.001). Fasting plasma glucagon concentration was markedly lower in total pancreatectomy than in type1 diabetes (P=0.00007), and was positively correlated with basal insulin requirement (P=0.038).
Conclusion: These data indicated marked difference in basal insulin requirement between total pancreatectomy and type 1 diabetes, suggesting the contribution of glucagon to basal insulin requirement and dawn phenomenon.
Supported by: the Ministry of Health, Labor and Welfare, Japan
Disclosure: **Y. Hiromine:** None.

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Efficacy of sensor-augmented insulin pump with predictive low glucose suspension: a multicentre clinical experience in adults and children in Spain

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Background and aims: A sensor-augmented pump with predictive low glucose suspend function (SAP-PLGS), the Medtronic MiniMed 640G®, has been commercially available in Europe since May 2015. The aim of the study was to analyse the effect of SAP-PLGS on glycaemic control in real-life clinical practice in adult and paediatric patients in 4 Paediatric and Diabetes Department at 3 referral hospitals in Spain.
Materials and methods: A retrospective evaluation of all the type 1 diabetes patients treated with SAP-PLGS was performed. Baseline HbA1c and HbA1c at 3, 6, 12 and 18 months were analysed.
Results: 170 patients were included, age: 32 ± 16 years, 48 (28%) patients < 18 years-old, 60% female, diabetes duration: 19 ± 13 years, median time on SAP-PLGS: 6 months [3-12]. 145 patients had at least 3 months of follow-up. Main indication for SAP-PLGS were problematic hypoglycaemia and/or hypoglycaemia unawareness (57%, n = 96), poor glycaemic control (18%, n = 31), high glycaemic variability (14%, n = 23), pregnancy planning (4%, n = 6) and need to improve quality of life (8%, n = 14). 28% (n = 48) of the patients had a previous history of severe hypoglycaemia. Previous treatment is shown in the Table. CSII was funded for all patient, 57 patients (34%) were self-funding the sensors and 113 (66%) were getting reimbursement for them. 7 patients had discontinued SAP-PLGS during follow-up, due to lack of benefit (n = 5), skin reaction (n = 1) and poor compliance (n = 1). In the group of patients with baseline HbA1c > 7% in adults and > 7.5% in children (n = 67), HbA1c was significantly lower than HbA1c before the start of SAP-PLGS at 3 months (7.9 ± 0.6% *vs* 7.5 ± 0.6%, n = 59), 6 months (7.9 ± 0.6% *vs* 7.5 ± 0.6%, n = 53), 12 months (8.2 ± 0.7% *vs* 7.6 ± 0.8%, n = 26) and 18 months (8.3 ± 0.7% *vs* 7.3 ± 0.3%, n = 8), all p = 0.001. Percentage of patients with HbA1c ≤ 7% in adults and ≤ 7.5% in children increased from 45% to 60% at 3 months (n = 45, p = 0.017) and from 57% to 61% at 12 months (n = 57, p = 0.001), without significant differences at 6 months (n = 63) or 18 months (n = 18).
Conclusion: Sensor-augmented insulin pump with predictive low glucose suspension improves glycaemic control in children and adults with type 1 diabetes in a real-world clinical setting.

Treatment prior to SAP-PLGS	n	(%)
CSII + SMBG	69	41
SAP-LGS	52	30
MDI + SMBG	25	15
CSII + FGM	11	7
MDI + CGM	6	3
SAP without LGS	3	2
MDI + FGM	2	1
Diabetes onset (< 20 months-old)	2	1

SAP-PLGS: sensor-augmented pump with predictive low glucose suspend, CSII: continuous subcutaneous insulin infusion, MDI: multiple daily insulin injections, SMBG: self-monitoring of blood glucose, CGM: continuous glucose monitoring, FGM: flash glucose monitoring, SAP: sensor-augmented pump, SAP-LGS: sensor-augmented pump with low glucose suspend

Disclosure: **P. Beato-Víbora:** None.

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Foetal outcomes in pregnancies complicated by type 1 diabetes treated with multiple daily injections of insulin and insulin pumps: a systematic review and meta-analysis

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Background and aims: An adequate glycemic control in pregnant women with type 1 diabetes mellitus (T1DM) constitutes the most important part of diabetes management, minimizing the risk of maternal or foetal complications. Apart from pre-pregnancy care and proper patient education, the key role in pregestational diabetes management constitutes the mode of insulin delivery. Randomized clinical trials has shown the advantage of continuous insulin infusion (CSII) over multiple daily injections (MDI) in the general T1DM population in terms of glycemic control and the reduction in severe hypoglycaemic episodes. However, it is not clear whether there are any differences in fetal outcomes in pregnancies complicated by type 1 diabetes (T1DM) that are treated with MDI and those treated with CSII. Therefore we performed a systematic review based on RCTs (randomized controlled trials) and non-RCTs to compare both methods of insulin delivery in pregestational T1DM.
Materials and methods: The systematic search of major medical databases (PubMed, EMBASE, CENTRAL) was performed to identify original papers regardless of their methodology (randomized and observational) and publication type (full text and conference abstract). Studies met the inclusion criteria if they compared CSII vs. MDI in pregnant women with T1DM. The studies were pooled in the meta-analysis to provide the point estimate with 95% confidence intervals as well as heterogeneity and publication bias assessment.
Results: The systematic search retrieved 38 original papers, including 15 conference abstracts. The majority of studies were open label, nonrandomized controlled trials with high risk of selection bias. Overall, the studies reported on 4499 pregnancies complicated by T1DM. Meta-analysis of available studies has shown that CSII and MDI provide similar results with respect to the risk of perinatal mortality (RR = 0.88 [0.52; 1.47]), congenital malformation (RR = 1.19 [0.90; 1.57]) and percentage of caesarian sections (RR = 1.04 [0.94; 1.15]). The risk of any kind of abortion did not differ significantly between the CSII and MDI group (RR = 1.16 [0.92; 1.46]), however, spontaneous

abortions were observed more frequently in the CSII group (RR = 1.54 [1.05, 2.25]), which could be due to reporting bias as women in the MDI group booked later (8-9 vs 6 weeks) to antenatal clinics and therefore early cases probably were detected less often than in patients in the CSII group. In the CSII group, we observed a higher percentage of neonates with birth weight large for gestational age defined as >90th percentile (RR = 1.20 [1.07; 1.35]) as well as percentage of macrosomic births defined as >4000 g (RR = 1.32 [1.05; 1.65). No significant difference between CSII and MDI was noticed for other neonatal complications, including respiratory distress syndrome, hypoglycemia, hyperbilirubinemia, shoulder dystocia and ICU admissions. No evidence of publication bias was observed.

Conclusion: In conclusion, the use of CSII in pregestational T1DM compared to MDI may increase the risk of macrosomia, but not other fetal complications.

Disclosure: **P. Rys:** None.

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A quality improvement project to reduce the prevalence of insulin omissions among adults with diabetes admitted to a Hospital NHS Trust

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Background and aims: Data from the national diabetes in-patient audit from 2016 reported that insulin errors are observed in 46% (nearly half) of adults patients treated with insulin. The national patient's safety agency issued a patient safety alert in 2011 following a review of patient safety incidents involving insulin over a six years period. Six deaths and 12 incidents resulting in severe harm were reported. 20% were due to drug omission particularly insulin. Inappropriate insulin omission in in-patients can result in serious harm. Insulin should never be stopped due to the risk of complications such as DKA and prolonged in-patient stay. The latest report from the NADIA 2016, showed that in England and Wales, the prevalence of insulin errors is high, we observed similar trend. The aims of our project are to review the prevalence of insulin omission among diabetes patients in our local hospital and to introduce measures to reduce the incidence by 50%.

Materials and methods: This is a 2-phase project, in the first phase; we retrospectively reviewed patient's case-files for all insulin-treated diabetic patients on 4 wards over 3 weeks for baseline data's. In the second phase, we introduced quality improvement pathways aim at reducing insulin omission; this was followed by prospective data collection similar to the first phase. The pathways are: coloured posters on the ward and drugs trolleys informing nursing staffs about insulin omission and implementation of a novel clinical reporting pathway on insulin errors. Data was also collected from a separate medical ward as a control. Result were analysed and the data was compared to control data.

Results: The prevalence of insulin omission on our wards was around 40%, p 0.001, similar to national average. Following quality improvement measures there was a statistically significant 81% decrease in insulin omissions p 0.0001. The control ward showed no statistically significant change in insulin omissions. p 0.117

Conclusion: Insulin omission is national safety issue that could result in serious harm to patients. The most common reason for insulin omission is the fear of hypoglycaemia. Introducing quality improvement measures in our hospital have shown significant reduction in insulin omission, therefore reducing the risk to patients. Similar quality measure could be adapted to other hospitals. Increasing awareness of the importance of timely insulin administration through pathways such as visual posters and educational reminders is very effective and inexpensive tool for reducing inappropriate insulin omission rates.

Disclosure: **V. Oguntolu:** None.

PS 051 Using technology in diabetes: from obesity to closed loop

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Trends in BMI, diabetes and obesity-associated comorbidities after explantation of the Duodenal-Jejunal Bypass Liner

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Background and aims: A novel approach for treatment of obesity and diabetes mellitus type 2 (T2DM) is represented by the temporary endoscopic duodenal-jejunal bypass liner (DJBL). Recent data from the German DJBL registry provided evidence for substantial efficacy of the DJBL during the implantation period in obese patients with T2DM. However, little is known about the trends of comorbidities and BMI after explantation of the DJBL, which have been investigated in the registry in this report.

Materials and methods: For this analysis, patients from the registry were selected if they had a data-set at implantation, explantation and at least one time-point after explantation of the DJBL. We analyzed a group of patients with data at at least 11 months after explantation of the DJBL (n = 35).

Results: HbA1c and BMI re-increased after explantation of the DJBL but stayed significantly below the postexplantation visit as compared to baseline. However, a concomitant increase of the mean number of antidiabetic drugs, which were reduced during the implantation period, was observed. There was a deterioration seen for blood pressure, total cholesterol, LDL-cholesterol and number of daily intake of antihypertensive and lipid lowering drugs over the postexplantation period.

Conclusion: With this data we show that improvement of HbA1c and BMI can be maintained over a time of nearly one year postexplantation of the DJBL. However, for HbA1c this may be biased by intensified medical treatment. However, effects deteriorate with time after explantation. These results suggest that implantation of the DJBL needs to be integrated in a long-term weight management program as most of other interventions in obese patients with T2DM.

Clinical Trial Registration Number: EBRD 001

Supported by: GID

Disclosure: **N. Riedel:** Grants; Establishment of the DJBL registry was supported by an unrestricted grant from GI Dynamics Inc., Lexington, MA, USA (GID).

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Efficacy, safety, tolerability and sustainability outcomes of endoscopic proximal intestinal exclusion with EndoBarrier: 1st UK NHS EndoBarrier service for diabesity

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Background and aims: Our institution leads a UK, multicentre, randomised controlled trial investigating the interaction of Endobarrier

therapy, a 60cm endoscopically implanted proximal intestinal liner, with glucagon-like peptide-1 drug therapy. Our aim was to evaluate whether acquired experience could translate into establishment of an effective and safe UK National Health Service (NHS) Endobarrier service for type 2 diabetes and obesity.

Materials and methods: i) We initiated the 1st NHS Endobarrier service for patients with suboptimally controlled type 2 diabetes and obesity. ii) We primed patients to maintain improvements after, by instituting behaviour changes during, the period of up to 1 year of Endobarrier. iii) We established a secure online registry to monitor outcomes.

Results: Since service initiation 65/153 (42%) referred have been accepted for treatment. 50 patients have had an Endobarrier implanted. 31 patients (see table) have had Endobarrier removed after treatment with the device. Mean±SD HbA1c fell by 24.7±24.1 mmol/mol from 81.4±23.4 to 56.7±11.6 mmol/mol (p<0.001), weight fell by 15.6±9.2 kg from 120.5 ±28.3 to 104.9±29.4 kg (p< 0.001) and systolic blood pressure from 137.3±13.7 to 124.9±15.8 mmHg (p<0.001). Serum alanine aminotransferase (ALT) reduced from 33.5±20.8 to 18.8±10.7U/L (p<0.001). In the 17 patients on insulin, median (IQR) total daily insulin dose reduced from 100 (40-130) to 30 (0-62) units (p=0.003) with 6/17 (35%) discontinuing insulin. Of 17 patients who have reached the 6 months post Endobarrier, 11/17 (65%) had sustained the substantial improvement achieved with Endobarrier. Of the 6 whose weight and/or HbA1c deteriorated after removal, 5/6 (83.3%) had big depression problems. 2/31 (6.5%) patients had early Endobarrier removal, one for gastrointestinal haemorrhage at 10 weeks having not complied with proton pump inhibitor therapy advice and one due to liver abscess at 7 months. Early removal led to resolution in both cases. All other patients achieved a full year of Endobarrier treatment. 93.8% patients would be extremely likely to recommend this service to friends and family.

Conclusion: This inaugural NHS service demonstrates Endobarrier to be highly effective in patients with refractory diabesity, with high patient satisfaction levels and an acceptable safety profile. After Endobarrier removal, 65% were able to sustain the benefits in HbA1c, weight, insulin dose and reduction in liver fat (as suggested by ALT reduction), and they reported considerable improvements in wellbeing, energy, and exercise ability. As endoscopy units are ubiquitous, our service could be readily disseminated, with the registry useful for on-going monitoring worldwide.

Parameter	Baseline	At Removal	Difference	P value (n=31)
Age (years)	51.3±7.3			
Sex (%male)	58.0			
Ethnicity (%Caucasian)	48			
Diabetes Duration (years)	13.3 (8.0-20.0)			
Taking insulin (%)	54.8			
Weight (Kg)	120.5±28.3	104.9±29.4	-15.6±9.2	<0.001
BMI (Kg/m^2)	41.5±8.7	35.8±8.8	-5.7±3.5	<0.001
HbA1c (mmol/mol)	81.4±23.4	56.7±11.6	-24.7±24.1	<0.001
HbA1c (%)	9.6±2.1	7.3±1.1	-2.3±2.2	<0.001
Systolic Blood Pressure	137.3±13.7	124.9±15.8	-12.4±17.1	<0.001
Total Daily Insulin Dose* [median(IQR), n=17]	100(40-130)	30(0-62)	-70	0.003

Table: Baseline characteristics of the 31 patients who completed treatment with Endobarrier along with outcomes at the time of removal as mean±SD or median(interquartile range [IQR]). P-values reflect change from baseline.
Removal at 1 year in 29/31 (94%) patients, with early removal due to complications in 2/31 (6%) patients.

Disclosure: **R.E.J. Ryder:** None.

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Performance of a non-invasive glucose monitoring device for people with prediabetes and type 2 diabetes

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Background and aims: Tight glycemic control is essential for delaying and even preventing the complications associated with type 2 diabetes mellitus (T2DM). Self-monitoring of glucose levels is a key component for achieving this goal. The positive effect of self-monitoring has also been demonstrated on pre-diabetic subjects whose state has deteriorated to T2DM (i.e., newly diagnosed T2DM individuals). Therefore, it is suggested that people with prediabetes may also benefit from self-monitoring and tight glycemic control. However, a major challenge in diabetes management is increasing the limited compliance with self-monitoring. This may be achieved by adopting a non-invasive approach, which aims to alleviate the pain associated with invasive devices. The present study evaluated the performance of GlucoTrack®, a truly non-invasive glucose monitoring device, in all intended use diabetes populations: people with prediabetes, persons newly diagnosed with type 2 diabetes and individuals with long-duration of type 2 diabetes.

Materials and methods: Device performance was evaluated on 32 subjects, including people with prediabetes (N=7), people with newly diagnosed type 2 diabetes (diabetes duration < 5 years; N=9) and people with long-duration type 2 diabetes (N=16). The accuracy of the device was clinically evaluated using Consensus error grid (EG) analysis and numerically evaluated using mean and median absolute relative difference (ARD) and mean absolute difference (MAD).

Results: Overall, 99.6% of 224 points were in the clinically accepted A and B zones of the Consensus EG. Similar proportions were found in the pre-diabetes, newly diagnosed and long-duration type 2 diabetes (100%, 98.4%, and 100%, respectively; Table 1). Mean ARD, median ARD and MAD results for all tested groups are presented in Table 1.

Conclusion: This study demonstrates high accuracy of GlucoTrack device. Clinical and numerical accuracies were comparable between all groups, indicating that the device is suitable for people with long and short durations of type 2 diabetes as well as prediabetes. Due to its non-invasive nature, the device has the potential to enhance compliance of glucose self-monitoring and improve glycemic control, and therefore reduce diabetes-related complications and in certain cases even prevent the development of diabetes in individuals with prediabetes.

Table 1: study results for all tested groups

	Consensus Error Grid			Mean ARD	Median	MAD
	A+B	A	B	(%)	ARD (%)	(mg/dL)
Pre-diabetes	100	93.9	6.1	18.3	13.9	20.5
Newly diagnosed diabetes (up to 5 years)	98.4	92.1	6.3	16.4	14.0	22.8
Long duration diabetes (over 5 years)	100	92.9	7.1	16.6	12.7	25.3

Clinical Trial Registration Number: NCT00889668
Disclosure: **K. Bahartan:** Employment/Consultancy; Integrity Applications Ltd.

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A prospective pragmatic clinical trial in type 2 diabetes to compare the V-Go insulin delivery device with standard treatment optimisation

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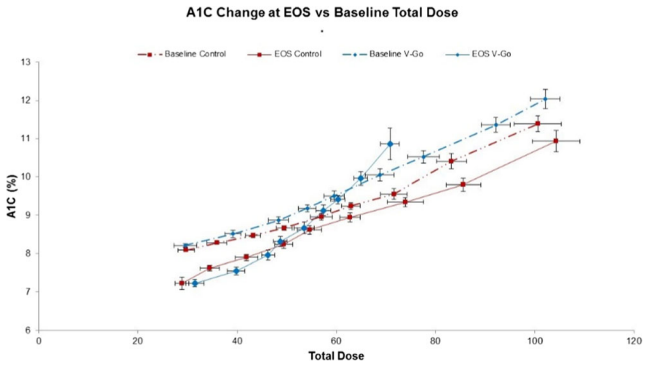
Background and aims: This prospective multicenter clinical study was conducted in a real world community-based practice setting. The study objective was to evaluate patients treated with the V-Go insulin delivery device compared to standard treatment optimization control (STO).

Materials and methods: The trial utilized cluster randomization where study sites rather than individual patients were randomized to V-Go or STO. Patients in the United States with type 2 diabetes taking insulin were enrolled and treated according to routine practice by their healthcare provider for up to 4 months. Patients initializing V-Go stopped using other insulin delivery devices while insulin and other diabetes treatments were continued and optimized in control patients. All treatments, medications and supplies were obtained by usual care utilizing patient insurance. The primary outcome was the difference in the change in A1C from baseline to end of study (EOS).

Results: The analysis population included 415 patients, 246 STO and 169 V-Go across 52 sites. Mean age was 59.8 years and weight was 220.7 lbs (100.1 kg). The study population was 62.4% Caucasian and 25.8% African American. The baseline A1C ranged from 7.9 to 14.2%. Nearly all patients (96.4%) had at least one comorbid condition. There were significant A1C decreases from baseline with V-Go (-0.95%, p<0.001) and STO (-0.46%, p<0.001) and for V-Go vs. STO (p<0.002) despite a significant reduction (p<0.001) in mean total daily insulin dose (TDD) with V-Go. V-Go baseline TDD was 71.3 U/Day (0.76 U/Kg) which decreased to 54.03 U/Day (0.57 U/Kg) at EOS. STO baseline TDD was 72.2 U/Day (0.72 U/Kg) which was unchanged at EOS (71.8 U/Day; 0.72 U/Kg). Baseline A1C was higher for V-Go vs. STO (9.88% vs. 9.34%; p<0.001) indicating a possible selection bias by study investigators randomized to the V-Go group of more advanced diabetes patients initiating V-Go given the a priori randomization of treatment sites. The figure describes the relationship between insulin dose (U/Day) and A1C response. Data were analyzed using a mixed model with site as a random effect. Treatment groups are presented by decile boundaries. The distribution graph shows the higher baseline A1C in the V-Go group compared to SOC at similar insulin doses. SOC had minor change in A1C with minimal change in TDD; V-Go had a resultant shift to lower A1Cs and TDD with the lower A1C deciles nearing guideline A1C goals at a modest TDD.

Conclusion: This prospective pragmatic clinical trial provides evidence of increased glycemic control coupled with decreased total insulin requirements in type 2 diabetes patients initiating V-Go.



Supported by: Valeritas

Disclosure: **M. Cziraky:** Grants; Valeritas. Other; Valeritas.

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Equivalent efficacy of intensive self-monitoring of blood glucose to real time continuous glucose monitoring in adults with type 2 diabetes

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Background and aims: Self-monitoring of blood glucose (SMBG) improves patients' adherence and glycemic control in diabetes. Use of real time continuous glucose monitoring (RT-CGM) in combination with SMBG brings even better glycemic control than SMBG alone. However, effectiveness of RT-CGM compared with more intensive SMBG is not clear. The aim of this study is to analyze the effectiveness of RT-CGM, retrospective CGM and, intensive SMBG in adults with type 2 diabetes.

Materials and methods: This is the interim analysis of the investigator-initiated randomized, open-labelled clinical trial. Patients were type 2 diabetes adults admitted to our hospital from August 1, 2016 to March 2, 2017. Patients of age over 85 years, pregnant, admission less than 1 week, or emergency hospitalization were excluded. Patients were randomized to SMBG with RT-CGM using the Medtronic MiniMed 620G, SMBG with retrospective CGM using Medtronic iPro2®, or SMBG alone. Randomization was performed by computer generated number according to predefined block size. Both CGMs were worn for the maximum period of 6 days. SMBG was done 6 times a day at every pre-meal and 2 hours post-meal. Primary outcome was the change in HbA1c from baseline to 12weeks. As the secondary outcomes, change in mean glucose level from the day of admission to discharge day, and frequency of in-hospital hypoglycemia (glucose level<70mg/dl from SMBG data) were evaluated. We also evaluated patients' satisfaction to treatment using Diabetes Treatment Satisfaction Questionnaire (DTSQ), Problem Area in Diabetes Survey (PAID).

Results: Fifty-seven patients were enrolled and 16 patients were allocated to RT-CGM group, 11 to retrospective CGM group, and 30 to SMBG group. Among these 57 patients, 35 (61.4%) were male, mean age was 65.8±10.8 years, diabetes duration was 7.7±7.6 years, BMI was 26.0±5.2 kg/m^2, and HbA1c was 9.1±1.9%. Overall, glycemic control was improved after treatment for both HbA1c and the mean glucose level; HbA1c from 9.1±1.9% to 6.8±1.1%, mean glucose level from 191 ±63mg/dl to 135±19mg/dl. However, the changes were not significantly different between the groups. (Table1) As the safety profile, hypoglycemia was rarely seen during hospitalization with no difference in frequency between the groups (P=0.54). There were no differences in patients' satisfaction to treatment assessed by DTSQ, PAID at hospital discharge date, either.

Conclusion: Among adults with type 2 diabetes, intensive SMBG may have the equivalent effect to RT-CGM and retrospective CGM.

(Table1)

		Overall (n=57)	RT-CGM (n=16)	Retrospective CGM (n=11)	SMBG (n=30)	Intergroup P value=
HbA1c (%)	baseline	9.1±2.2	9.1±2.2	10.1±2.4	8.7±0.7	
	3 month	6.8±1.1	6.9±0.8*	6.2±0.2*	6.8±1.5*	
	Change	-2.3±2.1	-2.1±2.4	-3.9±3.0	-1.9±1.4	0.41
Mean glucose level (mg/dl)	baseline	191±63	187±61	200±64	190±64	
	discharge	135±19	132±13*	128±16*	139±22*	
	Change	-57±69	-56±62	-73±72	-51±75	0.57
Frequency of hypoglycemia (times /day)		0.04±0.10	0.04±0.09	0.01±0.03	0.04±0.13	0.54
DTSQ	discharge	28.6±5.7	27.7±4.6	28.6±5.5	29.3±6.4	0.54
PAID	discharge	42±16	44±13	51±12	37±17	0.17

*P-value <0.05 vs baseline

Disclosure: **Y. Takano:** None.

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Insulin treatment and glucose patterns of 29,673 patients with diabetes using a tubeless insulin pump system and a cloud-based data management system

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Background and aims: Use of data management systems (DMS) by patients with diabetes can provide valuable insights for clinicians and patients to guide treatment decisions; however, there is little real world data surrounding their use. This study characterized blood glucose (BG) profiles and self-management patterns of a large cohort of patients with diabetes treated with a tubeless insulin pump using a cloud-based and mobile diabetes DMS.

Materials and methods: Anonymized data from patients with diabetes treated with a tubeless insulin pump and ≥3 mo data downloaded from their personal diabetes manager (PDM) to the DMS were analyzed. Analyses were also performed for subsets of patients with CGM use >15 days and with self-reported, de-identified demographic profiles. Variables included mean BG, percent time <70 mg/dL, total daily dose (TDD) of insulin, basal/bolus ratios, frequency of fingerstick BG testing,

percentage using advanced pump features >1x/mo and estimated A1c. Mean sensor glucose and percentage time 70-180 and >180 mg/dL over 24-hr and overnight (20:00 to 8:00) were also examined for CGM users.
Results: Data were available for 29,673 patients from January 2015 through February 2017. The mean±SD BG was 185.8±40.6 mg/dL, time <70 mg/dL 9.0%±11.0, TDD 43.2±21.6 U/d, 53% basal delivery, BG testing 4.2±2.4 x/d, 34.7% advanced pump feature use and estimated A1c 8.1%. Glycemic profiles for CGM users overall (n=2,509, 8.4%) and subsets of CGM patients stratified by age group are presented in the Table. CGM users reported BG testing 4.6±2.5 x/d and 63.8% used advanced pump features. Estimated mean A1c was 7.8%, 7.6% and 7.1% for pediatric, adolescent and adult CGM users, respectively. The type 1 diabetes cohort with self-reported demographic data (n= 4,239, 14.3%) included 25% pediatric, 18% adolescent and 57% adult patients. Overall the type 1 diabetes cohort was age 28.0±18.6 yr, 55% female, mean BG 184.3±39.6, TDD 38.9±19.3 U/d, 50.1% basal delivery, BG testing 4.8 ±2.4 x/d, 42.9% advanced feature use, estimated A1c 8.0%, with insulin pump and DMS use of 28.0 and 11.7 mo, respectively. Patients with type 2 diabetes (n=393) were age 56.8±10.9 yr, 46% female, mean BG 173.3 ±40.5, TDD 57.4±25.4 U/d with 59.8% basal delivery, blood glucose testing 3.4±1.4 x/d, 21.9% advanced feature use, estimated A1c 7.7%, with insulin pump and DMS use of 22.0 and 12.8 mo.
Conclusion: These real world data characterizing a large cohort of patients with diabetes provide insight to self-management patterns and glycemic profiles with current technology use. For patients with type 1 and type 2 diabetes across all age groups the use of a tubeless insulin pump and a cloud-based DMS with or without CGM use was associated with frequent fingerstick BG testing and a high prevalence of advanced insulin pump feature use. The mean BG levels in the cohort overall compared favorably to U.S. national averages with the lowest BG levels observed in the subset of patients using CGM.

Table. Glycemic Profile of the CGM Cohort

	Total (n=2,504)	Pediatric 0 to <13 yr (n=192)	Adolescent 13 to <18 yr (n=83)	Adult ≥18 yr (n=481)
Age, yr	33.4±19.8	8.3±2.7	14.1±1.2	42.7±13.6
TDD insulin, U/d	38.7±19.6	22.8±12.4	43.0±16.2	40.9±19.7
Basal insulin, U/d (%)	19.3±10.9 (50)	9.9±5.7 (43)	19.8±8.6 (46)	21.1±11.0 (52)
Bolus insulin, U/d (%)	19.4±11.7 (50)	13.0±7.5 (57)	23.3±10.0 (54)	19.8±11.2 (48)
Sensor BG, mg/dL	163.5±30.6	177.8±27.7	171.4±29.5	157.1±30.3
Time 70-180 mg/dL, % (24-hr)	61.6±16.5	53.8±13.8	58.3±16.2	64.8±16.6
Time <70 mg/dL, % (24-hr)	3.9±3.9	3.1±3.2	2.5±2.5	4.4±4.1
Time >180 mg/dL, % (24-hr)	33.9±11.9	39.9±11.5	38.0±10.7	31.6±11.6
Time 70-180 mg/dL, % (20:00-8:00)	60.9±17.2	53.7±15.0	58.5±17.1	63.2±18.1
Time >180 mg/dL, % (20:00-8:00)	35.3±18.6	43.5±16.7	39.0±18.2	32.4±19.4
Time <70 mg/dL, % (20:00-8:00)	3.9±4.2	2.8±3.4	2.5±3.4	4.4±4.3

Disclosure: **J.E. Layne:** Employment/Consultancy; Insulet Corporation.

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Frequency of blood glucose monitoring and glycaemic outcomes with adoption of Continuous Glucose Monitoring (CGM)

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Background and aims: Diabetes treatment-related decisions, such as determining an insulin dose, have historically relied on self-monitored blood glucose (SMBG) results. Due to improved accuracy, one CGM system is now indicated (CE Mark and FDA approval) for non-adjunctive use – it can replace SMBG measurements for routine diabetes management decisions. Numerous studies have associated CGM use with reductions in both hypoglycemia and HbA1c. In six of these studies (Table), SMBG frequency was found to decrease with CGM use. However, none of the studies reported the association of SMBG reduction and glycemic benefit or risk. We examined the relationship between the extent of SMBG frequency reductions and glycemic outcomes in the DIaMonD Study.
Materials and methods: The DIaMonD randomized controlled trial introduced CGM to subjects who were using multiple daily injections. In this group, there were 175 subjects (99 with T1D, 76 with T2D) for whom SMBG meter data were available at baseline and followup. Data from this study were retrospectively analyzed to relate reductions in SMBG testing frequency and changes in HbA1c. We compared HbA1c changes observed with larger vs. smaller reductions in SMBG frequency using an analysis of covariance model adjusted for baseline HbA1c and clinical site as a random effect.
Results: Subjects' mean±SD baseline HbA1c was 8.6±0.6% and age was 52±14 years. Baseline SMBG frequency was 4.7±1.6/day and decreased to 3.3±1.5/day at week 24, a 30% reduction. Mean HbA1c reduction from baseline was similar in the 75 subjects who reduced their SBMG frequency by >1 test/day compared to the 100 subjects who reduced it by ≤1 test/day (-0.9 percentage points in both groups; p=0.36). Further, when comparing subjects in the top quartile of SMBG reduction (mean decrease of at least 2.2 tests/day; n=43) to subjects in the lowest quartile of SMBG reduction (mean decrease of no more than 0.4 tests/day; n=43), mean HbA1c reduction was similar (-0.9 percentage points in both groups; p=0.56). Findings were similar in participants with T1D and T2D (p=0.39). There was only 1 severe hypoglycemic event in each SMBG group (SMBG reduction of >1 and ≤1 test/day).
Conclusion: These data demonstrate that reduction of SMBG frequency with CGM use does not impact glycemic control, suggesting that nonadjunctive use of CGM does not diminish the safety and effectiveness of CGM.

Study Name	Reference	Population	CGM System	Duration of CGM use	SMBG reduction
DirecNet	Diabetes Care 31:525	n=27, T1D	FreeStyle Navigator	3 mo	41% (p=0.16)
EVADIAC	Diabetes Care 35:965	n=178, T1D	FreeStyle Navigator	12 mo	16% (p<0.001)
SWITCH	Diabetologia 55:3155	n=153, T1D	Paradigm	6 mo	11% (p<0.001)
ASPIRE In-Home	NEJM 369:224	n=247, T1D	Veo, Revel	3 mo	10%
GLADIS	Diabetic Med. 32:609	n=160; T1D and T2D	FreeStyle Navigator	100 days	>50% (p<0.0001)
GOLD	JAMA 317:379	n=161, T1D	Dexcom G4P	26 weeks	27%

Clinical Trial Registration Number: NCT02282397
Disclosure: **T.C. Walker:** Employment/Consultancy; Dexcom.

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Efficacy and safety of an insulin decision support system in the treatment of steroid hyperglycaemia in patients with graft-versus-host-disease: a randomised pilot trial

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Background and aims: Graft-versus-host-disease (GvHD) is a common and life-threatening complication in patients undergoing allogeneic stem cell transplantation. Since high-dose systemic glucocorticoids are the established first-line therapy, about half of these patients develop steroid-induced hyperglycaemia, which has been associated with adverse outcome in this patient population. Adequate glucose control in these patients proved to be challenging. Over the last decades, decision support systems (DSS) were developed to standardise and facilitate insulin treatment for patients during hospitalisation. To date, there is still only sparse data available on the performance of decision support systems in patients with steroid-induced hyperglycaemia. We aimed to investigate whether glucose control with an electronic algorithm-based decision support system for insulin therapy (GlucoTab®) is effective and safe in this patient group.

Materials and methods: We randomly assigned 10 patients with acute GvHD and hyperglycaemia (2 independent fasting glucose values >7.8 mmol/L) while receiving corticosteroid therapy to either an electronic algorithm-based decision support system (GlucoTab®, GT) or conventional treatment (CON) according to local standards. Glycaemic control was assessed by frequent capillary blood glucose (BG) measurements during hospital stay. Follow-up period was 6 months.
Results: Mean total daily insulin dose was 36.8 ± 28.0 U (GT) vs 11.6 ± 12.0 U (CON). Mean daily prednisolone or equivalent dose was 74.1 ± 44.3 mg (GT) vs. 95.2 ± 65.3 mg (CON). The percentage of glucose values within the target range (3.9-10.0 mmol/L) was 68.2% for GT vs. 64.2% for CON. Numbers of hypoglycaemic events (BG <3.9 mmol/l) were 3 (GT) and 30 (CON) (p=0.026), respectively.
Conclusion: Insulin treatment guided by GlucoTab® was effective and safe in GvHD patients with steroid induced hyperglycaemia during hospital stay. As compared to standard care, the DSS based regimen showed a higher percentage of values in target range and was associated with lower hypoglycaemia rates. Further optimisation of the algorithm for high-dose steroid treatment is about to be processed. To explore whether good glycaemic control might also improve patients' outcome, larger studies are needed in this Population.
Disclosure: **F. Aberer:** None.

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Carbohydrate-to-insulin ratio: a major factor that could influence daytime glucose control of a hybrid closed-loop system

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Background and aims: The MiniMed™ 670G hybrid closed-loop (HCL) system was designed to automatically adjust basal insulin delivery every 5 minutes based on sensor glucose (SG) values and insulin delivery history. The system includes the MiniMed 670G pump, Guardian® Sensor 3, and a proprietary control algorithm. During the HCL pivotal trial, subjects needed to calibrate the sensor, notify the system of exercise, and enter mealtime carbohydrate estimates in order to deliver meal-boluses. The HCL pivotal trial data was analyzed to study the effect of a more aggressive carbohydrate-to-insulin (CHO:I) ratio (i.e., more insulin for a given carbohydrate estimate) on daytime glucose control during the study period.
Materials and methods: A ten-center pivotal trial was conducted with 124 type 1 diabetes patients. The study protocol consisted of a 2-week Manual Mode (run-in phase) followed by a 3-month in-home Auto Mode (study phase) of HCL control. Percentage of daily insulin administered as meal bolus (which is a reliable indicator of the aggressiveness of meal bolus) and SG data during the daytime period (6:00am-11:59pm) was analyzed.
Results: The figure represents the daytime mean SG value with respect to the percentage of daily insulin administered as meal bolus, during the study period. A Pearson's correlation coefficient shows a significant (p<0.001) inverse correlation between daytime mean SG value and percentage of daily insulin administered as meal bolus (R=−0.5), thus demonstrating a higher daily percentage of meal bolus due to the more aggressive CHO:I ratio resulting in lowered daytime mean SG values.
Conclusion: The post-hoc analysis of subjects in the HCL pivotal trial demonstrates a significant effect of a more aggressive CHO:I ratio on automatic insulin delivery that impacts overall daytime glucose values. Overall, the 670G system was safe for unsupervised at-home use by adults and adolescents. Additionally, the system resulted in a significant decrease in HbA1C levels, especially when the initial HbA1C at baseline was >7.0%.

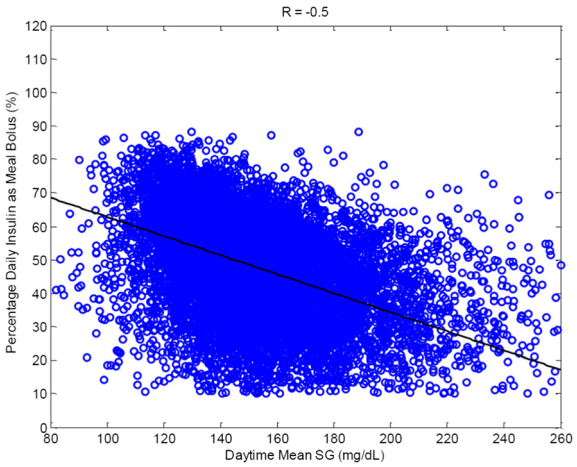

Figure: The x-axis represents daytime mean sensor glucose values and the y-axis represents percentage of daily insulin administered as meal bolus. The blue circles represent each day in the study period for all the 124 subjects. The black solid line is a linear fit of the data.

Clinical Trial Registration Number: NCT02660827
Disclosure: **A. Roy:** Employment/Consultancy; Employee and stock holder of Medtronic plc.

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Determining duration of closed-loop studies: evidence from a randomised day-and-night three month trial

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Background and aims: Randomised clinical trials of closed-loop insulin delivery have ranged from a single day to 3 months. The time required by closed-loop systems to achieve stable glucose control including behavioural adaptation to take place is currently unknown. We addressed this aspect by comparing outcomes after 1 week, 4 weeks, and 12 weeks (reference) of day-and-night closed-loop insulin delivery.
Materials and methods: Analysis was performed retrospectively on data from an open-label, three-centre, multi-national randomised two-period crossover day-and-night hybrid closed-loop study. Thirty-three adults with type 1 diabetes on insulin pump therapy (age 40.0±9.4years, duration of diabetes 20.9±9.3years, baseline HbA1c 8.5±0.7%, duration of pump therapy 7.8±5.9years) were recruited and underwent automated hybrid closed-loop for 12 weeks within a randomised clinical trial. Sensor-based glucose outcomes during closed-loop intervention after 1 week and 4 weeks were compared with outcomes after 12 weeks using paired samples T-tests or Wilcoxon signed rank tests.
Results: Compared with 12 weeks, there was no difference in time spent hypoglycaemia <3.9 mM at 1 and 4 weeks (Table). In contrast, a small increase in time spent <3.0 mM was noted between 4 and 12. Nadir of mean glucose and time spent >10mM were shown at 1 week, whilst modest increase noted at 4 and 12 weeks. Total daily insulin and time in target were not statistically different between time points.
Conclusion: Glucose control settles rapidly after start of closed-loop, with comparable outcomes at 1, 4 and 12 weeks. An increase in mean glucose and time spent >10mM of borderline clinical significance was

present at 12 weeks compared to 1 and 4 weeks. A trend towards more time spent hypoglycaemia <3.0mM at 12 weeks was observed although absolute percentages were low. We conclude that closed-loop trials of 4 weeks may be sufficient to provide representative data for majority of glucose outcomes, but assessment of time spent hypoglycaemia <3.0mM may warrant longer trial duration. System use over longer periods may change which warrants longer studies to take place.

Table: Closed-loop performance over 12 weeks (reference) versus 1 week, and 12 weeks (reference) versus 4 weeks

	12 weeks	1 week	P Value[1]	4 weeks	P Value[2]
Percentage of time with sensor glucose					
3.9 to 10mM	67.7±9.2	69.6±11.6	0.091	68.6±11.6	0.078
>10mM	29.2±11.4	27.0±12.4	0.050	28.4±11.6	0.12
<3.9mM	2.9 (1.4, 4.5)	2.9(1.4, 4.9)	0.64	2.5(1.5, 4.6)	0.64
<3.0mM	0.4 (0.1, 1.0)	0.2 (0, 1)	0.33	0.2 (0.1, 0.8)	0.04
Mean glucose (mM)	8.7± 1.1	8.5±1.1	0.014	8.6±7.6	0.059
Total daily basal Insulin (U/day)	48.8±16.1	47.3±16.2	0.091	47.9±15.6	0.219

[1] Paired Samples T-test or Wilcoxon Signed Ranks Test between 12 weeks and 1 week
[2] Paired Samples T-test or Wilcoxon Signed Ranks Test between 12 weeks and 4 weeks

Clinical Trial Registration Number: NCT01961622
Supported by: Seventh Framework Programme of the European Union (ICT FP7- 247138)
Disclosure: **L. Leelarathna:** None.

PS 052 CGM in type 1 diabetes

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FreeStyle Libre use for self-management of diabetes in children and adolescents

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Background and aims: The impact of using the FreeStyle Libre™ Flash Glucose Monitoring System has been reported in adults with diabetes. This study aimed to evaluate the use of FreeStyle Libre as a replacement for self-monitoring of blood glucose (SMBG) in young people (4-17 years) with type 1 diabetes (T1D).
Materials and methods: Patients (n=76, 58% CSII users, 46% males) enrolled in a 10 week single arm European study (UK, Ireland and Germany) and underwent 2 weeks baseline masked (blinded) wear, followed by 8 weeks open use according to labelling. The primary endpoint was non-inferiority of glycaemic control assessed using time in range. Patients were aged 10.3±4.0 years (mean±SD) with baseline HbA1c 62.9±11.1 mmol/mol (7.9 ±1.0%), T1D duration 5.4±3.7 years and self-reported SMBG tests 7.3±2.7/day.
Results: Time in range (3.9-10.0 mmol/L) significantly improved vs. baseline by 1.0±2.8 hours/day (mean±SD), p=0.0056. HbA1c significantly improved vs. baseline, -4.4±5.9 mmol/mol (-0.4±0.6%), p<0.0001. Time in hyperglycaemia (>10.0 mmol/L) significantly reduced vs. baseline by -1.2±3.3 hours/day, p=0.0038, while no statistically significant changes were observed for time in hypoglycaemia (time <3.9 mmol/L, baseline, 1.1±1.2 hours/day). Total daily insulin dose increased by 1.4 ±3.5 units (p=0.0010). Scan frequency of FreeStyle Libre was on average 12.9 times daily, whereas SMBG tests dropped from a median of 8.0 (baseline) to 1.0 per day during open use. Diabetes Treatment Satisfaction Questionnaire showed increased overall treatment satisfaction for parents (n=70), 21.7±6.6 (mean change score±SD), p<0.0001 and teens (13+years) (n=23), 18.7±5.6, p<0.0001. Occurrences of mild or moderate sensor insertion site symptoms were as expected with device use (96 from 42 patients). Three device-related adverse events (AEs), all mild (dry collection, dry flaky skin and redness) were reported by 3 patients. There were no device-related serious AEs.
Conclusion: Use of FreeStyle Libre as a replacement for SMBG by children and adolescents with T1D improved glycaemic control, reduced HbA1c with no change in hypoglycaemia and enhanced treatment satisfaction.
Clinical Trial Registration Number: NCT02821117
Supported by: Abbott Diabetes Care
Disclosure: **F. Campbell:** None.

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Intermittent scanned glucose (FGM) in pediatrics with typ 1 diabetes: correlation with traditional metabolic control parameters, a multicentre DPV analysis of 476 patients

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Background and aims: The method of flash glucose monitoring (FGM) is a novel alternative to finger-prick blood glucose measurements (SMBG).Current FGM devices provide actual glucose values, trend arrows and a retrospective continuous curve of interstitial glucose during the last 8 hours. Combining anonymized profiles with registry data allows real-world analysis of the impact of this new technology in pediatric patients, an age-group characterized by instable glucose values.
Materials and methods: The DPV-Register ("Diabetes-Patienten-Verlaufsdokumentation"),a longitudinal surveillance of patients in Austria, Switzerland, Luxemburg and Germany since 1995, has implemented a method to upload glucose profiles from real-time CGM and FGM devices.In this cross-sectional analysis, FGM data are compared to traditional parameters of glycemic control. Until March 20 2017, we documented flash glucose profiles from 476 patients with T1DM (74,517 total days, 134.9 days per patient). Median age of patients was 12.1 years (Q1-Q3: 8.8 - 14.9),duration of Diabetes 3.4 years, 54.0 % male, 67.2 % with insulin pump therapy (CSII)
Results: Median glucose value was 176.5 mg/dl (9.8 mmol/L). Time in range: 50.9% of individual glucose values were in-between 70 and 180 mg/dl (3.9 and 10 mmol/L), 2.2% <54, 6.2 % < 70, 42.9 % > 180 (<3.0, <3.9, >10). In 20.9 % of the documented time, interval glucose was > 250 mg/dl (> 13.9 mmol/L),in 3.1 % of the time even above 400 mg/dl (22.2 mmol/L).The estimated HbA1c from these profiles was 7.8%, the corresponding median from blood based measurement 7.1 % (Spearman-correlation: r=0.66). n=320 of these patients were on insulin pump therapy, n=156 had MDI. Median Glucose was similar in patients with CSII or MDI (176mg/dl - 9.8 mmol/L vs. 179 mg/dl - 9.9 mmol/L p=0.92 ; Time in Range 50.7% vs. 51.3%, p=0.87). During adolescence (age >= 12 years, n= 245) FGM profiles demonstrated significantly worse glucose control: median glucose 183.4 versus 171.6 mg/dL, time-in-range 47.2 % versus 54.6%.No significant differences between boys and girls were present.On weekends, median glucose values were slightly higher than during weekdays: 179 vs. 176 mg/dl (9.9 vs. 9.7 mmol/L), p<0.001; time-in-range 50.6% versus 51.2 %, p<0.001).
Conclusion: Intermittent continuous measurement of tissue glucose allows a more in-depth analysis of glycemic control and variability.Subgroup analysis revealed clear differences between age-groups, as well as according to treatment modality.Correlation to measured HbA1c seems to be lower compared to adult patients, probably reflecting the lower stability of metabolic control in pediatric patients.Real-world data will help to determine the value of FGM/CGM profiles to improve clinical and patient related outcomes in pediatric diabetology.
Supported by: Abbott
Disclosure: **T. Biester:** Lecture/other fees; Medtronic.

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Prediction quality of glucose trend indicators in current tissue glucose monitoring systems for use in therapeutic decisions

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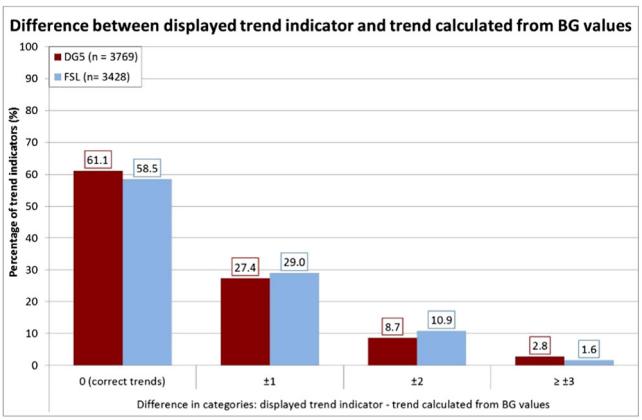
Background and aims: Tissue glucose monitoring systems (TGMS) typically show glucose curves in addition to current glucose readings, and they often indicate glucose trends (e.g., trend arrows). When using TGMS for diabetes therapy, therapeutic decisions may not only be based on glucose readings alone, but also on glucose curves and trend indicators. In this investigation, the quality of trend indicators of 2 TGMS Dexcom® G5® (DG5) and FreeStyle Libre (FSL) was assessed in 2 different ways.
Materials and methods: Over a span of 14 days, each of 20 patients wore 2 sensors of each TGMS in parallel. Because DG5 is indicated for 7 days wear time, it was routinely replaced after approx. 7 days. On 3 separate occasions, patients visited the study site for 48 h each. On average, 115 blood glucose (BG) measurements were performed per visit with a FreeStyle Freedom Lite system. In parallel to each BG measurement, trend indicators of all TGMS were recorded. DG5 and FSL showed 7 and 5 different trend categories, respectively. On average, a difference of 1 trend category equals to a rate-of-change difference of more than 1 mg/dl/min. In analysis A, the trend displayed by the TGMS was compared to the trend calculated from the continuously stored, linearly interpolated TGMS values over a 15-minute interval. DG5 and FSL stored values every 5 and 15 min, respectively. In analysis B, the trend displayed by the TGMS was compared to the trend calculated from BG measurements with approx. 1 BG measurement per hour. In both analyses, glucose rate of change (based on TGMS or BG values) was calculated and converted to trend indicators according to the respective TGMS labeling.
Results: In analysis A, the displayed trend did match the trend calculated from TGMS values in 63.2%/62.1% (DG5/FSL) of cases. Trends indicating non-stable glucose were mostly overestimating rate of change compared to the calculated trend: When the displayed trend was negative, the indicated rate of change was mostly more rapidly decreasing than the calculated rate of change, whereas when the displayed trend was positive, the indicated rate of change was mostly more rapidly increasing than the calculated rate of change. A difference of at least 2 trend categories was found in 9.5%/8.4% (DG5/FSL) of cases. In analysis B, the displayed trend did match the trend calculated from BG values in 61.1%/58.5% (DG5/FSL) of cases. Again, trends indicating non-stable glucose were mostly overestimating calculated rate of change. A difference of at least 2 trend categories was found in 11.4%/12.5% (DG5/FSL) of cases (see Figure).
Conclusion: More than a third of displayed trend indicators in this study were found to be different from the trend calculated from TGMS or BG values, and slightly more than 10% of trend indicators differed from the calculated BG trend by at least 2 categories. When therapeutic decisions are based on TGMS readings, it should be kept in mind that displayed trends may differ from actual glucose changes.



Supported by: Roche Diabetes Care GmbH
Disclosure: **S. Pleus:** None.

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Increase in continuous glucose monitoring among adults with type 1 diabetes: international comparison from the T1D Exchange and the DPV initiative

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Background and aims: In previous analyses, only a small minority of adults with T1D in the T1DX and DPV registries were using continuous glucose monitoring (CGM). In the past five years, new generations of CGM have shown considerable improvements in accuracy and usability. In this analysis we assessed increase in CGM use over the past 5 years, and compared glycemic control (HbA1c %) among current CGM users and non-users by insulin delivery status (pump or injection) in the US T1DX and Germany/Austria DPV registries.

Materials and methods: Registry data from 15,831 adult patients (age ≥ 18 years) with diabetes duration ≥ 1 year and registry updates from January 1, 2016 to December 31, 2016 (8090 participants from 72 T1DX sites, and 7741 participants from 365 DPV sites) were compared with data collected from both registries on 19,225 participants between September 1, 2010 to August 1, 2012 for T1DX (N=10,956) and between January 1, 2011 to December 31, 2011 for DPV (N=8269). CGM use (including both real-time and intermittent scanning CGM) and most recent HbA1c at each data collection time point were obtained from clinic medical records.

Results: Frequency of CGM use among T1D adults increased from 9% in 2011 to 11% in 2016 in DPV and from 11% in 2010-2012 to 26% in 2016 for T1DX. Increase in CGM use was observed across all age groups for T1DX but only in the young adult (18-<26 years) age group for DPV (Table). In 2016 CGM use was higher among insulin pump users in T1DX vs. DPV (35% vs. 12%, P<0.001) but similar among injection users (12% vs. 10%, P=0.01). For DPV mean HbA1c was lower among Pump+CGM (N=350, HbA1c 7.7%) users compared with injection+ CGM (N=460, HbA1c 8.1%, P=0.009), pump only (N=2538, HbA1c 8.0%, P=0.008) and injection only (N=4032, HbA1c 8.0%, P=0.002) users. For T1DX mean HbA1c in 2016 was lower among both injection+CGM (N=333, HbA1c 7.8%) and Pump+CGM (N=1616, HbA1c 7.6%) users compared with pump only (N=2944, HbA1c 8.1%, P<0.001) and injection only (N=2448, HbA1c 8.6%, P<0.001) users.

Conclusion: CGM use has increased dramatically among adults with T1D in T1DX over the past few years likely due to improved CGM performance but less so in DPV with the exception of the young adult age group. CGM use was associated with improved glycemic control in both pump and injection users for T1DX but only in pump users for DPV. The differences in CGM uptake and glycemic control among CGM users between the two countries may be reflective of differences in registry populations, availability of devices, provider beliefs, diabetes education and CGM training practices and insurance coverage.

	DPV		T1DX	
	2011 N=8269 # (%) CGM Use	2016 N=7741 # (%) CGM Use	2010-2012 N=10956 # (%) CGM Use	2016 N=8090 # (%) CGM Use
Overall	9%	11%	11%	26%
Age Group				
18-<26 years	5%	12%	4%	18%
26-< 50 years	12%	12%	15%	33%
50-<65 years	11%	9%	16%	33%
≥ 65 years	8%	8%	10%	23%
Insulin Delivery Method				
Injections	8%	10%	5%	12%
Pump	11%	12%	16%	35%

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Disclosure: **K. Miller:** None.

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Glycaemic outcomes in subjects with and without prior CGM experience, in the MiniMed™ 670G system pivotal trial

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Background and aims: At-home use of the MiniMed 670G hybrid closed-loop system by 124 adolescents and adults, for 3 months, reduced HbA1c and increased overall percentage of sensor glucose (SG) values in target range (71-180 mg/dL), compared to a 2-week baseline run-in phase. Per protocol, both the run-in and study phases required use of the system's continuous glucose monitoring (CGM) feature. Given complexities associated with CGM onboarding, glycemic outcomes of subjects with and without prior CGM experience were evaluated.

Materials and methods: Study subjects (adolescents: n=30, aged 14-21 years and adults: n=94, aged 22-75 years) had T1D for ≥2 years, HbA1c <10%, and used insulin pump therapy for >6 months with or without CGM experience. Exploratory analyses between the run-in and study phases for subjects with previous CGM experience (Prior CGM) and those without previous CGM experience (No Prior CGM) were conducted. Outcomes included: HbA1c, the percent of overall SG values across ranges (≤50 mg/dL, ≤70 mg/dL, 71-180 mg/dL, >180mg/dL and >300mg/dL) and within-day variability of SG values (SD and CV).

Results: From run-in to study phase for adolescents, mean HbA1c fell from 7.5±1.0% to 7.0±0.6% and from 8.0±0.6% to 7.1±0.6% for Prior CGM (n=16) and No Prior CGM (n=14), respectively. The mean percent of in-target SG values increased from 62.2±11.8% to 68.6±8.8% and 58.4±9.8% to 65.5±7.5% for Prior CGM and No Prior CGM, respectively (Table). For adults, HbA1c dropped from 7.3±0.9% to 6.9±0.7% and 7.3±0.8 to 6.7±0.4 for Prior CGM (n=62) and No Prior CGM (n=31), respectively. The percent of in-target SG values increased from 68.1±12.5% to 73.6±8.9% and 70.1±10.7% to 74.2±7.6%, respectively. Comparisons of entire study phase SG values across ranges and within-day variability of SG values, between cohort Prior CGM and No Prior CGM groups, revealed no significant differences (Table, p-values). The change in HbA1c between Prior CGM and No Prior CGM groups also did not differ (adolescents: p=0.1953, adults: p=0.4052, Wilcoxon rank-sum test).

Conclusion: Use of the MiniMed 670G System, at home, improved HbA1c and in-target SG values regardless of study participant prior CGM experience. These findings suggest that the system may benefit current CGM, as well as CGM-naive, users.

	Adolescents Prior CGM (16)		Adolescents No Prior CGM (14)		Prior CGM vs No Prior CGM (Study Phase only)	Adults Prior CGM (62)		Adults No Prior CGM (32)		Prior CGM vs No Prior CGM (Study Phase only)
	Run-in	Study	Run-in	Study	p	Run-in	Study	Run-in	Study	p
Percent of sensor glucose values across ranges, mg/dL										
≤50	0.6±0.5	0.6±0.5	0.7±0.7	0.4±0.3	0.1934[a]	1.1±1.3	0.7±0.7	1.0±1.0	0.6±0.5	0.9778[a]
≤70	4.0±2.3	3.0±1.5	4.5±3.6	2.5±0.9	0.2313	6.1±4.4	3.5±2.3	7.0±4.0	3.3±1.7	0.9210[a]
71-180	62.2±11.8	68.6±8.8	58.4±9.8	65.5±7.5	0.3099	68.1±12.5	73.6±8.9	70.1±10.7	74.2±7.6	0.9842[a]
>180	33.8±12.5	28.3±8.3	37.0±10.1	32.0±7.4	0.2141	25.9±14.2	22.9±9.5	22.9±11.8	22.6±7.8	0.9336[a]
>300	4.1±5.3	2.6±1.9	3.4±2.9	2.9±2.2	0.8218[a]	1.9±4.4	1.4±1.8	1.7±3.4	1.3±1.5	0.6538[a]
Variability of sensor glucose values										
Within-day SD of SG, mg/dL	56.4±11.7	51.3±8.2	56.5±10.1	51.5±7.4	1.0000[a]	48.3±8.9	45.2±6.8	47.8±8.6	45.1±6.2	0.9618
Within-day CV of SG, %	34.9±4.3	32.6±3.5	34.6±5.2	31.7±2.5	0.4502	32.9±4.2	30.3±3.3	33.6±3.9	30.4±3.1	0.8683

The run-in phase duration was 2 weeks and the study phase duration was 3 months.
Values are shown as mean±SD.
SD=Standard deviation.
CV=Coefficient of variation.
[a]Wilcoxon rank-sum test.

Clinical Trial Registration Number: NCT02660827

Disclosure: **S.W. Lee:** Employment/Consultancy; Medtronic. Stock/ Shareholding; Medtronic.

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Impact of continuous glucose monitoring (CGM) on hypoglycaemia in type 1 diabetes adults on multiple daily insulin injections (MDI)

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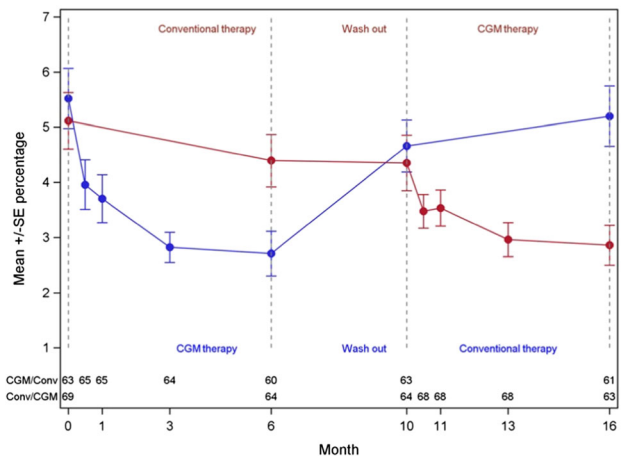
Background and aims: Conventional intensive insulin therapy helps decrease HbA1c but with increased risk of hypoglycaemia. Recent studies show that CGM can decrease HbA1c for persons treated with MDI but how this affects hypoglycaemia is not fully understood. The aim of this study was to evaluate the impact of CGM on hypoglycaemia in persons with type 1 diabetes (T1D) treated with MDI and evaluate hypoglycaemic confidence.
Materials and methods: Evaluations were performed from the GOLD study, an open-label multicentre cross-over randomized trial (n=161) over 69 weeks comparing CGM to self-measurement of blood glucose (SMBG) in persons with T1D treated with MDI. Masked CGM and the Hypoglycaemic Confidence Scale (HCS) were used for evaluations.
Results: Mean age was 43.7 years, 45.3% were female, mean HbA1c was 70mmol/mol and was reduced by 5mmol/mol (p<0.001) more by CGM than SMBG. The hypoglycaemia levels used were <3mmol/L and <3.9mmol/L. The mean time spent in nocturnal hypoglycaemia (00-05:59) for SMBG was 8.9 min (95% CI 6.3-11.5 min) and 19.2 min (95% CI 15.1-24.2 min) respectively. For CGM the mean was 3.1 min (95% CI 1.9-4.2 min) and 10.2 min (95% CI 7.9-12.5 min) respectively. For daytime hypoglycaemia (06:00-23:59) the corresponding values for SMBG were 18 min (95% CI 14.3-21.9 min) and 48.8 min (95% CI 41.3-56.4 min). For daytime hypoglycaemia with CGM the corresponding values were 8.2 min (95% CI 5.8-10.6 min) and 29.5 min (95% CI 23.5-35.4 min) respectively. All differences were significant at significance level, p< 0.001. The improvement on time spent in hypoglycaemia disappeared when CGM was not used (figure 1). The total HCS score improved significantly from 3.27 to 3.40 (p<0.001) during CGM treatment and when analysed separately 4 out of the 9 HCS items improved significantly: staying safe from serious problems with hypoglycaemia when in social situation (p = 0.016), confidence in catching and responding in time to hypoglycaemia (p = 0.0033), avoiding serious problems due to hypoglycaemia (p = 0.002), and continue to do the things you really want despite the hypoglycaemia risk (p = 0.022).
Conclusion: CGM reduces time spent in nocturnal and daytime hypoglycaemia in T1D adults on MDI. CGM also increases confidence in avoiding hypoglycaemia and problems related to them.

Figure 1 Mean percent time (+/-SE) of glucose levels below 3.9 mmol/l per treatment sequence over time



Clinical Trial Registration Number: NCT02092051
Supported by: Dexcom Inc. funded parts of the study
Disclosure: **A.F. Ólafsdóttir:** Non-financial support; CGM systems from Dexcom Inc.

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CGM combined with either MDI or CSII is superior to standalone MDI or CSII in type 1 diabetes: 2 years of follow-up in the COMISAIR study

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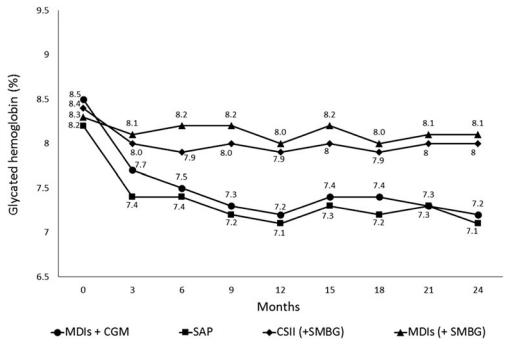
Background and aims: To compare different treatment modalities for patients with type 1 diabetes (T1D) based on one of real-time continuous glucose monitoring (RT-CGM) or self-monitoring of blood glucose (SMBG) in combination with one of multiple daily injections (MDIs) or continuous subcutaneous insulin infusion (CSII).
Materials and methods: In this prospective, real-life study, 65 patients with T1D were followed up for 2 years. Of these, 27 started to use RT-CGM; within this CGM group, 15 subjects started sensor augmented pump (SAP) therapy and the remaining 12 continued with MDIs (MDIs + RT-CGM). A second group of 20 patients initiated CSII without RT-CGM, while a third group of 18 subjects continued on MDIs and SMBG. At the baseline, all subjects were monitored by blinded CGM and underwent a structured 4-day training program. In addition, those groups of patients without RT-CGM were monitored for a week by blinded CGM every 3 months and again at the end of the study. The main endpoints were reduction of HbA_{1c}, glycemic variability (GV), and incidence of hypoglycemia.
Results: After 2 years, the baseline mean HbA_{1c} in the CGM group (8.3%) decreased to 7.1% (p<0.0001); both CGM subgroups, SAP and MDIs + RT-CGM, showed comparable improvement. In contrast with the CGM group, at the end of the study no significant reduction of HbA_{1c} was seen either in the MDIs or CSII groups. Improvement of HbA1c in the CGM group was superior to MDIs (p=0.002) and CSII (p=0.002). Importantly, the superiority of a CGM strategy in comparison with CSII alone held not just for SAP, but also for MDIs +RT-CGM; the MDIs + RT-CGM vs CSII comparison favoured the former by -0.86% (95% CI, -1.5% to -0.22%; p<0.01). GV was also lowered both in the CGM (p<0.0001) and CSII (p<0.05) groups. Reduced incidence of hypoglycemia was observed only with CGM (8%±4% vs. 6%±3%; p< 0.01).
Conclusion: To the best of our knowledge, this is the first prospective, 2-year real-life study simultaneously comparing four different treatment strategies based on different combinations of insulin delivery and monitoring systems. In our study, both CGM modalities, SAP and MDIs + RT-CGM, provided significant and comparable decrease of HbA1c with concurrent reduction of hypoglycemia. This improvement was greater than that seen with CSII. The combination of RT-CGM and MDIs can be a suitable alternative to SAP for some patients.



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Disclosure: **J. Šoupal:** None.

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Flash glucose monitoring in over 50,000 users: a favourable relationship between frequency of testing and glycaemic measures

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Background and aims: Optimising glycaemic control in diabetes involves a combination of reducing high glucose levels while avoiding hypoglycaemia. Our aim was to assess the use of flash glucose monitoring (FreeStyle Libre®) in real life clinical practice across European countries. In particular, glucose testing frequency and relationship with glycemic parameters was analysed together with regional differences in exposure to hyper and hypoglycaemia.
Materials and methods: When flash glucose readers are connected to an internet ready PC, 90-day memory of the device is de-identified and uploaded onto a database after user's consent. For analysis, sensors were required to have at least 120 hours of operation, and all sensors were grouped per reader, resulting in 50,831 readers with 279,446 sensors (86.4 million monitoring hours by 63.8 million scans). Six regions were identified, five countries having the highest device use (Germany, Spain, France, UK and Italy), and a sixth "region" grouped all remaining. Scan rate per reader was determined and twenty equally-sized rank-ordered groups, categorized by scan frequency, were evaluated. Glucose scan frequency was analysed together with relationship to glycaemic markers in each of these regions.
Results: Average scan frequency was 16.3/day [median (IQR) of 14 (10-20)] but this varied significantly across regions. Highest scan frequency was found in the UK at 18.0 [15 (11-23)] while individuals in France showed the lowest scan rate at 13.6 [12 (8-17); p<0.001]. All countries demonstrated strong correlations between frequency of glucose scans and glycaemic markers. Time spent in hyperglycemia (>10 mmol/l) was reduced from (mean±SD) 10.5±5 to 5.9±5 hours/day (p<0.001) in lowest compared with highest frequency scanning groups, while time in hypoglycaemia (<3.1 mmol/l) was reduced from 43±60 to 26±47 minutes/day (p<0.001). However, hypoglycaemic exposure showed regional differences with individuals in France spending the longest time in hypoglycaemia 58±65 to 40±62 minutes/day in lowest and highest frequency scanning groups, respectively, whereas patients from Italy spent the least time in hypoglycaemia 33±59 and 20±35 minutes/day in the lowest and highest frequency scanning groups. Increased scan frequency was also associated with longer time spent in range (defined as 3.9-10.0 mmol/l) and lower estimated HbA1c.
Conclusion: In real-world clinical practice, frequency of glucose testing with the flash monitoring system is high across countries, although regional differences were observed. Increased scan frequency is universally associated with reduced time spent in both hyper and hypoglycaemia but total exposure to low glucose levels showed differences between countries. These findings have implications for the use of flash glucose monitoring to improve glycaemia, whereas the documented regional differences warrant further research.
Supported by: Abbott Diabetes Care
Disclosure: **R.A. Ajjan:** Grants; Abbott Diabetes Care. Honorarium; Abbott Diabetes Care.

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Type 1 diabetes at high altitude: performance of medical devices

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Background and aims: High altitude trekking can expose people to extreme environmental conditions, like low temperatures and hypobaric hypoxia. Such extreme conditions make it more difficult for people with type 1 diabetes mellitus (T1DM) to achieve glycemic control. While frequent blood glucose monitoring is imperative in these cases, it remains unclear whether altitude impacts blood glucose meter accuracy. In this observational study, we examined the performance of various insulin pumps at high altitude, including continuous glucose monitoring (CGM) systems and a new flash glucose monitoring (FGM) system (FreeStyle Libre).
Materials and methods: All 19 patients with T1DM included in this study participated in trekking Damavand Mountain (Iran) to an altitude of 5671 meters above sea level. The mean age of the patients was 32.5 years, with a mean body mass index (BMI) of 23.8 kg/m^2, and a mean HbA_{1c} level of 6.6%.
Results: Statistical analysis showed a difference in blood glucose values obtained using the different glucose monitoring systems at day 1 (mean BGM values vs. mean FGM values, 137 vs. 169 mg/dl, p=0.0000), day 2 (mean CGM values vs. mean FGM values, 163 vs. 219 mg/dl, p=0.0014; and mean BGM vs. mean FGM values, 181 vs. 219 mg/dl, p=0.02), and day 3 (mean CGM values vs. mean FGM values, 202 vs. 264 mg/dl, p=0.0035; and BGM vs. FGM, 187 vs. 218 mg/dl, p=0.0000). The SmartGuard technology of insulin pump Minimed 640G (used by 6 patients during expedition) was activated on average 3.3 times per patient per day. We found that, without extreme weather conditions, high altitude trekking is safe for insulin pumps and CGM/FGM systems and causes no clinically significant problems. All pump models worked well without any disruption, and no cases of diabetes decompensation or severe hypoglycemia occurred.
Conclusion: To conclude, despite the risks, healthy, physically fit and experienced individuals with type 1 diabetes can be encouraged to participate in mountain trekking activities and attain their summit goals. Modern personal insulin pumps and continuous glucose monitoring systems appear to work properly even at high altitudes at least in the absence of extreme winter conditions.

Table 1. Glucose values during the three expedition days obtained via the different glucose monitoring methods.

	Mean BGM values ± SD [mg/dl]	Mean CGM values ± SD [mg/dl]	Mean FGM values ± SD [mg/dl]	Number of blood glucose measurements per day ± SD [mg/dl]
Day 1 (3200–4200 m.a.s.l.)	153 ± 33	153 ± 19	168 ± 36	12.4 ± 4.3
Day 2 (4200–4700 m.a.s.l.)	183 ± 40	163 ± 19	219 ± 42	12.4 ± 6.4
Day 3 (4200–5671 m.a.s.l.)	202 ± 31	202 ± 30	264 ± 45	14.6 ± 7.6
P value	0.0004	0.0004	0.00005	0.48

BGM, blood glucose measurement with a glucometer; CGM, continuous glucose monitoring; FGM, flash glucose monitoring.

Supported by: Sanofi, Bayer, Ascenscia Diabetes Care, Diabetyk24.pl, Vitrum, PTD
Disclosure: **T. Klupa:** None.

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Positive clinical outcome of Belgian reimbursement of real-time continuous glucose monitoring for type 1 diabetes patients on insulin pump therapy

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Background and aims: Improving glycemic control, while reducing hypoglycemia, has been achieved in randomized controlled trials evaluating real-time continuous glucose monitoring (RT-CGM) in patients with type 1 diabetes (T1D). However, studies investigating real-life use of RT-CGM are sparse, even though they are of major interest to healthcare providers. This study evaluates the impact of RT-CGM reimbursement on glycemic control and quality of life of patients with T1D treated with continuous subcutaneous insulin infusion (CSII) therapy, followed in one of 17 high volume diabetes centers.
Materials and methods: Over a period of 12 months, the observational RESCUE trial analyzes the impact on predefined clinical outcome parameters of a Belgian RT-CGM reimbursement system in patients with T1D on CSII. Data were collected during standardized clinical follow-up and from questionnaires (SF-36, Problem Areas In Diabetes-Short Form, Hypoglycemia Fear Survey-II) between September 2014 and December 2016. The primary endpoint was defined as evolution of HbA1c from baseline to 12 months. Secondary outcome measures included evolution from baseline to 12 months of hypoglycemic events, quality of life and hospitalization rate for severe hypoglycemia and ketoacidosis. Data are reported as median (interquartile range) unless otherwise stated.
Results: 515 adult patients entered the study of which 421 (82%) used RT-CGM for at least 12 months and 52 (10%) terminated RT-CGM earlier, mainly because of alarm fatigue. The majority of patients used Medtronic sensors (75%), mostly with low glucose suspend features (74%), while others used Dexcom (24%) or Abbott Navigator (1%) sensors. Patients were 41 years (32 - 51) old, 59% female, had diabetes for 21 years (14 - 30) and 47% had complete or partial hypoglycemia unawareness. HbA1c at baseline was 7.5% (7.1 - 8.2) (58 mmol/mol [54 - 66]) and decreased to 7.3% (6.8 - 7.9) (56 mmol/mol [51 - 63]) at 12 months (P<0.001). Patients in the highest HbA1c quartile dropped from 8.7% (72 mmol/mol) to 8.0% (64 mmol/mol) at 12 months (P<0.001), with simultaneous reduction of 26% (-5 - 49) in glucose values < 70 mg/dL (3.9 mmol/L) (P<0.001). For patients in the lowest HbA1c quartile, hypoglycemic glucose values also dropped by 36% (11 - 56) at 12 months (P<0.001), while maintaining an unchanged HbA1c level. Quality of life improved significantly (P<0.001) in all but two sub measures of SF-36 (bodily pain and vitality). One year before reimbursement, 16% of patients were admitted to the hospital for severe hypoglycemic or ketoacidosis episodes in contrast to 3% (P<0.001) the year after, corresponding to a decrease in hospitalization days from 54 to 19 days per 100 patient-years.
Conclusion: Reimbursement of RT-CGM in patients with T1D on CSII results in lower HbA1c and decreased risk of hypoglycemia under real-life conditions and leads to fewer diabetes-related hospitalizations and improvement in quality of life.
Clinical Trial Registration Number: NCT02601729
Disclosure: **S. Charleer:** None.

PS 053 Hypoglycaemia: identifying people at increased risk

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Impact of genetic polymorphism in the beta-2-receptor gene on the risk of severe hypoglycaemia in patients with type 1 diabetes

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Background and aims: The frequency of severe hypoglycaemia in T1 diabetes is not equally distributed and a fraction of the patients tends to have recurrent severe hypoglycaemia which makes a genetic influence probable. A potential candidate is the beta-2-receptor gene (ADRB2) that has several common polymorphisms and of which the Arg^{16} allele is associated with agonist-mediated down-regulation. This could be of importance for attenuation of symptoms, counter regulation and promotion of severe hypoglycaemia after repeated hypoglycaemia in patients with T1 diabetes.
Materials and methods: A cohort of 314 patients with T1 diabetes was recruited in an outpatient clinic. Severe hypoglycaemic events were reported retrospectively in a validated questionnaire. The patients were further characterized by diabetes history, degree of hypoglycaemia awareness (Clarke, Gold, and Hillerød methods), C-peptide status, haemoglobin A1c (HbA1c), and determination of ADRB2 genotype.
Results: The ADRB2 Gly16Arg genotype distribution was in Hardy-Weinberg equilibrium and was similar to the distribution in the general population. There was no association between genotype and degree of awareness. There was a significant difference in rate of severe hypoglycaemia between all genotypes (p=0.011) and patients homozygous for the Arg^{16} genotype (AA, n=60) had a relative rate of severe hypoglycaemia of 2.19 (95% CI 1.31 - 3.64) compared to patients homozygotes for the Gly^{16} genotype (GG, n=117) (p=0.003). In patients with impaired awareness the difference was even more pronounced with a relative rate of severe hypoglycaemia of 3.18 (95% CI 1.67 - 6.04) in patients with the AA genotype (p<0.0001). The difference was not explained by other risk factors (HbA1c, diabetes duration, C-peptide).
Conclusion: Genetic polymorphism in the beta-2-receptor gene is associated with risk of severe hypoglycaemia in patients with T1 diabetes, especially in those with impaired hypoglycaemia awareness.
Disclosure: **K.Z. Rokamp:** None.

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Hypoglycaemia awareness and the risk of severe hypoglycaemia in the global HAT study of 27,585 patients from 24 countries with type 1 and type 2 diabetes

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Background and aims: Impaired awareness of hypoglycaemia (IAH) is an important predictor for severe hypoglycaemia, particularly in type 1 diabetes (T1D). IAH and its effects are largely unknown outside Europe and North America. The global Hypoglycaemia Assessment Tool (HAT) study aimed to assess hypoglycaemia incidence and awareness in patients with T1D and type 2 diabetes (T2D) in Northern Europe, Canada, Eastern Europe, the Middle East, Latin America, South East (SE) Asia and Russia.
Materials and methods: Hypoglycaemia awareness was assessed at baseline using a validated method based on the question: 'Do you have

symptoms when you have a low sugar level?'. The answer 'always' implied normal awareness, 'usually' impaired awareness, 'occasionally' and 'never' unawareness. Severe hypoglycaemia was reported in 6-month retrospective and 1-month prospective periods.

Results: In T1D 45% declared normal awareness, 39% impaired awareness and 17% unawareness. People with unawareness had the highest estimated annual incidence rates (IRs) for severe hypoglycaemia both retrospectively and prospectively (Table). Patients with unawareness were older (47.6 years), had longer diabetes duration (21.3 years), and lower HbA_{1c} (62.3 mmol/mol) than those with awareness. Regionally, in T1D, the association between severe hypoglycaemia and IAH was most pronounced in Eastern Europe (retrospective IRs: always 1.36; usually 1.71; occasionally 2.18; never 3.08), but an inverse association was uniquely observed in SE Asia (retrospective IRs: always 2.90; usually 1.92; occasionally 0.91; never 0.27). In T2D, 45% reported normal awareness, 30% impaired awareness and 25% unawareness. Slightly lower IRs for severe hypoglycaemia were observed with reduced awareness particularly in the retrospective period (Table). In contrast to T1D, patients with unawareness were younger (60.6 years) with a shorter duration of diabetes (12.9 years) and insulin use (5.2 years) and had higher HbA_{1c} (65.1 mmol/mol) than those with awareness. In T2D, an inverse association of that seen in T1D was observed in all regions apart from Russia, where there was no association.

Conclusion: The HAT study shows the expected prevalence of hypoglycaemia unawareness and its association with increased risk of severe hypoglycaemia in T1D in some but not all parts of the world. In T2D, an unexpected higher prevalence of unawareness was found and an inverse association with severe hypoglycaemia. Further research is needed in order to explore factors associated with awareness across regions and why contrasting associations occur in T2D.

Table. Severe hypoglycemia in the overall population reported in retrospective and prospective periods.

Period	Hypoglycaemia Awareness	T1D		T2D	
		N (%)	IR	N (%)	IR
All regions retrospective (6 months)	Normal	3501 (23.4)	1.87	6713 (19.8)	1.30
	Impaired	2990 (26.7)	2.00	4592 (22.1)	1.21
	Unawareness	1296 (77.1)	3.40	3972 (23.1)	0.66
All regions prospective (4 weeks)	Normal	3097 (13.0)	4.30	6284 (9.7)	2.90
	Impaired	2631 (14.8)	4.79	4394 (12.2)	3.04
	Unawareness	1170 (42.2)	6.81	3972 (12.9)	2.48

IR, incidence rate; N, number of patients in the hypoglycaemia awareness class; T1D, type 1 diabetes; T2D, type 2 diabetes; %, number (%) of patients having a severe hypoglycaemic event

Clinical Trial Registration Number: NCT01696266
Supported by: Novo Nordisk A/S
Disclosure: **U. Pedersen-Bjergaard:** None.

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Impaired awareness of hypoglycaemia and severe hypoglycaemia impact on executive functions, hypoglycaemic symptoms, quality of life and distress in type 1 diabetes adults

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Background and aims: Impaired awareness of hypoglycaemia (IAH) increases risk of severe hypoglycaemia (SH) 6-fold in adults with type 1 diabetes (T1D). We hypothesised that IAH and SH may be associated with executive dysfunction, increased distress, and reduced quality of life (QoL).

Materials and methods: We recruited 85 adults with T1D. Hypoglycaemia awareness status was determined using the 1-item Gold Score (GS) and the 8-item Clarke score (CS), with ≥4 = IAH for each. SH was evaluated over two different time frames (6 and 12 months) by 2 questions (3 and 4, respectively) in the CS. Participants also completed the Edinburgh Hypoglycaemia Scale (assessing autonomic, neuroglycopenic, and nonspecific hypoglycaemic symptoms); the Montreal Cognitive Assessment (MoCA, global cognition: visuospatial abilities, executive functions, language, delayed recall, and orientation); the INECO Frontal Screening (IFS, executive functions: inhibition and set shifting, abstraction, and working memory); the Diabetes Health Profile (DHP, QoL: barriers to activity [BA], psychological distress [PD], and disinhibited eating [DE]); and the Hospital Anxiety and Depression Scale (HADS). Mann-Whitney U test was used to compare mean ranks of groups: those with intact awareness of hypoglycaemia (AH) *vs* IAH; SH *vs* noSH; SH&AH *vs* noSH&AH.

Results: Participants' mean±SD age and diabetes duration were 38.4±12.5 and 19.1±11.7 years, respectively; 54.1% were male; 31.8% had IAH by GS and 16.5% by CS. 86% of IAH patients by CS *vs* only 41% with IAH by GS had had SH in the past 6 months (CS question 3), with 50% *vs* 19% reporting seizure, or parenteral therapy for SH in the last 12 months (CS question 4). IAH patients by CS had significantly ($p<0.05$) lower performance on MoCA executive functions domain, higher neuroglycopenic symptoms when hypoglycaemic, more BA, and depression than AH patients by CS. No differences were found between IAH and AH assessed by GS. Furthermore, patients with SH in the past 6 months had significantly worse performance on MoCA executive functions domain, and higher neuroglycopenic symptoms, BA, psychological distress, anxiety and depression than those with noSH. Patients with SH in the past year had worse performance on MoCA language domain, and higher neuroglycopenic symptoms and higher depression scores than noSH patients (all $p<0.05$). SH&AH patients had higher BA and depression than those with noSH&AH. Also, SH&AH had worse performance on MoCA executive functions domain ($p=.004$), and motor series ($p=.034$), conflicting instructions ($p=.009$) and Go-No-Go ($p=.007$) IFS subtests, tending to show poorer performance on IFS total scores ($p=.058$).

Conclusion: Adults with T1D and either IAH or SH show evidence of impaired executive functions, depression, neuroglycopenic symptoms when hypoglycaemic, and higher BA. The evidence for impaired executive functioning (programming, interference sensitivity, inhibitory control), higher depression and BA related to SH, even in people with intact awareness, suggest that SH may be necessary for these dysfunctions. The contribution of IAH on executive dysfunction needs to be further explored.

Disclosure: **E. Sepúlveda:** None.

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Unhelpful cognitions about hypoglycaemia in adults with type 1 diabetes

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Background and aims: Severe hypoglycaemia (SH) is a barrier to optimising glycaemic control and continues to affect a significant proportion of people with Type 1 Diabetes (T1D). Growing evidence suggests that cognitive factors play a role in the ability to avoid SH. We explored these using the Attitudes to Awareness Questionnaire (A2A) in a cross sectional survey of the US T1D Exchange Clinic Registry adult population. The A2A was developed from qualitative research into hypoglycaemia beliefs in hypoglycaemia-prone adults with T1D in the UK.

Materials and methods: The A2A was sent to 6200 adults in the T1D Exchange Clinic Registry. Each of the 14 A2A questionnaire items (rated

on a likert scale) relates to an unhelpful belief about hypoglycaemia. The structure of the A2A was examined using exploratory factor analysis with eigenvalues >1.0 and factor loadings >0.3 used in interpretation. Responses were compared between those with and without impaired awareness of hypoglycaemia (IAH) and those with and without SH.
Results: 1978 responses were received (response rate 32%) from participants, mean age 40±16 yrs, 62% female, T1D duration 23±14yrs, HbA1c 7.8±1.4%; 62% female, 37% had IAH; 14% recurrent SH (≥2 episodes of SH in past year). Of those with IAH, 25% had recurrent SH. Data supported the use of factor analysis (Kaiser-Meyer-Olkin = 0.785, Bartlett's test of sphericity χ^2 (91) 3399.71 p<0.001). A forced 3-factor analysis generated 3 strong factors, explaining 40.1% of the variance. Parallel analysis confirmed the retention of 3 factors. The first factor (eigenvalue: 3.12, Cronbach's α=0.64) explained 22.3% of the variance in responses and comprised 5 items relating to minimising concern about negative effects of SH. It was labelled Minimising Hypoglycaemia. The second factor (eigenvalue: 1.31, α=0.49) explained 9.4% of the variance and comprised 5 items describing cognitions relating to excessive emphasis on avoiding high blood glucose. It was labelled Hyperglycaemia Prioritised. The third factor (eigenvalue: 1.17, α=0.51) explained 8.4% of the variance and contained 4 items. Its theme was a lack of concern about hypoglycaemia in the absence of symptoms and was labelled Asymptomatic Hypoglycaemia Normalised. Differences in prevalence of factors were found by awareness status and frequency of SH. Those with IAH minimised concern about hypoglycaemia less (p<0.001) and prioritised avoiding hyperglycaemia more (p=0.002) than those with intact awareness. Those with recurrent SH minimised concern about hypoglycaemia less (p<0.001) and normalised asymptomatic hypoglycaemia more (p=0.019) than those without recurrent SH.
Conclusion: This study identified 3 distinct unhelpful beliefs which appear to present a barrier to reducing hypoglycaemia risk. Prevalence of the beliefs varied with awareness status and frequency of hypoglycaemia. These problematic beliefs may have utility to identify people at high risk of recurrent SH and could represent areas to address in supporting those at risk to protect themselves from SH.
Disclosure: **A. Cook:** None.

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Under-reported hypoglycaemia: detection of burden and clinical interventions in a real-life setting using text mining and electronic health records

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Background and aims: Appropriate glycaemic control is a foundation of type 2 diabetes (T2DM) management while presence of treatment-induced hypoglycaemic episodes (HEs) and anxiety linked to or fear of HEs may limit proper use of therapies targeting to reduce the risk of progressive vascular complications. Detection of subclinical and potentially under-reported HEs in patients with T2DM is challenging in the absence of frequent blood glucose monitoring or patient but also, physician awareness. We explored the real-world burden of HEs, the rate of subclinical HEs and presence of symptoms indicative of potentially undiagnosed HEs, and clinical interventions using computerised electronic health records (EHRs).
Materials and methods: A convenience population of 347,348 patients from the Northeast states of U.S., and associated 2 million longitudinal records from year 2010 to 2013 with clinical documentation and structured data from the EHRs were used. An analytics funnel for comparison of subgroup combinations of HEs was created using standard database query and natural language processing techniques of 20 pre-defined descriptive signs or symptoms and 14 projected, different physician intervention options related to HE management. The difference in action taken and presence of pre-defined symptoms was assessed between those with and without documented HEs.
Results: The EHR population comprised of 7.8% (n=27,175) patients with a diagnosis of T2DM of those, 5.6% (n=1,522) had documented hypoglycaemia: 91.8% with symptoms and, of those, 84.8% led to an intervention. Yet, out of 25,653 subjects with no documented hypoglycaemia, 80.8% reported at least one symptom of potentially under-reported HEs and, of those, 65.7% had a documented intervention. Subjects with potentially under-reported HEs had a mean age of 61.8 years and multiple comorbidities: 19.8% had renal impairment, 38.8% a history of acute myocardial infarction, and approximately 20.0% had congestive heart failure or hypertension. The subjects were mostly treated with insulin (43.8%), biguanides (metformin) (25.7%) or sulphonylurea (23.6%). Pre-defined potential indicators of HEs such as chills, confusion, tachycardia, dizziness, hunger, sleepiness, blurred vision, numbness, headache, weakness, lack of coordination and seizure were documented more often among those with a documented HE. These symptoms of HEs also more often lead to a therapy switch (OR=1.6; 95% CI: 1.4, 2.0), change in dose or frequency adjustment (OR=2.3; 95% CI: 1.9, 2.7), a consultation (OR=1.6; 95% CI: 1.4, 1.8), or a referral to a diabetes educator (OR=2.2; 95% CI: 1.3, 3.6).
Conclusion: In our real-world dataset, the prevalence of confirmed HEs among the patients with T2DM was around 5-6%. Pre-defined symptoms of HEs lead more often to an intervention when the HE had been well-documented while the same symptoms frequently occur in patients with comorbidity- or therapy-related established risk factors for HEs. Providers with modern and well-supported EHR systems worldwide have potential for proactive detection, testing and documentation for HEs. Quality surveillance of the patient records can alert providers and also include suggestions for an intervention in cases where symptoms occur which are indicative of HEs.
Supported by: Novartis
Disclosure: **P.M. Paldánius:** Employment/Consultancy; Novartis Pharma AG. Stock/Shareholding; Novartis.

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HbA_{1c} and occurrence of severe hypoglycaemias in patients with type 1 diabetes. Results from the disease management programmes in North Rhine-Westphalia, Germany

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Background and aims: To prevent the incidence and progression of complications caused by diabetes is one of the central aims in the therapy of type-1-diabetes, so a tight glycaemic control is intended. But tight glycaemic control can increase the risk for severe hypoglycaemias. Therefore the association between HbA1c and the occurrence of severe hypoglycaemias was analysed within a population of patients inscribed in disease management programmes (DMP) for type-1-diabetes.
Materials and methods: All analyses are based on documentations of a total of 44 995 patients with type-1-diabetes who were participating in 2014 in the DMPs in the German federal state of North Rhine-Westphalia. In a subgroup of 41 924 patients documentation with regard to the incidence of severe hypoglycaemias was available. Stratified by age and sex HbA1c values and frequencies of severe hypoglycaemias, i.e. cases when patients needed external help and / or an intravenous glucose injection, are presented. Predictors for the incidence of severe hypoglycaemias were estimated by logistic regression analysis (odds ratios, OR, and 95-percent confidence intervals, CI 95%, are given).
Results: In 48.4% of the patients HbA1c is <= 7.5% (58.5 mmol/mol) and in 23.4% of the patients HbA1c is > 8.5% (69.4 mmol/mol). In the time period from early adolescence until approximately the age of 30 low HbA1c levels are reached seldom. From the age of c. 50 yrs. female patients show higher HbA1c levels than male patients. In 2014 severe hypoglycaemias are documented in n = 1.601 (3.8%) of the patients, most frequently in the n = 8 356 patients > 60 yrs. (n = 389, 4.7%). Prevalence

of severe hypoglycaemias is associated with the HbA1c level in 2013 (<= 7.5% = 4.9%; > 10% / 85.8 mmol/mol = 2.9%). Previous severe hypoglycaemias are the most significant predictor of severe hypoglycaemias in 2014 (OR = 10.6; 95-%-CI = 9.3-12.0). As well risk for severe hypoglycaemias increases when diabetic complications (OR = 1.4; 95-%-CI = 1.3-1.6) or cardiovascular comorbidities are documented (OR = 1.2; 95-%-CI = 1.0-1.4). Risk of severe hypoglycaemias decreases when the HbA1c level in 2013 was > 8.5% (OR = 0.6, 95-%-CI = 0.5-0.8) or in case of a body-mass-index >= 30 kg/sqm (OR = 0.8; 95-%-CI = 0.7-0.9).

Conclusion: Approximately half of the patients with type-1-diabetes reach HbA1c levels recommended by clinical guidelines. Nevertheless in around 4% of these patients severe hypoglycaemias are documented in 2014. The strong association found between the previous and the current incidence of severe hypoglycaemias confirms results which have been published earlier by other authors.

Disclosure: **B.K. Hagen:** None.

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Can CGM discriminate between type 1 diabetes patients with and without severe hypoglycaemia. Results of the baseline assessment from the HypoDe study

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Background and aims: Patients with type 1 diabetes on a multiple daily insulin injections (MDI) regimen and hypoglycaemia issues were eligible for the HypoDE study. Hypoglycaemia issues were defined as having suffered a severe hypoglycaemic (SH) episode (third party assistance for recovery needed) during the past 12 months or having reduced hypoglycaemia awareness (unawareness-score ≥4). We analysed whether CGM enables identification of patients which reported at least one episode of SH or which reported reduced hypoglycaemia awareness without SH.

Materials and methods: 149 patients (age 46.5±11.9 yrs, 40.3% female, HbA1c 7.5±1.0%, diabetes duration 21.3±11.9 yrs) were included and randomised to this RCT. Participants wore blinded CGM (DexCom Gen 4) for 28 days and completed the Hypoglycaemia Awareness Questionnaire (HAQ). From these, 90 patients reported at least one episode of SH and 59 reported reduced hypoglycaemia awareness without SH.

Results: Patients wore blinded CGM during 26.9±2.1 days. Patients with SH spent longer time (minutes per day) in the low glucose ranges in comparison to those without SH (≤70 mg/dl: 130.5±98.9 vs. 72.4±56.1 min per day, p<.001; ≤55 mg/dl: 59.4±60.0 vs. 27.3 ±26.5 min per day, p<.001; ≤40 mg/dl: 13.5±25.8vs. 6.7±9.3 min per day, p=.001). However, they spend less time in the hyperglycaemic range (≥180 mg/dl: 441.0±220.8 vs. 568.0±244.4 min per day, p=.001). The number of hypoglycaemic events ≤55 mg/dl was also higher in the SH group than in the group without SH (14.5±11.8 vs. 8.1±7.8 events per 28 days, p<.001). The area under the Receiver Operating Characteristics (ROC) curves of different low glucose ranges or number of hypoglycaemic events ≤55 mg/dl was different from chance assignments (≤70 mg/dl: ROC 0.68 (95% CI 0.60 to 0.77, p<.001); ≤55 mg/dl: ROC 0.68 (0.60 to 0.77, p<.001); number of events ≤55 mg/dl: ROC 0.67 (0.58 to 0.76, p<.001)). Time in range (>70 to <180 mg/dl) does not enable identification of patients with type 1 diabetes and SH in the past (ROC 0.59 (0.49 to 0.69, p=.062)) significantly better than chance.

Conclusion: Time spend in low glucose range during CGM assessment or number of hypoglycaemic events ≤55 mg/dl can discriminate between patients with type 1 diabetes and previous occurrences of SH respectively hypoglycaemia unawareness without occurrence of SH. For the identification of patients with SH events, duration of low glucose values or number of low glucose events appears to be more important than time spent in euglycaemic range. The potential of CGM to identify patients with and without SH is not fully exhausted, since different targets might be applicable to both groups. In patients with SH prevention of re-occurrence of SH should be the primary aim (secondary prevention), whereas in patients without SH, but with hypoglycaemia unawareness the primary prevention of SH should be the primary objective.

Clinical Trial Registration Number: NCT02671968

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Disclosure: **L. Heinemann:** Employment/Consultancy; Consultant for Dexcom, Roche Diagnostics, Abbott, Medtronic, Sanofi. Lecture/other fees; Sanofi, Novo Nordisk. Stock/Shareholding; Profil Institute for Clinical Research, Profil Institut for Metabolic Research.

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Day-to-day variability of fasting self-measured plasma glucose correlates with risk of hypoglycaemia in adults with type 1 and type 2 diabetes

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Background and aims: The relationship between hypoglycaemia and day-to-day variability of glycaemic control has not yet been well established. This *post hoc* analysis aimed to investigate the correlation between the day-to-day variability of fasting self-measured plasma glucose (SMPG) and hypoglycaemia in patients with type 1 (T1D) and type 2 diabetes (T2D).

Materials and methods: A *post hoc* analysis was performed correlating day-to-day variability of fasting SMPG with hypoglycaemia in two double-blind, treat-to-target, crossover trials that compared insulin degludec once daily (OD) with insulin glargine U100 OD in adults with T1D (SWITCH 1, n=501) or insulin-experienced adults with T2D (SWITCH 2, n=721). Available pre-breakfast SMPG measurements were used to determine a weekly variance for each patient, using the log SMPG values to allow for relative comparisons. For each patient and treatment, the geometric mean of the weekly variance was calculated and these values were categorised into low, medium and high tertiles, as a measure for day-to-day variability. The effect of having low or high variability compared with medium variability was analysed in relation to overall symptomatic (severe or blood glucose [<3.1 mmol/L (56 mg/dL)] confirmed), nocturnal symptomatic (00:01-05:59, both inclusive), and severe (requiring third-party assistance and confirmed by a blinded adjudication committee) hypoglycaemia.

Results: Day-to-day SMPG variability was a significant predictor for the risk of overall and nocturnal hypoglycaemia in T1D and T2D, and severe hypoglycaemia in T1D (Table).

Conclusion: In conclusion, day-to-day glycaemic variability is associated with a risk of hypoglycaemia.

Table. Effect of fasting SMPG variability on hypoglycaemia in SWITCH 1 and 2: low and high tertiles compared with medium tertile

Hypoglycaemia	Variability tertiles	SWITCH 1			SWITCH 2		
		Events	Estimate [95% CI]	*p*-value	Events	Estimate [95% CI]	*p*-value
Overall	Low	1113	0.71 [0.62; 0.81]	*p*<0.0001	63	0.32 [0.23; 0.45]	*p*<0.0001
	Medium	1971	1.00		199	1.00	
	High	2790	1.17 [1.05; 1.29]		587	2.25 [1.81; 2.81]	
Nocturnal	Low	129	0.40 [0.30; 0.54]	*p*<0.0001	16	0.27 [0.15; 0.48]	*p*<0.0001
	Medium	335	1.00		74	1.00	
	High	428	1.31 [1.04; 1.64]		190	2.15 [1.55; 2.98]	
Severe	Low	39	1.16 [0.68; 1.98]	*p*<0.0001	5	0.74 [0.23; 2.35]	*p*=0.1835
	Medium	34	1.00		8	1.00	
	High	130	2.52 [1.60; 3.96]		14	1.85 [0.74; 4.61]	

CI, confidence interval; SMPG, self-measured plasma glucose

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Within-day variability based on 9-point profiles correlates with risk of overall and nocturnal hypoglycaemia in adults with type 1 and type 2 diabetes

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Background and aims: Higher glycaemic variability has previously been linked to an increased risk of hypoglycaemia. This *post hoc* analysis investigated the correlation between clinical within-day glycaemic variability, based on 9-point profiles, and hypoglycaemia in patients with type 1 (T1D) and type 2 diabetes (T2D).
Materials and methods: The correlation between within-day variability, based on 9-point profiles, and hypoglycaemia was investigated as a *post hoc* analysis in two double-blind, treat-to-target, crossover trials comparing insulin degludec once daily (OD) with insulin glargine U100 OD in adults with T1D (SWITCH 1, n=501) or insulin-experienced adults with T2D (SWITCH 2, n=721). Within-day glycaemic variability was calculated as the relative fluctuation of the 9-point profile, defined through the integrated absolute distance from the mean. Variabilities were subsequently categorised into low, medium and high tertiles based on the geometric mean of the two 9-point profiles available per patient and treatment. Hypoglycaemia was defined as overall symptomatic (severe or blood glucose [<3.1 mmol/L (56 mg/dL)] confirmed), nocturnal symptomatic (00:01-05:59, both inclusive) and severe (requiring third-party assistance and confirmed by a blinded adjudication committee) events.
Results: Within-day variability was a significant predictor for the risk of overall and nocturnal hypoglycaemia in patients with T1D or T2D (Table). However, no correlation was found for severe hypoglycaemia in this dataset.
Conclusion: In conclusion, within-day glycaemic variability is associated with a risk of overall and nocturnal hypoglycaemia.

Table. Effect of within-day glycaemic variability (9-point profile) on hypoglycaemia in SWITCH 1 and 2: low and high tertiles compared with medium tertile

Hypoglycaemia	Variability tertiles	SWITCH 1			SWITCH 2		
		Events	Estimate [95% CI]	*p*-value	Events	Estimate [95% CI]	*p*-value
Overall	Low	1318	0.92 [0.82; 1.03]	*p*=0.0008	140	0.79 [0.61; 1.03]	*p*<0.0001
	Medium	1675	1.00		258	1.00	
	High	2234	1.15 [1.04; 1.27]		430	1.49 [1.20; 1.86]	
Nocturnal	Low	166	0.76 [0.59; 0.97]	*p*<0.0001	44	0.76 [0.49; 1.20]	*p*=0.0002
	Medium	247	1.00		79	1.00	
	High	365	1.45 [1.14; 1.84]		149	1.72 [1.22; 2.41]	
Severe	Low	54	0.78 [0.49; 1.25]	*p*=0.5388	8	1.46 [0.52; 4.13]	*p*=0.7756
	Medium	55	1.00		9	1.00	
	High	53	0.81 [0.50; 1.31]		10	1.18 [0.45; 3.08]	

CI, confidence interval

Clinical Trial Registration Number: SWITCH 1 [NCT02034513]; SWITCH 2 [NCT02030600]
Supported by: Novo Nordisk A/S
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Decreased glucose intestinal absorption lowers 2 hour postprandial glycaemia after gastric bypass

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Background and aims: Postprandial hypoglycemia is a frequent concern after Roux-en-Y gastric bypass (RYGB). This condition occurs generally 2 hours after a meal. It is favored by postoperative weight loss in patients with high preoperative beta cell function. However, it is rarely observed after ajustable gastric banding (AGB). The aim of the study was to determine the specific mechanisms responsible for lowering two hour postprandial blood glucose after RYGB.
Materials and methods: We enrolled in a parallel arm, observational study, 109 patients undergoing RYGB (n=89) or AGB (n=20). All patients were submitted to a standardized mixed meal test (MMT) prior to, and again 3, 12, or 60 months after surgery. We measured glucose, insulin, and glucagon like peptide-1 (GLP-1) postprandial profiles during 3 hours after the mixed meal, as well as intestinal glucose absorption (D-xylose test), and calculated validated indices of insulin sensitivity (Matsuda index), and postprandial beta cell function (Insulinogenic index).
Results: The shape of postprandial blood glucose curve remained unchanged after AGB. After RYGB an early rise at 30 minutes was followed by a rapid fall beyond 60 minutes. When adjusted to fasting level, 2 hour postprandial blood glucose was significantly lower after RYGB than after AGB, independent of BMI, insulin sensitivity, and beta cell function (linear mixed model, $p < 0.0001$). Two hour postprandial blood glucose was significantly associated with lower intestinal glucose absorption (area under the curve of D-xylose), independent of BMI, insulin sensitivity, beta cell function, and GLP1 response (linear mixed model, $p < 0.01$).
Conclusion: Two hour postprandial blood glucose decreases after RYGB in close relation with intestinal glucose absorption, independent of BMI, insulin sensitivity, beta cell function, and GLP1 response.
Supported by: FFRD
Disclosure: **V. Raverdy:** None.

PS 054 Hypoglycaemia: treatment and consequences

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High one year mortality rate following admissions with severe hypoglycaemia to the hospital: Which group of patients is most at risk?

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Background and aims: Intensification of glycaemic control in diabetes patients increases the risk of severe hypoglycaemia. Hence, hypoglycaemia is recognised as a barrier to attaining good diabetes control. Severe hypoglycaemia is associated with increase mortality but there is a lack of evidence to link hypoglycaemia and mortality directly. Self - reporting of severe hypoglycaemia is associated with increased mortality at least in the medium term of 5 years. However, much less is known about the 1 year short term mortality rate following hospital admissions with severe hypoglycaemia.
The aims of the study are: 1.To ascertain the 1 year short term mortality rate following admission with severe hypoglycaemia to an acute tertiary care hospital. 2.To determine the factors which influence the 1 year mortality rate.
Materials and methods: Clinical, biochemical and 1 year mortality data from diabetes patients who were admitted with severe hypoglycaemia in the year of 2014 were extracted from the institutional medical record. Comparison of the patients' characteristics was made between the patients who survived a year following admission with those who did not survive a year following admission. Logistic regression was used to determine the risk of death within 1 year which is expressed as odd ratio and 95% confidence interval (CI).
Results: Three hundreds and eleven patients (185F: 126M) had been admitted with severe hypoglycaemia and the mean capillary blood glucose on admission was 2.3±0.7 mmol/l. Mean age, glycated haemoglobin and Charlson Comorbidity index (CCI) of the cohort were 70.7 ±11.2 year old, 6.9±1.4% and 4.5±2.1 respectively. Seventy patients or 22.5% of the cohort died within a year of admission. Those who deceased were older (69.2±11.0 vs 75.3±11.0 year old, $p<0.05$) with higher CCI (4.1 ±1.9vs6.0±2.3, $p<0.05$) and longer length of stay (5.0±7.4vs 10.0±14.5 days, $p<0.05$). However, there was no significant difference in glycated haemoglobin level between the 2 groups (7.0±1.4vs 6.7±1.6%, $p>0.05$). The patients who deceased within a year of admission also had lower albumin count on admission (36.2±5.4vs 30.3±6.8G/L, $p<0.05$). Age, length of stay and CCI were associated with increased in 1 year mortality risk with odd ratio of 1.06 (95% CI: 1.03 -1.09, p =0.01), 1.05 (95% CI: 1.02-1.08, p=0.01) and 1.57 (95% CI: 1.34-1.83, $p<0.001$) respectively.
Conclusion: Alarmingly high 1 year mortality rate of 22.5% following admissions with severe hypoglycaemia. Older diabetes patients with more comorbidities and longer length of stay were at increased risk of dying within a year of admission with severe hypoglycaemia. The degree of glycaemic control did not influence the 1 year mortality rate significantly. There was insufficient evidence in the current data set to draw the definitive link between hypoglycaemia and the cause of death. The high 1 year mortality rate also highlighted the vulnerabilities of certain individuals to adverse outcomes. Hence, admission with severe hypoglycaemia has important prognostic implications. Admission with severe hypoglycaemia offers the window of opportunity for healthcare professionals to intervene by comprehensively addressing the issue of hypoglycaemia and other health issues especially in the high risk group of patients.
Disclosure: **M.M. Teh:** None.

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Psychological impact of severe hypoglycaemia on fear of hypoglycaemia and diabetes-related distress: baseline assessment in participants of the HypoDe study

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Background and aims: Patients with type 1 diabetes on a multiple daily insulin injections (MDI) regimen were eligible to participate in the HypoDE study if they reported reduced hypoglycaemia awareness (unawareness-score ≥4) or the occurrence of severe hypoglycaemia (SH) in the past 12 months. We analysed the psychological profile of participants who had experienced at least one episode of SH (third party assistance for recovery) vs. participants with hypoglycaemia unawareness, but without a recent SH episode.
Materials and methods: Blinded CGM recordings (DexCom Gen 4; 28 days) of 149 study participants (age 46.5±11.9 yrs, 40.3% female, HbA1c 7.5±1.0%, diabetes duration 21.3±11.9 yrs) which had completed the Hypoglycaemia Awareness Questionnaire (HAQ), the Hypoglycaemia Fear Survey (HFS) and the Diabetes Distress Scale (DDS) were analysed.
Results: Participants who experienced SH and participants without SH did not differ in age (46.1±11.5 vs. 47.2±12.5 yrs, p=.578), diabetes duration (22.6±13.9 vs. 19.1±13.7 yrs, p=.126) or gender (female 42.2% vs. 37.3%, p=.551). Patients who experienced SH had a lower HbA1c (7.2±1.0% vs. 7.8±0.9%, p<.001) and a lower HAQ score (4.6±0.6 vs. 5.0±1.4, p=.018) compared to the group of patients without SH. Patients with SH also spent more time in the hypoglycaemic range (≤70mg/dl: 131±99 vs. 72±55 min per day, p<.01) and less time in the hyperglycaemic range (≥180 mg/dl: 441 ±221 vs. 568±244 min per day, p=.001) during the baseline blinded CGM phase. These participants also reported more worries about hypoglycaemia (34.6±14.4 vs. 29.2±15.2, p=.030) and more avoidance behaviour towards low glucose values (22.2±9.1 vs. 18.7±8.3, p=.020) than participants without SH. Overall, diabetes distress was higher in subjects with SH (2.7±0.8 vs. 2.3±0.7, p=.003) than in patients without SH. Participants with SH also reported more feelings of powerlessness (3.3±1.2 vs. 2.8±1.1, p=.010), more hypoglycaemia-related distress (3.9±1.2 vs. 3.3±1.2, p=.007), more social problems in public (2.2±1.1 vs. 1.8±0.9, p=.045), more family arguments (2.7±1.1 vs. 2.1±1.1, p=.003) and physician-related distress (1.4 ±0.7 vs. 1.2±0.4, p=.014) as well as eating-relating distress (2.7±1.2 vs. 2.3±1.1, p=.025). Overall diabetes-related distress and negative family arguments showed significant correlations with duration of hypoglycaemic episodes (r=.19, p=.022 respectively r=.20, p=.012).
Conclusion: Occurrence of SH affects many areas of living with diabetes. The psychological impact of SH was strongest with regard to family arguments and the overall distress levels. Avoidance of hypoglycaemic glucose values was also more prominent in participants with SH. Diabetes distress and family arguments was not only correlated with occurrence of SH but also with the duration of low glucose values. Besides the reduction of biochemical and clinical hypoglycaemia, also hypoglycaemia-related distress should be addressed in this specific group of patients with diabetes.
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Supported by: Unrestricted grant from DexCom, San Diego, California. U.S.A.
Disclosure: **D. Ehrmann:** Lecture/other fees; Berlin-Chemie AG, Roche Diabetes Care.

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Association of hypoglycaemia with insulin titration and body weight in people with type 2 diabetes commencing insulin glargine 100 U/ml and achieving different HbA_{1c} levels

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Background and aims: To assess the relationship between hypoglycaemia frequency, insulin dose titration, and body weight in people with type 2 diabetes (T2D) commencing Gla-100 at bedtime in combination with oral agents, and achieving different HbA_{1c} levels at Week 24.
Materials and methods: Standardized patient-level data were pooled from 16 treat-to-target randomized controlled trials (≥24 week duration, fasting plasma glucose [FPG] target <5.5 mmol/l). Glycaemic and hypoglycaemia outcomes were assessed descriptively to Week 24, stratified according to endpoint HbA_{1c} (<7.0, 7.0-8.0, >8.0%) and number of episodes (0, 1-3, ≥4) of confirmed hypoglycaemia (PG <3.9 mmol/l).
Results: Overall, 3,415 participants (54% male) were included. Baseline characteristics in the <7.0, 7.0-8.0, and >8.0% HbA_1 categories were (mean ± SD): age 58 ± 9, 58 ± 10, and 57 ± 10 years; disease duration 8.1 ± 5.6, 9.4 ± 6.5 and 10.0 ± 6.7 years; BMI 30.8 ± 5.0, 30.5 ± 5.4 and 29.9 ± 5.4 kg/m^2; C-peptide level 1.16 ± 0.55, 1.13 ± 0.57, and 1.16 ± 0.77 nmol/l, respectively. More participants (46%) achieved endpoint HbA_{1c} <7.0% than 7.0-8.0% (37%) or >8.0% (17%) and more people achieved FPG target (≤ 5.5 mmol/l) in the <7.0% group (44%) vs other groups (30% and 22%) despite similar reductions in FPG (−4.1 ± 0.1 mmol/l) from baseline to Week 24. HbA_{1c} response was lower in the >8.0% group (−0.5%) vs other groups (−2.0 and −1.4%), associated with longer diabetes duration, higher baseline FPG and HbA_{1c}. In the <7.0% group, more people (54%) had anytime hypoglycaemia (PG <3.9 mmol/l), had the lowest mean final Gla-100 dose (0.42 U/kg vs 0.44 U/kg and 0.51 U/kg, respectively), and the smallest dose increment (0.27 U/kg vs 0.28 U/kg and 0.32 U/kg, respectively). FPG change was greater in patients with ≥4 hypoglycaemia events vs those with no hypoglycaemia events in the <7.0% group (−4.5 vs −4.1 mmol/l) and the >8.0% group (−5.7 vs −3.5 mmol/l). Hypoglycaemia was associated with lower baseline C-peptide levels and lower final Gla-100 dose across all HbA_{1c} groups in people experiencing hypoglycaemia (**Table**). Weight gain was more pronounced in people with increasing numbers of hypoglycaemia events and with higher endpoint HbA_{1c} (1.1 ± 4.1 kg, 2.0 ± 3.5 kg, 2.4 ± 3.5 kg) in those with no hypoglycaemia vs 1.8 ± 3.7 kg, 2.5 ± 3.0 kg, and 3.7 ± 3.8 kg in those with ≥4 hypoglycaemia events and HbA_{1c} <7.0, 7.0-8.0, and >8.0%, respectively).
Conclusion: This pooled analysis of people with T2D confirms that baseline HbA_{1c} and FPG levels are important predictors for achieving HbA_{1c} <7.0%. Hypoglycaemia apparently has little or no influence on HbA_{1c} achievement and is mainly associated with insulin dose titration and weight gain over the first 6 months after beginning Gla-100 therapy.

Table. Clinical outcomes in people with type 2 diabetes starting Gla-100, stratified by endpoint HbA_{1c} range

		<7.0% n=1,584	7.0–8.0% n=1,262	>8.0% n=569
Hypoglycaemia[a]	no events (% people)	46	53	65
	1–3 events (% people)	30	26	20
	≥4 events (% people)	24	21	15
	event rate (events/person-year)	6.5 (0.3)	5.3 (0.3)	4.4 (0.5)
Hypoglycaemia[a] no events	C-peptide baseline (nmol/l)	1.22 (0.62)	1.20 (0.55)	1.28 (0.86)
	FPG week 24 (mmol/l)	6.0 (1.4)	6.8 (1.8)	8.3 (3.1)
	Gla-100 dose Week 24 (U/kg)	0.45 (0.24)	0.46 (0.24)	0.56 (0.31)
≥4 events	C-peptide baseline (nmol/l)	1.08 (0.54)	1.00 (0.48)	0.75 (0.38)
	FPG Week 24 (mmol/l)	5.9 (1.7)	6.2 (1.9)	6.3 (1.9)
	Gla-100 dose Week 24 (U/kg)	0.37 (0.20)	0.39 (0.23)	0.41 (0.21)

Mean (SD) or percent people; Group numbers may vary if missing values
[a] *overall-anytime confirmed plasma glucose <3.9 mmol/l*

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Disclosure: **B.M. Frier:** Honorarium; Eli Lilly, Novo Nordisk, Sanofi, MSD, Roche and Boehringer Ingelheim.

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Recurrent insulin-induced hypoglycaemia leads to weight gain in association with increased adiposity and reduced basal metabolic rate

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Background and aims: Intensification of insulin therapy in type 1 diabetes is associated with significant weight gain. This study examined the hypothesis that recurrent hypoglycaemia (RH) might contribute, at least in part, to this phenomenon. This hypothesis was tested in rodents subjected to RH or control injections over 12 weeks.
Materials and methods: Male C57BL6J mice (n=12/group) received either RH (1mu/g insulin i.p.; 3 per week for 12 weeks to achieve glucose of approx.3.0mmol/l) or Vehicle (Saline i.p. in equivalent volume). Body composition and energy homeostasis were assessed using EchoMRI and CLAMS system respectively. Transcript abundance was assessed using Taqman real time PCR. Data are expressed as mean±SEM.
Results: RH animals were significantly heavier and had increased fat mass compared to Control animals (8.49±0.64 v 16.56±1.38% body weight) despite comparable food intake (4.83±0.81 v 4.89±0.44 g/mouse/24hr). Energy expenditure was significantly lower at baseline in RH animals (0.414±0.006 v 0.218±0.004 kcal/min) and was associated with a decrease in the respiratory exchange ratio (RER; 0.98±0.01 v. 0.96 ±0.01). There was no difference in locomotor activity. These changes were associated with the suppression of genes involved in thermogenesis, mitochondrial energetics and lipolysis within the brown adipose tissue.
Conclusion: Consistent with our hypothesis, RH leads to increased accumulation of fat stores which is associated reduced energy expenditure and RER. These results suggest that RH induces a state of hypometabolism that may contribute to weight gain observed with intensification of insulin therapy in Type 1 diabetes
Supported by: DUK project grant
Disclosure: **A.D. McNeilly:** None.

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Fear of hypoglycaemia was not associated with glycaemic variability in patients with type 1 diabetes: the VARDIA study

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Background and aims: In type 1 diabetes (T1D), treatment efficacy is limited by unpredictability of blood glucose results and glycemic variability. This unpredictability strongly and negatively impacts patients' appreciation of their disease. Fear of Hypoglycemia (FOH) remains associated with significant issues for managing insulin treatment. The main goal of our study was to assess the relationship between glycemic variability and FOH in patients with T1D in an observational cross-sectional study performed in 9 centers from Western France.
Materials and methods: Main recruitment criteria were the following: men or women with T1D diagnosed for ≥ 5 years, aged 18 - 75 years, on stable insulin therapy (type of insulin and number of injections/pump) for ≥ 3 months, no severe renal failure (eGFR ≥ 30 ml/min/1.73 m^2) and no acute condition possibly leading to unusual glycemic

variability.Glycemic variability was determined using the coefficient of variation of three 7-point self-monitoring blood glucose (SMBG) profile determined in 2 weeks: 3 pre-meal /3 post-prandial/ 1 night (4:00) determinations. FOH was assessed using the validated French version of the Hypoglycemia Fear Survey-II (HFS-II) questionnaire.
Results: A total of 571 patients were recruited, with 527 fulfilling all recruitment criteria. Among them, 393 returned their SMBG profiles: 55% women, 55% on insulin pump, mean age 48 ± 12 years, mean diabetes duration of 26±13 years, HbA1c of 7.5±1.0 % and 12% with severe hypoglycemia (requiring assistance from another person) in the previous 6 months. HFS-II score was 34±18. SMBG glycemic variability was not significantly different between patients treated with continuous subcutaneous insulin infusion and multiple daily injections (39.3±9.4 and 40.9±10.1 % respectively).SMBG variability did not significantly correlate with HFS-II score (R= 0.022; *P*=0.69), and was not associated with the presence or absence of long-term complications (retinopathy, nephropathy and cardiovascular disease). HFS-II score was not different according to the distribution of quintiles of SMBG variability.The SMBG variability did not differ between patients with or without history of severe hypoglycemic episode in the previous 6 months (42±11 vs. 40 ±9 *P*=0.12), but HFS-II score was higher in patients with history of severe hypoglycemia: 39±20 vs. 33±18; *P*=0.03, respectively regarding anxiety (18±13 vs. 23±15; *P*=0.02), but not behavior (15±8 vs. 16±8; *P*=0.263).
Conclusion: FOH determined using the HFS-II questionnaire was not associated with SMBG variability in patients with T1D, but was associated with the occurrence of severe hypoglycemia in the previous 6 months.
Clinical Trial Registration Number: NCT02790060
Supported by: AADAIRC / DINNOSANTE/NOVONORDISK
Disclosure: **S. Hadjadj:** Grants; Novonordisk, AADAIRC, DinnoSanté.

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A pilot study of mini-dose glucagon for treatment of non-severe hypoglycaemia in adults with type 1 diabetes

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Background and aims: The objective of this analysis is to evaluate the use of low-dose glucagon to treat mild hypoglycemia in ambulatory adults with type 1 diabetes.
Materials and methods: Twenty adults with type 1 diabetes using an insulin pump and continuous glucose monitor (CGM) and experiencing frequent mild hypoglycemia participated in a crossover trial (two 3-week periods) comparing non-aqueous mini-dose glucagon (MDG) (150 μg, Xeris Pharmaceuticals, Inc.) versus oral glucose tablets (TABS) (16 g) to treat hypoglycemia (blood glucose [BG] 40-69 mg/dL). BG meter measurements were made prior to treatment and again 15 and 30 minutes post-treatment. Successful treatment was defined as BG ≥50 mg/dL 15 minutes and ≥70 mg/dL 30 minutes after intervention. Two authors, blinded to treatment arm, independently judged the outcome of each event as a clinical success or failure.
Results: Sixteen participants (mean age 39 years, 75% female, mean diabetes duration 23 years, mean HbA1c 7.2%) had 118 analyzable events with initial BG 50-69 mg/dL. Successful treatment criteria were met for 58 (94%) of 62 events during the MDG period and 53 (95%) of 56 events during the TABS period (adjusted p=0.99). Clinical assessments of success for these events were 97% and 96%, respectively. CGM-measured time 70-180 mg/dL data did not differ between treatment groups during the 2 hours post-events but TABS resulted in higher maximum glucose (116 vs 102 mg/dL; p=0.01) over the first hour.
Conclusion: Small doses of glucagon can successfully treat mild-to-moderate hypoglycemia and may be a useful alternative to treatment with oral carbohydrate.
Clinical Trial Registration Number: NCT02411578
Supported by: Funding was provided by the Leona M. and Harry B. Helmsley Charitable Trust
Disclosure: **M. Haymod:** None.

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Nasal glucagon for the treatment of moderate to severe hypoglycaemia episodes in children and adolescents with type 1 diabetes in home or school settings

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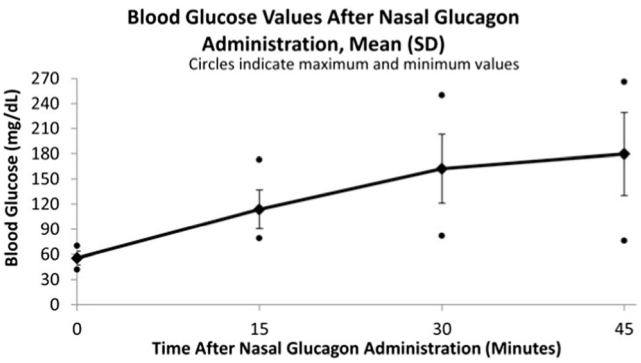
Background and aims: This multicenter, open-label study evaluated NG for effectiveness and ease of use in treating moderate or severe hypoglycemia episodes in patients with T1D, age 4 to <18 years.
Materials and methods: Patients and caregivers were taught how to use NG. During naturally occurring symptomatic episodes of moderate or severe hypoglycemia in real-world settings, caregivers administered 3 mg NG and measured blood glucose (BG) over time. Adverse events (AEs), recovery of symptoms, and ease of use were solicited by questionnaires.
Results: Fourteen patients, who experienced 33 moderate hypoglycemia episodes with neuroglycopenic symptoms and a BG ≤70 mg/dL, were included in the efficacy and main safety analyses. The mean number of episodes per pt was 2.4 (range 1-4). In all episodes, patients returned to normal status within 30 minutes after NG dose. No calls to 911 (emergency medical services) were needed. Mean baseline BG was 56 (range 42-70) mg/dL. Within 15 minutes after NG dose, mean BG rose to 114 (range 79-173) mg/dL, and continued to rise (figure). No serious AEs occurred. For most episodes (61%), caregivers administered NG in <30 seconds; in all cases administration took <2 minutes. Caregivers were satisfied or very satisfied with NG after most episodes (91%).
Conclusion: NG raised BG and resolved symptoms in all reported episodes of hypoglycemia among children and adolescents with T1D. The majority of caregivers were highly satisfied with NG. Data suggest NG is a viable alternative to currently available injectable recombinant glucagons.



Mean blood glucose at time 0 was 56 mg/dL

Clinical Trial Registration Number: NCT02402933
Supported by: Eli Lilly and Company

Disclosure: **C.B. Guzman:** Employment/Consultancy; Eli Lilly and Company.

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A pilot study in adults with type 1 diabetes to examine the efficacy of stable non-aqueous liquid glucagon for treatment of severe hypoglycaemia

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Background and aims: Severe hypoglycemia remains a significant problem in patients with diabetes. Currently approved rescue products are based on lyophilized formulations which require reconstitution at time of use, complicating administration in emergency situations. A premixed, ready-to-use, non-aqueous liquid glucagon formulation in a prefilled syringe and auto-injector is in development. This glucagon formulation is expected to be stable for at least 24 months at room temperature. A pilot study was conducted in adults with type 1 diabetes mellitus (T1DM) to explore whether subcutaneously injected non-aqueous glucagon (0.5 or 1 mg) could rescue subjects from insulin-induced hypoglycemia.
Materials and methods: On treatment days subjects arrived at the clinic following an overnight fast and were given IV insulin to induce moderate hypoglycemia with plasma glucose concentration < 2.8 mmol/l. After a 5-minute wait and a confirmatory plasma glucose < 2.8 mmol/l, subjects were given a subcutaneous injection of non-aqueous liquid glucagon in the upper arm. The product was stored in a vial for approximately 18 months at controlled room temperature before it was used in the study. Plasma glucose was assessed every 15 minutes via glucometer (YSI Life Sciences) during the hypoglycemia induction phase. Once plasma glucose was < 2.8 mmol/l, glucose was assessed every 5 minutes until 90 minutes post-treatment. At the same time points during the induction phase and through 30 minutes post-treatment, subjects completed a hypoglycemia symptom questionnaire. Pharmacodynamic characteristics of plasma glucose were assessed with particular attention to time to reach > 3.9 mmol/l. Subjects with return of plasma glucose to > 3.9 mmol/l by 30 minutes post-dosing were considered a primary (objective) responder. Secondary efficacy endpoints included hypoglycemia symptom relief as assessed based on aggregate scores for 4 autonomic and 4 neuroglycopenic symptoms. Safety endpoints included: incidence/severity of adverse events, clinically significant out-of-range vital signs, and tolerability.
Results: Across 7 adult subjects and 13 hypoglycemia induction procedures, IV insulin resulted in a mean plasma glucose of 2.5 ± 0.18 mmol/l just prior to treatment. For the 1 mg dose, 7/7 subjects achieved primary response with plasma glucose > 3.9 mmol/l in a mean time of 11.9 minutes (range: 8.1 to 15.9 minutes). For the 0.5 mg dose, 6/6 subjects achieved plasma glucose > 3.9 mmol/l in a mean time of 14.4 minutes (range: 9.5 to 19.7 minutes). Symptomatic relief began within 5 minutes of dosing, and all subjects experienced complete resolution of hypoglycemia symptoms in a median time of 20 minutes (range 10-30). Overall, no significant safety concerns were noted in this pilot study. For the 1 mg dose, 3/7 subjects reported a total of 8 mostly mild treatment-emergent adverse events, including 3 cases of nausea/vomiting.
Conclusion: The results of this study provide support for Phase 3 clinical development of this room-temperature stable non-aqueous liquid glucagon in a ready-to-use auto-injector as a rescue treatment for severe hypoglycemia.
Clinical Trial Registration Number: NCT02423980
Disclosure: **M. Christiansen:** None.

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Dasiglucagon, a novel soluble glucagon analogue, successfully restores blood glucose levels after hypoglycaemia in people with type 1 diabetes

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Background and aims: Currently available glucagon formulations for rescue treatment of severe hypoglycaemia need to be reconstituted before use which delays its use and may lead to underutilization.
Materials and methods: In this single centre, randomised, double-blind trial we investigated the pharmacodynamic (PD) and pharmacokinetic (PK) characteristics of dasiglucagon (DASI), a novel stable soluble glucagon analogue, in comparison to GlucaGen® (GLUCA). Fifty-eight patients with type 1 diabetes received subcutaneous injections of 0.1, 0.3, 0.6 or 1 mg DASI or 0.5 or 1 mg GLUCA after induction of hypoglycaemia (55 mg/dl) through intravenous insulin infusion.
Results: DASI demonstrated a dose-dependent and rapid increase in PK reaching maximum levels at ~35 min with a half-life of ~0.5 hours. DASI rapidly increased plasma glucose (PG) by ≥20 mg/dl (9-14 min) and to PG≥70 mg/dl (within 6-10 min), similar to GLUCA (table). All patients on DASI reached these endpoints within 30 min (predefined success criteria). Both treatments were well tolerated. Nausea was the most frequent adverse event occurring at a similar rate of 44-56% patients.
Conclusion: DASI was well tolerated and showed similar early PD response to GLUCA at equivalent doses suggesting they have comparable clinical effects. Dasiglucagon may be an effective and reliable emergency treatment for severe hypoglycaemia in a ready-to-use pen.

Table 1

	Dasiglucagon*				GlucaGen®	
Dose	0.1 mg	0.3 mg	0.6 mg	1.0 mg	0.5 mg	1.0 mg
N	5	16	17	16	15	31
Time to reach plasma glucose levels ≥70 mg/dl [min]						
Median (min-max)	10.0 (2.0-17.0)	6.0 (0-13.0)	6.0 (0-9.0)	6.0 (0-9.0)	6.0 (0-9.0)	7.0 (0-10.0)
Time to increase in plasma glucose levels ≥20 mg/dl [min]						
Median (min-max)	14.0 (11.0-27.0)	10.0 (7.0-20.0)	9.0 (6.0-16.0)	9.0 (7.0-15.0)	10.0 (6.0-13.0)	10.0 (5.0-15.0)
*proposed int. non-proprietary name						

Clinical Trial Registration Number: NCT02660008
Supported by: Zealand Pharma A/S
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Nasal glucagon for the treatment of moderate-to-severe hypoglycaemic episodes in real-world settings in adults with type 1 diabetes

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Background and aims: This study evaluated nasal glucagon (NG) for efficacy and ease-of-use in moderate or severe hypoglycaemic episodes (HEs) in real-world settings in adult patients (pts) with type 1 diabetes (T1D).
Materials and methods: Pts and caregivers (CGs) were taught to administer NG 3 mg for symptomatic HE and to assess for return to normal status over time. In addition, Pt/CG-reported HE symptoms, blood glucose (BG), adverse events (AEs), and ease-of-use were evaluated through questionnaire.
Results: In the efficacy population (EP) 69 pts experienced a total of 157 HEs (mean [SD], 2.3 [1.77] events/pt). In 96.2% of HEs, pts met the primary objective, return to normal status within 30 minutes. There were 6 HEs in which the recovery did not occur within 30 minutes. In 5 of these 6 events, pts recovered within 30 to 45 minutes and in 4 events, BG was ≥3.9 mmol/L at 30 minutes. Mean BG at HE onset was 2.7 (range 1.2 to 4.1) mmol/L and rose to 6.3 (range: 2.4 to 14.8) mmol/L by 30 minutes and continued to rise with progressive time. No emergency service calls were made. Twelve severe HEs in 7 pts were observed in the EP. All severe HEs resolved; and pts awoke or returned to normal status within 15 minutes. NG administration time was <30 seconds for most HEs (70.4%) and was <2 minutes in nearly all (97.7%) HEs. The safety population included 74 pts who had a total of 179 HEs. At least 1 AE was experienced by 87.8% of pts, with the most common being nasal irritation (82.4%) and headache (54.1%). Most AEs during HEs lasted ≤1 hour (59.5%) and were of mild or moderate severity. There were no serious drug-related AEs, and CGs were satisfied or very satisfied with NG after most HEs (82.7%).
Conclusion: NG showed real-world effectiveness when administered to treat moderate or severe HE in pts with T1D. For most HEs (96.2%), pts recovered within 30 minutes and there were no emergency calls. The majority of CGs were satisfied with NG. NG is a potential alternative to currently available injectable recombinant glucagon.
Clinical Trial Registration Number: NCT01994746
Supported by: Eli Lilly and Company
Disclosure: **E.R. Seaquist:** Employment/Consultancy; Eli Lilly and Company, Locemia. Grants; JDRF, NIH, Eli Lilly and Company. Other; International hypoglycemia study group funded by Novonordisk.

PS 055 Surveys, barriers and solutions to improve education in diabetes

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Glycaemic index, extended bolusing and diabetes education in insulin pump therapy

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Background and aims: Advice given to insulin pump users on how to manage slowly digested food is varied. There is no published evidence on whether educating patients on glycaemic index, extended bolusing and super bolusing can affect glycaemic control, quality of life or treatment satisfaction. The aim of this study was to evaluate the clinical and psychosocial efficacy of a clinical education programme on rates of digestion and bolusing.
Materials and methods: The literature on glycaemic index, extended bolusing and superbolusing was reviewed and consolidated to produce a three hour, interactive education session aiming to enable effective self-management. At baseline, participants undertook one week of blinded continuous glucose monitoring (CGM) and kept a food diary. During this period participants were provided with two test meals: a low GI meal (pizza with extra cheese) and a high GI meal (white bread with jam). Participants were asked to bolus in whatever way they would usually for these foods. Participants completed validated "Problem Areas in Diabetes" (PAID) and "Diabetes Quality of Life" (DQOL) questionnaires and blood samples were taken to measure HbA1c. The participants then attended the education programme. Four weeks after attending the educational intervention, the CGM and test meals week were repeated. 8 and 12 weeks after the intervention, participants underwent another week of CGM. At the end of week 12, HbA1c, DQOL and PAID were repeated. Glucose AUC were calculated at 1 hour, 2 hours, 4 hours and 8 hours after the test meals. Time spent in hyperglycemia and hypoglycaemia were calculated.
Results: Eleven participants were recruited (mean age 42.3 (SD 12.8) years, mean baseline HbA1c 57.3 (SD 10.0) mmol/mol, duration of diabetes 19.5 (SD 9.9) years). HbA1c did not change over the treatment period, nor did quality of life assessed by DQOL and PAID. %time spent in hypoglycaemia (<3.9mmo/L and <3.3mmol/L) fell at week 12 compared to baseline from 5.8 (IQR 2.1-8.3)% to 4.3(IQR 2.1-5.4)%, p=0.013, for <3.9mmol/L, and from 2.9 (IQR 1.2-3.9)% to 1.6 (IQR 0.7-2.4)%, p=0.029, for <3.3mmol/L. Area under the curve for glucose following test meals did not differ significantly following education except for in the first two hours after the high GI meal (pre course 83.1 (0.23-88.9), post course 5.38 (-16.2- 50.8).
Conclusion: An education programme to support type 1 diabetes self-management using extended and super boluses does not impact on overall glucose or quality of life but does reduce exposure to hypoglycaemia in this pilot study suggesting further work is warranted.
Supported by: NIHR BRC based at Imperial College Healthcare NHS Trust & Imperial College
Disclosure: **S.K. Rilstone:** None.

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The smartphone application "Pregnant with Diabetes" communicates antenatal health information and reaches pregnant women with diabetes and those planning pregnancy

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Background and aims: To evaluate the awareness and use of the smartphone application "Pregnant with Diabetes" locally, nationally and internationally.
Materials and methods: In 2013, a teamwork between a patient and the staff at The Center for Pregnant Women with Diabetes, Rigshospitalet, succeeded in developing the smartphone application (app) "Pregnant with Diabetes". The app communicates clinically important antenatal health information to women with diabetes, based on recommendations from our center. To obtain local data, women with type 1 or type 2 diabetes completed an anonymous, structured questionnaire at first antenatal visit in early pregnancy, at our center. National and international data on numbers of downloads were obtained from Google Play, App Store and Google analytics.
Results: Among 139 pregnant women with diabetes (96 with type 1 diabetes, 43 with type 2 diabetes), 99% had a smartphone and 75% had downloaded the app, whereof 48% had obtained information from the app before pregnancy. For women with type 1 diabetes, the topics reported of greatest interest in the app were "blood glucose", "the fetus", and "insulin dose", and for type 2 diabetes; "diet and carbohydrates", "blood glucose" and "what is diabetes". In December 2016, the app had been downloaded 3,810 times in Denmark and 21,640 times in 177 different countries worldwide. Internationally, the topics of greatest interest were "diet and carbohydrates", "blood glucose" and "possible complications".
Conclusion: Easily accessible patient information, via app technology, reaches the patients and may contribute to improved pregnancy planning and outcome in women with diabetes - locally, nationally and internationally.
Disclosure: **E.R. Mathiesen:** None.

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Close reading and creative writing in a group care intervention to manage type 2 diabetes: a randomised trial

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Background and aims: The use of group dynamics for patients with diabetes is a recognized practice with positive outcomes in terms of metabolic control, quality of life parameters and knowledge acquisition about the condition. This study attempts to determine if the same group dynamic strategies using narrative and reading produce the same positive outcomes.
Materials and methods: A total of 49 patients with type 2 Diabetes, aged < 85 years old, with a follow-up in our outpatient clinic for at least 6 months and > 6 years of formal education were randomized to two different Group Care Dynamics. One group, with 17 patients, designed 'control group', was presented with a classical structured educational approach of six monthly sessions where different issues of diabetes management were discussed (chronic disease, nutrition, exercise, complications, self-management, and diabetic foot). Another group, with 32 patients, designed 'intervention group', subdivided in 2 similar subgroups, was presented with six monthly sessions with close reading and creative writing using literary narratives that parallel the issues focused in the control group. Participants were evaluated before the first session and after the third session. Two final evaluation will be performed at the sixt session and one month later. Patient evaluation includes anthropometrical measures (weight, fat mass, waist circumference), A1c and specific questionnaires about quality of life, locus of control, empathy and group satisfaction (respectively DQOL, SF36, DSLOC, JSPE, GSS).
Results: We present the baseline results and the interim analysis at the third session. The entire sample had an initial BMI of 29,16 (SD-4,67) and A1c of 7,51% (SD- 1,13). The two groups were very similar either in the physical and in the psychological variables at baseline. An interim analysis at the moment of the second evaluation was performed on a sample of 39 patients. The intervention group showed a significant reduction of HbA1c of 0,35% (initial: 7,55% (DP-1,13) vs. 7,20% (DP-1,06); p<0,001 while the control group maintained the A1c (initial: 7,44 %(DP-1,23) vs 7,42% (DP-1,49).
Conclusion: To our knowledge this is the first randomized trial designed to evaluate a Group Care intervention to manage type 2 diabetes using close reading and creative writing. At the interim analysis, a significant reduction of A1c was observed in the intervention group. The final appraisal of all the anthropometric and biochemical variables as well of the psychological domains will be made after the 6th month session.
Disclosure: **F.S. Rosário:** None.

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Assessment of inpatient diabetes education throughout an structured questionnaire applied by a team for diabetes education (TED)

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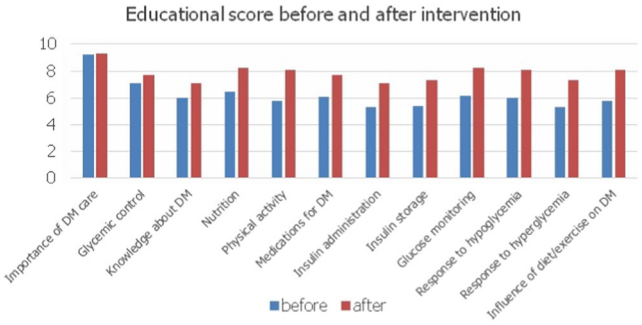
Background and aims: To describe the work and methodology for the implementation of a team for diabetes education in hospital settings and to test the efficiency of patient education using a adapted questionnaire based on American Association of Diabetes Educators.
Materials and methods: In August 2014, a project of diabetes education inspired by STAR Practical Diabetology Course was started, a questionnaire was adapted from American Association of Diabetes Educators to evaluate patient's knowledge. Eight professionals (6 nurses and 2 nutritional therapists) were selected, trained and designated to dedicate three hours weekly exclusively to diabetes education in collaboration with TED. From November 2014 to December 2014, the assigned professionals were trained in weekly sessions and initiated educational activities in January 2015.
Results: From January 2015 to January 2017, we included 474 patients; they were educated during hospitalization and were considered in this analysis. The questionnaire evaluated the perceived importance of DM care and the knowlwdge about DM, nutrition, physical activity, glycemic control and medications. For those on insulin, there were questions concerning administration and storage, glucose monitoring, response to hypo/hyperglycemia and foot care. Each item was scored form 0 (no knowledge) to 10 (full knowledge). The same questionnaire was applied as a pretest and after the educational process as a posttest. On average, age was 66+17 yrs and time of diagnosis was 16+2 yrs and 172 patients were on insulin. The average number of sessions were 1,9 (range 1-8). Previous A1c were available for 322 patients on the average: 8,1%. From all the patients 48 unaware of the diagnosis of DM and 55 with admissions primarily related to diabetes (15 ketoacidosis). Some of these patients did not agree or were not able to respond to the pretest (242). 175 (37%) answered the pretest and were reevaluated by the posttest. The major reasons for no reevaluation were discharge during weekend or without notification.
Conclusion: Using TED, we were able to develop a methodology to better assess the knowledge and skill of diabetic patients and to improve the education for complex patients By performing a structured evaluation, we documented an improvement in diabetes knowledge of hospitalized patients. Besides every patient has some knowledge of the disease, after the education guided by the question form, all patients increase their grades for each subject.



Disclosure: **T.B.M. Cardim:** None.

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Family dialogue tools: how can healthcare professionals facilitate mutual involvement in families dealing with type 2 diabetes

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Background and aims: Family involvement plays a key role in management of type 2 diabetes. Self-management interventions rarely target family dynamics or family education and support. Through an extensive design based research process including needs assessment, ideation, prototype-testing and feasibility study we have developed dialogue tools designed to generate mutual family involvement in daily family life with type 2 diabetes. To implement the tools in healthcare practice we have trained 70 diabetes educators in how to use the tools.
Materials and methods: 126 family members from 54 families participating in family education sessions filled in the Family functioning and change questionnaire before the session and then again 3-4 weeks after the session. 40 of these families were randomly chosen to take part in semi-structured family interviews that took place in their home. The combined data were analysed using radical hermeneutics.
Results: The analysis disclosed that the families were affected in 5 primary areas: 1) Knowledge: Many families found it easier to share knowledge and search for common knowledge after they had worked with the tools. Before working with the tools the relatives often felt that they lacked knowledge about the disease and how to support. Families often felt more involved in each other's life after the education session, 2) Communication: Before the sessions most families found it difficult to balance communication about type 2 diabetes. Some families do not talk about it at all and others talk too much about it. Many families seemed to find it easier to find that balance after working with the tools, 3) Roles: Accepting the role as a person with type 2 diabetes as well as the role as a relative to a person with type 2 diabetes is difficult. The dialogue generated by the tools make it easier to understand the new roles and to relate existing role structures to life with type 2 diabetes, 4) Everyday life: Most families do not want type 2 diabetes to dominate life and therefore try to maintain a perceived normality. After working with the tools families found it easier to accept type 2 diabetes in their lives and did not feel the need to create difficult practices designed to ignore the disease, 5) Support: Before the session many relatives found it difficult to be supportive without being controlling. Many families had experienced conflicts about support that had resulted in the relatives completely opting out of supporting and thereby significantly reducing mutual involvement. After working with the tools several families reported that they had gained a common understanding of each other's needs and worries and that this made it significantly easier to support each other in appropriate ways.
Conclusion: The family dialogue tools provide healthcare professionals with a novel approach to facilitate mutual involvement in families. The study shows that the tools manage to generate communication and dialogue in the families and that significant problem areas in daily life are positively affected.
Disclosure: **D. Grabowski:** None.

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Barriers to uptake of education by adults with type 1 diabetes: the BUDiE study

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Background and aims: Structured diabetes education transfers clinical knowledge to empower people to self-manage their long-term condition. Type 1 diabetes requires multiple daily decisions about self-management to maintain glycaemic control and avoid complications. However, attendance at high-quality courses run by the National Health Service (NHS) is poor. We explored reasons for poor attendance rates in an adult population in south London, UK.
Materials and methods: There were three parts to this mixed-methods study using exploratory sequential design with quantitative dominance. Adults with, or providing care for people with, type 1 diabetes, in two London boroughs were included. In part 1, we compared education course attenders with non-attenders as recorded in an NHS service-use database. In part 2, a survey of adults with type 1 diabetes and semi-structured interviews were done, and a provider survey conducted. Focus groups were conducted in part 3. Analysis included exploratory regression models, thematic analysis of interviews, and quantisising data using a mixed methods matrix.
Results: In part 1, 303 (27%) of 1121 adults had attended courses. In univariate analysis, non-attendance was significantly associated with older age, male gender and greater social deprivation. In part 2 (n=496, 34% response rate), there were 233 non-attenders (47%), 59 (31%) of whom had not heard of the course. Univariate analysis corroborated part 1 findings, with significant differences (all $p<0{\cdot}05$) in attendance according to employment status, ethnic origin, and other socioeconomic factors. Exploratory regression analysis identified four key variables associated with attendance: a positive health-care professional message (odds ratio for positive *vs* negative message 2·77, 95% CI 1·54-5·01; p=0·001), female sex (0·56, 0·36-0·86, p=0·009), educational attainment (university *vs* secondary school 0·49, 0·3-0·81; p=0·005), and glycaemic control (glycated haemoglobin <7·5% (<58 mmol/mol) *vs* 7·5-8·9% (58-75 mmol/mol): 1·83, 1·06-3·14; p=0·03). Four typologies were identified and labelled according to individuals' coping strategies: "go-getters" (high educational attainment, high thirst for knowledge, and internal locus of control leading to self-education and perceived low benefit from the course); "not yetters" (long-standing diagnosis, previous or current judgmental relationships, unable to prioritise attendance); "trodden downers" (low numeracy, low self-worth, nervousness of taking control); and "diabetes downers" (denial of diabetes or avoidant behaviour making it difficult to self-manage). These typologies were integrated with the patient survey to quantify barriers. In part 3 (five focus groups) potential solutions were proposed, such as modular or blended learning options and taster sessions for both healthcare professionals and patients.
Conclusion: Social determinants of health such as educational attainment and gender influence attendance at structured education, but health-care professional attitude to courses is key. Identifying and quantifying typologies on the basis of psychological constructs supports recommendations for service redesign— for example; patient champions, motivational interviewing, and clear clinical pathways integrating education courses.
Supported by: CLAHRC & HIN (south London)
Disclosure: **S. Harris:** Employment/Consultancy; Health Innovation Network (AHSN). Grants; South London CLAHRC (NIHR).

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Changing HbA_{1c} units: a hindarance, not a help to patients

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Background and aims: Patient knowledge of HbA1c has been reported as being associated with better glycaemic outcomes. However, despite opposition from some clinicians, in 2009 the way in which HbA1c was reported altered, and for 2 years from June 2009 to May 2011 HbA1c units were dual reported, before % was phased out. In this study we aimed to assess patient knowledge of HbA1c either in terms of mmol/mol or %, to determine how well this change has been received from a patient perspective

Materials and methods: The study was undertaken using data collected as part of the Health and Self-Management in Diabetes (HASMiD) project. This project aims to develop a Patient Reported Outcome Measure to determine the utility of self-management in people with diabetes in the UK, in both patients with type 1 diabetes (T1DM) and type 2 diabetes (T2DM). As part of this we conducted a survey, Oct '16 to Jan '17, and two of the questions related to knowledge of HbA1c, "What is your current HbA1c, either in mmol/mol or %?" and "What do you think is the ideal number for HbA1c? Please choose a number for either mmol/mol or %". Ethics approval for this research was provided by the Coventry and Warwick Research Ethics Committee, 16/WM/0345PR. The demographics of the 2973 total cohort, 835 with T1DM, and 2088 with T2DM were 53.4; 67.4; 47.8 %female, mean+SD: age 56.3+/-15.9; 42.6+/-16.0; 61.8+/-12.1 years, and duration of diabetes 12.0+/-10.8; 20.1+/-14.3; 8.8+/-6.8 years respectively. Statistical significance was determined using paired Student's t-tests for continuous data and Chi-squared tests for categorical data, where a p value of <0.05 was considered to be statistically significant.

Results: Only 41.9% (1247) of participants said they knew their current HbA1c, and this was more common in those with T1DM as opposed to T2DM, 59.8% vs 34.9%, p<0.00001. Participants self-reported a plausible HbA1c more often in mmol/mol vs %, 60% vs 40% respectively. The new units, mmol/mol, were more often adopted in those with T2DM, as opposed to T1DM, 62.6% vs 55.7%, p=0.004. In comparison, knowledge of the ideal target HbA1c was less for the new units of mmol/mol, as opposed to %, 79.8% vs 85.5% respectively. Whilst knowledge of the ideal HbA1c in mmol/mol was consistent across both types of diabetes, 79.9% and 79.8% for T1DM and T2DM, more T1DM patients correctly re-called the target HbA1c in % units, 100% vs 74.9%. In terms of duration of diabetes, those diagnosed since 2011, the new units (mmol/mol), were equally adopted in those with T1DM, as opposed to T2DM, 71.4% vs 66.0%, p=0.15, but patients with T1DM were less able to reliably recall the correct mmol/mol target, than those with T2DM, 81.0 vs 70.9% respectively.

Conclusion: Patient recall of their HbA1c is poor, and understanding of target HbA1cs is sub-optimal for those using either mmol/mol or still using the old units of %. There were differences in knowledge, patients with T1DM were more likely to be able to recall their HbA1c. Whilst the newer units are more often used, especially amongst those diagnosed since the change in units, patient understanding of the ideal target is less reliable than for those still using % for HbA1c. Therefore this study highlights a significant gap in patient knowledge that ought to be addressed in order to try to improve glycaemic outcomes in the UK.

Supported by: The Health Foundation

Disclosure: **J. Elliott:** Grants; The Health Foundation.

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Glycaemic control and associated factors in a population of children and adolescents living with type 1 diabetes in Cameroon

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Background and aims: Free access to insulin in the context of Changing Diabetes in Children (CDiC) program has improved glycemic control in children living with type 1 diabetes in Cameroon. Despite this improvement, glycemic control is still largely insufficient

Materials and methods: We carried out a cross-sectional study on a 5-month period in a group of children living with type 1 diabetes and provided free of charge management in seven different centers of diabetic children's care pertaining to the CDiC program. HbA1c was used to assess glycemic control. For each participant, the last value of HbA1c on a period of 6 months was considered. The analyzed parameters were related to sociodemographic data, history of diabetes, patients knowledge about diabetes, self monitoring of blood glucose level and dietary adherence. Good glycemic control was defined as the achievement of specific HbA1c targets according to age as defined by ADA. Logistic regression analysis was used to identify factors associated with glycemic control. Data was analyzed using SPSS 19.0 and STATA 9

Results: A total of 95 children and adolescents (50 boys) were included in our study. The mean age was 16±3 year with a greater proportion of the study population being aged from 13 to 19 years (85/95). The mean diabetes duration was 4.1± 2.9 (1-14 years). The mean insulin dose per day was 0.79±0.32 U/kg and 68.1% of the study population received 3 or more insulin injections a day. The mean HbA1c was 9.5%±2.8 and only 29.5% of the participants met their glycemic targets. Participants with a level of at least upper secondary education had a better glycemic control ($\chi2$=5.59; p=0.018). Conventional insulin regimen was associated with achievement of HbA1c targets ($\chi2$=16.8; p<0.001). Leaving with both parents was associated with a better glycemic control but this association was not statistically significant ($\chi2$=3.44; p=0.063). Glycemic control seems to be inversely correlated to diabetes duration, but this was not significant ($\chi2$= 1.49; p=0.475). In multivariate analysis, only the conventional insulin regimen was associated with achieving glycemic targets (OR=0.15; p=0.018). That may be explained by the residual secretion of insulin in those with shorter duration of diabetes (the majority of patients with conventional insulin regimen), but also by the less frequent blood glucose monitoring observed in this study

Conclusion: Despite free access to care, only 29.5% of participants achieve their glycemic targets. Conventional insulin regimen has been identified as a determinant of good glycemic control

Disclosure: **C.D.D. Djonou:** None.

PS 056 Economic and social factors contributing to outcomes in diabetes care

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Real-world clinical and economic outcomes of liraglutide among patients with type 2 diabetes at high risk of cardiovascular events, Canadian perspective

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Background and aims: Diabetes affects 3.5 million Canadians, or 9.2% of the population. The prevalence is rising mostly due to an aging population. Annual direct healthcare costs for diabetes are $3-4 billion, with much of it spent on managing macro- and microvascular complications. The Liraglutide Effect and Action in Diabetes Evaluation of cardiovascular outcome Results (LEADER) trial reported a 13% risk reduction in time to first cardiovascular event with liraglutide 1.8 mg compared to placebo (both added to standard of care [SOC]) among subjects with type 2 diabetes mellitus at high cardiovascular risk over 3.5-5 years.
Materials and methods: A state-transition analysis estimated the effects of adding liraglutide or placebo to SOC on clinical and economic outcomes over 25 years from a Canadian public payer perspective. The analysis represented multivariate causal relationships among patient demographics, disease characteristics, management pathways, and endpoints. Endpoint-rate equations for complications were derived from LEADER findings. Price and quality-of-life vectors were from published literature. A 5% annual discount rate was applied to health benefits and costs accrued in the simulation. Univariate and multivariate sensitivity analyses were conducted to assess robustness of the outcomes.
Results: Liraglutide was associated with longer overall survival and quality-adjusted survival compared to placebo (0.75 and 0.46 years, respectively). Cumulative rates for all macrovascular events and nephropathy were lower with liraglutide. The drug/administration costs of liraglutide were partly offset by lower costs to manage complications. The incremental cost-effectiveness ratio per quality-adjusted year gained was $12,808. The sensitivity analyses confirmed the robustness of the findings across a large range of input values, including the hazard ratios for complications.
Conclusion: Adding liraglutide to SOC improves survival and is cost-effective from a Canadian public payer perspective.
Clinical Trial Registration Number: NCT01179048
Supported by: Novo Nordisk A/S
Disclosure: **N. Kragh:** Other; Employee, Author, Stock/Shareholder: Novo Nordisk A/S.

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Low income predicts cardiovascular event risk independently from the presence of type 2 diabetes and pre-existing coronary artery disease

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Background and aims: A low socioeconomic status has been associated with an increased cardiovascular event risk. Whether low income predicts cardiovascular event risk independently from the presence of type 2 diabetes (T2DM) and pre-existing coronary artery disease (CAD) is not known and is addressed in the present study.
Materials and methods: We assessed the annual net income through a standardized questionnaire in a consecutive series of 389 patients referred to coronary angiography for the evaluation of established or suspected stable coronary artery disease (CAD). Prospectively, we recorded cardiovascular events over a mean follow-up period of 8.0±3.7 years.
Results: Annual net income was <€20.000 in 58%, €20.000-35.000 in 33.1% and >€35.000 in 8.9% of our patients. It was significantly lower in women (<€20.000 in 70.4%, €20.000-35.000 in 25.6%, >€35.000 in 4.0 %) than in men (<€20.000 in 53.0%, €20.000-35.000 in 36.1%, >€35.000 in 10.9%; p<0.001) but did not differ significantly between patients with T2DM (n=109) and nondiabetic subjects (p=0.237) nor between patients with CAD (n=225) and those who did not have CAD at angiography (p=0.995). During follow-up, the incidence of cardiovascular events significantly increased with decreasing income: it was 62.4%, 32.4%, and 5.3 % in patients with net incomes of <€20.000, €20.000-35.000 and >€35.000, respectively; p=0.042). Annual net income significantly predicted the incidence of cardiovascular events both univariately (HR 0.77 [0.60-0.98]; p=0.037) and after adjustment for age, gender, smoking, LDL cholesterol, HDL cholesterol, hypertension, BMI, waist circumference, T2DM and angiographically determined baseline CAD (HR 0.68 [0.51-0,92]; p=0.011).
Conclusion: We conclude that a low net income predicts cardiovascular event risk independently from the presence of T2DM and pre-existing coronary artery disease.
Disclosure: **R. Saely:** None.

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Costs and patient outcomes associated with adherence to basal insulin therapy for people with type 2 diabetes

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Background and aims: Studies have suggested that people with type 2 diabetes (T2D) experience improved outcomes when they take their medication as prescribed. However, there is little evidence specifically focusing on adherence to basal insulin therapy or examining the effects of such adherence over an extended time horizon. The goal of this research is to compare costs, resource utilisation, and complications between adherent and non-adherent patients over the 3 year period post initiation on basal insulin therapy.
Materials and methods: US based Truven Health MarketScan® Research Databases from 2011 through 2015 were utilised. Adults age 18 or older identified with T2D who initiated therapy on basal insulin in 2012 were included. Patients were excluded if they were pregnant at any time during the 3 years post-period, if they filled their index basal insulin prescription via mail order, or if they were not continuously insured from 1 year prior through 3 years post initiation on basal insulin. Instrumental variables were used to control for selection bias and general linear models with a gamma distribution and log link were used to examine costs, while logistic and negative binomial regressions were used to examine resource utiliz\sation and acute complications. Adherence was measured by the proportion of days covered (PDC) and patients were considered adherent if they achieved a PDC threshold of 80%. Analyses controlled for patient characteristics, pre-period comorbidities, general health, medication use, visits to specialists, and number of A1c laboratory tests ordered.
Results: There were 21,363 individuals who fit the study criteria. Over the 3 year post-period, patients who initiated therapy on basal insulin and were adherent to such therapy (33.7% of patients) had significantly higher diabetes-related drug costs. However, adherence was also associated with significantly lower diabetes-related outpatient, acute care, and total diabetes-related costs. Results for all-cause costs fit the same general pattern. Patients initiating basal insulin who were adherent had significantly higher all-cause drug costs ($22,267 v $21,030; P<0.0001) and lower total costs ($73,687 v $78,778; P<0.0001). Patients initiating basal insulin who were adherent also had significantly fewer all-cause and diabetes-related hospitalisations and ER visits and were significantly less

likely to be diagnosed with an acute complication (Odds Ratio = 0.766; 95% Confidence Interval 0.713 - 0.823).
Conclusion: Results of this study illustrate that despite higher drug costs, there are both disease specific and all-cause cost offsets and improved patient outcomes associated with adherence to basal insulin therapy for people with T2D. These results suggest that it is important for healthcare providers and payers to create interventions and to work with their patients to improve adherence to basal insulin therapy.

Three Year Outcomes	Non-Adherent PDC < 80% (N=14,149)		Adherent PDC ≥ 80% (N=7,214)		P Value
	Mean	Std Dev	Mean	Std Dev	
Diabetes-Related Outpatient Costs	$6,759	$3,795	$6,319	$3,518	<0.0001
Diabetes-Related Acute Care Costs	$9,789	$6,946	$8,290	$5,709	<0.0001
Diabetes-Related Drug Costs	$10,316	$3,769	$11,898	$4,163	<0.0001
Diabetes-Related Total Costs	$26,800	$9,953	$26,488	$9,176	0.0027
Number of Diabetes-Related Hospitalisations	0.64	0.58	0.53	0.45	<0.0001
Diabetes-Related Hospital Length of Stay	3.68	4.56	2.91	3.51	<0.0001
Number of Diabetes-Related Emergency Room Visits	1.21	1.09	1.00	0.86	<0.0001

Diabetes-Related Costs and Resource Utilisation by Adherence Status

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Ethnic and socioeconomic disparities in type 2 diabetes care: a trend analysis

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Background and aims: The prevalence of type 2 diabetes (T2D) is more common in people of lower socioeconomic status (SES) and those of Black and Asian ethnic groups, who are also more likely to experience complications. It is unclear if disparities in diabetes management contribute to complication rates. We analysed the monitoring of diabetes and complications in England to identify possible disparities in healthcare provision.
Materials and methods: We performed a retrospective cohort analysis using a primary care sentinel network in England (Royal College of General Practitioners Research and Surveillance Centre). We identified people with T2D diagnosed before 2012 and registered for a five year follow up period (2012 - 2016 inclusive). We analysed the monitoring of HbA1c, blood pressure (BP), estimated glomerular filtration rate (eGFR), retinopathy, and neuropathy annually for the period. We ran logistic regression models to identify factors associated with complete monitoring (annual checks for all five years). Factors included were: age, gender, ethnicity, SES, duration of diabetes, and comorbidities. SES was assessed using a governmental measure: the index of multiple deprivation.
Results: We identified 50,615 adults with T2D registered for the follow up period. The proportion monitored has improved annually for HbA1c (82.6% in 2012 to 92.8% in 2016), eGFR (83.3% in 2012 to 92.4% in 2016), and neuropathy (65.5% in 2012 to 72.1% in 2016). A similar trend was seen for BP (86.0% in 2012 to 92.9% in 2015) except a slight reduction in 2016 (91.3%). The monitoring rates for retinopathy peaked in 2013 (68.6%) and have fallen annually since to 59.3% in 2016. The proportions with complete monitoring over these five years were; HbA1c (69.0%), eGFR (66.1%), BP (71.5%), neuropathy (27.7%), and retinopathy (25.8%). People from Black ethnicity groups were less likely to have complete monitoring for HbA1c, eGFR, retinopathy, and neuropathy; those of Mixed ethnicity less likely for HbA1c, eGFR, and retinopathy; and those of Asian ethnicity were more likely for BP and less likely for retinopathy (Table 1). The people in the most deprived SES quintile were less likely to have complete monitoring in all domains investigated.
Conclusion: Monitoring rates for diabetes complications are high and have broadly improved over the last five years with the exception of retinopathy. People are less likely to have consistent monitoring if they are of Black or Mixed ethnicities, or of lower SES. These differences may exacerbate or otherwise contribute to wider ethnic and socioeconomic disparities in diabetes outcomes and should be urgently addressed.

	HbA1c OR (95% CI)	BP OR (95% CI)	eGFR OR (95% CI)	Retinopathy OR (95% CI)	Neuropathy OR (95% CI)
White	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Asian	1.00 (0.94-1.08)	1.08 (1.01-1.16)	1.03 (0.96-1.10)	0.89 (0.83-0.96)	0.94 (0.87-1.01)
Black	0.79 (0.71-0.86)	0.92 (0.83-1.02)	0.75 (0.68-0.82)	0.51 (0.45-0.58)	0.62 (0.55-0.69)
Mixed	0.69 (0.56-0.85)	0.84 (0.67-1.04)	0.76 (0.61-0.93)	0.62 (0.47-0.81)	0.84 (0.67-1.07)
Other	0.71 (0.57-0.87)	0.68 (0.55-0.84)	0.75 (0.60-0.92)	0.69 (0.53-0.90)	0.59 (0.45-0.78)
IMD Quintile 5 (least deprived)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
IMD Quintile 1 (most deprived)	0.78 (0.73-0.83)	0.91 (0.85-0.97)	0.87 (0.82-0.93)	0.67 (0.63-0.72)	0.82 (0.77-0.87)
IMD Quintile 2	0.84 (0.79-0.89)	0.88 (0.83-0.94)	0.94 (0.88-1.00)	0.78 (0.73-0.83)	0.75 (0.70-0.80)
IMD Quintile 3	0.90 (0.85-0.96)	0.95 (0.89-1.01)	0.97 (0.91-1.03)	0.71 (0.67-0.76)	0.88 (0.83-0.93)
IMD Quintile 4	0.89 (0.83-0.94)	0.92 (0.86-0.98)	0.95 (0.90-1.01)	0.91 (0.86-0.97)	0.91 (0.86-0.97)

Table 1. Odd ratios for monitoring between 2012 and 2016, by ethnicity and SES.

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Disclosure: **W. Hinton:** Other; University of Surrey-Eli Lilly and Company Real World Evidence (RWE) Centre in Diabetes.

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Healthcare resource utilisation in patients with diabetes and elevated potassium levels, a Danish population based cohort study

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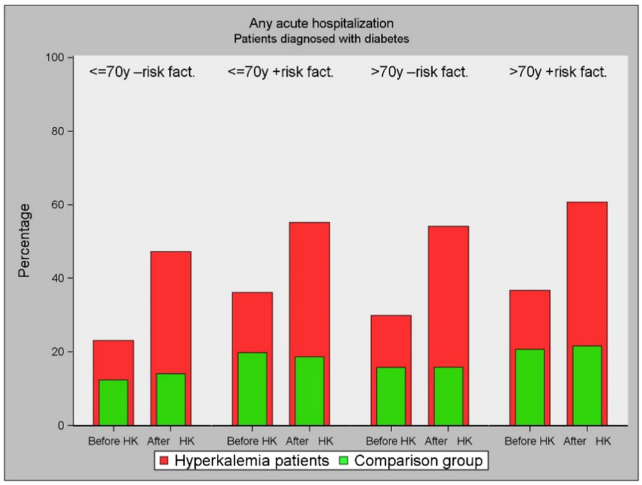
Background and aims: The aim was to investigate healthcare resource utilization (HRU) before and after a hyperkalemia event (HK, i.e. elevated potassium (K+) levels) in patients with diabetes in the Danish clinical practice setting.
Materials and methods: We identified 76,942 patients with a first incident record of DM using population-based health databases between 2000 and 2012, covering the entire population of Northern Denmark (1.8 million). Of these patients, 13,530 were identified with incident HK, i.e., a first blood test in primary care or hospital with K+ level >5.0 mmol/L. HRU was estimated based on patient level information on hospital care, general practitioner consultations, and use of prescription drugs, and was compared 6 months before and 6 months after the HK event (event date of HK included). HRU changes were also explored among age-, gender-, and diabetes duration- matched diabetes patients without HK. Results were stratified by age and presence of kidney disease or chronic heart failure.
Results: Among 13,530 patients with diabetes and incident HK, median age was 71 years, 42% were female, and 49% had a history of chronic kidney disease, 19% chronic heart failure, and 71% hypertension. The *Figure* shows that the proportions with any acute hospitalization increased substantially from 6 months before to 6 months after the HK event, most profoundly among younger diabetes patients without kidney disease or heart failure (increase from 23% to 47%). In comparison

patients without HK, risks were lower and more stable throughout the follow-up. In patients with HK, the mean number of acute hospitalization bed days increased from 3.5 before the HK episode to 6.0 after the HK episode (+72% increase). The corresponding bed days for the matched comparison cohort without HK were 1.3 and 1.4. Non-acute hospitalization bed days increased from 1.0 before HK to 1.9 (+84% increase) after HK (comparison cohort 0.5 before and after), whereas ICU treatments increased from 1 in 33 persons to 1 in 5 persons after the HK event. Mean hospital outpatient visits per patient increased from 1.4 before to 1.6 (+16% increase) after the HK event (comparison cohort, 0.8 before and after). Mean number of GP consultations was rather stable at 4.9 before HK and 4.7 after HK (4% decrease) (comparison cohort 4.2 and 4.1). The average number of individual medication prescriptions filled declined from 30 to 23 prescriptions after the HK event (21% decrease) (comparison cohort 24 and 23) potentially related to drug discontinuation, transfer to hospital care, or mortality associated with the HK event.

Conclusion: The burden of hospitalization is greatly increased after an event of HK in patients with diabetes. As hospital admission costs constitute the largest healthcare cost component, societal costs associated with HK are likely to be high.



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Diabetes and Opiate Replacement Therapy (ORT): a retrospective cohort study of health care usage and clinical outcomes

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Background and aims: People with problem drug use can have chaotic lives and often have high risk health behaviours. Diabetes is a common comorbidity in this population with significant potential impact of excess complications and mortality. We investigated the hypothesis that people with both diabetes and problem drug use engage less with services and have worse outcomes.

Materials and methods: All adults prescribed methadone or suboxone opiate replacement therapy (ORT) between 2011 and 2016 in our region who also appeared in national diabetes database were included. An age, gender and diabetes duration matched case control cohort was identified without ORT use from national database and a comparative analysis performed.

Results: 393 people (age 41.0 (35.9-46.1), male 64%) on ORT and an equally sized matched cohort were included for analysis. ORT group included 389 people who had been prescribed methadone and 38 who had been prescribed suboxone. People on ORT were offered more diabetes clinic review appointments/year than those not on ORT (0(0-1.13),0(0-0.6)p=0.009). Non-attendance at diabetes clinic and retinal screening (proportion appointments not attended/year) was higher in ORT group versus matched cohort ((median(IQR)0.53 (0.33-0.83)0.17(0-0.5)p<0.001)(0.33 (0-0.8),0 (0 -0.2) p<0.001)). In ORT cohort unscheduled hospital attendance rate/year was higher (0.6(0-1.64), 0(0-0.52), p=<0.001). HbA1c levels showed no significant difference (p=0.47) but number of HbA1c measures/year were lower (1.2(0.4-2.0), 1.6(0.8-2.5), p<0.001). 5-year all cause mortality was increased in ORT cohort (Hazard Ratio = 5.14, p<0.001).

Conclusion: People with diabetes and problem drug use engage less with scheduled diabetes services and have more unscheduled care attendances resulting in potentially significant excess healthcare costs. HbA1c is measured less often, but glycaemic control is similar, in ORT patients compared to diabetes only cohort. The similarity in HbA1c may be secondary to acquisition bias in those who do attend but could also suggest that better control is possible when there is engagement with diabetes services. Mortality in ORT using group is much higher than in patients with diabetes alone. This cohort presents a potential target for enhanced case management facilitated through their regular attendance at ORT treatment and care clinics.

Disclosure: **H. Druce:** None.

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Factors influencing non-adherence to diabetes self care activities among diabetic patients in rural Bangladesh

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Background and aims: This study was designed to determine the extent of non-adherence to the 7 components of diabetes self care activities including drug, diet, physical exercise, follow-up visit, risk behaviors, blood glucose test and foot care and to identify the influencing factors.

Materials and methods: A cross-sectional study was conducted among 990 diabetics aged between 18-64 years residing in Thakurgaon district of Bangladesh. Data were collected by face to face interview method.

Results: The proportion of non-adherence was found for drug (65.8%), diet (91.8%), physical exercise (70.6%), follow-up visit (82.5%), risk behaviors (50.6%), blood glucose test (82.5%) and foot care (92.1%). Factors which were found to be significantly influencing to the non-adherences of drug were >2 number of rooms (OR=1.591), >10,000 Tk of monthly income (OR=1.353), residing in their own house (OR=8.059) and using electricity (OR=5.342); to dietary non-adherences were education >5 class (OR=2.609), >10000 Tk monthly income (OR=2.740), >2 number of rooms (OR=1.699), negative family history DM (OR=0.619), had complications (OR=1.679); to physical exercise were having diabetes >2 years (OR=0.756), more cost need to come to hospital (OR=0.714), nuclear family (OR=1.480); to follow-up visits and blood glucose test were >10000 Tk monthly income (OR=1.950), age >25 years (OR=0.313), education >5 class (OR=1.448), >2 number of rooms (OR=1.772), negative family history DM (OR=0.719); to tobacco quitting were education >5 class (OR=0.467), >10,000 Tk monthly income (OR=0.630), parity >2 (OR=1.388), nuclear family (OR=1.381), >2 number of rooms (OR=0.768), having >200 decimal of land (OR=0.763), having diabetes >2 years (OR=0.727), negative family history DM (OR=1.486), more distance (OR=1.380) and more time (OR=2.010) to reach hospital; to foot care were education >5 class (OR=3.493), >10,000

Tk monthly income (OR=3.539), parity >2 (OR=0.639), nuclear family (OR=0.114), >2 number of rooms (OR=1.742), negative family history DM (OR=0.508). Reasons for non-adherences to 7 self care activities were also identified.

Conclusion: An alarmingly high proportion of diabetics' are non-adherent to self care management. Major factors for these non-adherences are found under socio-economic and health care services related characteristics. Long term health educational programs including monitoring and reminder system could motivate patients for proper management of diabetes.

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Disclosure: **B. Banu:** None.

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Long term sickness absence and diabetes: a Danish register-based longitudinal study with up to 17 years of followup

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Background and aims: The number of individuals with diabetes mellitus within the working age range is expected to rise. Type 1 and type 2 diabetes mellitus (diabetes) can have work related consequences such as decreased productivity, increased risks of sickness absence and early retirement. Sickness absence has been used as a central indicator of work disability, but has mainly been examined by short term spells of sickness absence in studies with limited follow-up time. This study aimed to identify the risk of long term sickness absence (LTSA) in individuals with diabetes within the first year of diagnosis and in subsequent years.

Materials and methods: The study was based on registers with up to 17 years of follow-up. In a working population (n=102,746) individuals with the diagnoses of type 1 or type 2 diabetes (n=3,325: women, n=1,987, men=1,338) and individuals without diabetes (n=99,421: women, n=73,332, men=26,089) were identified by Danish national registries of diagnosis (ICD-10 codes: E10.0-10.9, E11.0-E11.9, E12-E14) and prescribed medicine (ATC codes: A10A, A10B, A10BA02), in the period 1994 to 2011. We estimated the hazard ratios of transitions from work to LTSA (>3 consecutive weeks) in Cox proportional multi-state models with separate estimates for the risk within the first and subsequent years after diagnosis. The underlying time axis for the analyses was age. Analyses were stratified by gender and controlled for immigrant status, highest attained education, marital status, calendar year, and job type. Results were reported in terms of incidence rates (LTSA events per 1,000 person years: PY), hazard rates (HR) with a 95% confidence interval (CI) and relative risks for LTSA in subsequent years compared to the first year after diagnosis.

Results: Within the first year of diagnosis, the incidence rates for persons with diabetes were 126 in women (138 events / 1,091 PY) and 100 in men (78 events / 780 PY) with significantly elevated risks of LTSA (HR=1.60, CI: 1.35-1.91 in women and HR=1.57, CI: 1.24-1.98 in men). In the subsequent years after diagnosis the incidence rates for persons with diabetes were 137 in women (784 events / 5,718 PY) and 132 in men (506 events / 3,821 PY) with significantly elevated risks of LTSA (HR=1.49, CI: 1.36-1.63 in women and HR=1.82 CI: 1.60-2.06 in men). The relative risks of LTSA in subsequent years were 0.93 in women and 1.16 in men.

Conclusion: Women and men with diabetes had elevated risk of LTSA, compared to those without diabetes. After the first year of diagnoses the risk decreased slightly in women and increased slightly in men. Studies of other chronic diseases have found substantial decrease in risk of LTSA after the first year of diagnosis - presumably due to a stabilization of disease with treatment. The absence of such an effect in diabetes may be due to diabetes-related comorbidities or poor treatment adherence. Future studies need to consider the impact of diabetes type, co-morbidities, and treatment adherence on LTSA.

Disclosure: **M.A. Nexo:** None.

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Work matters: accounts of the relationship between diabetes and work life in the second Diabetes Attitudes Wishes and Needs (DAWN 2) study

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Background and aims: In previous analyses of qualitative data obtained in the second Diabetes Attitudes Wishes and Needs study (DAWN2), discrimination at work was identified as a theme associated with negative psychosocial experiences for people with diabetes (PWD). We undertook a more specific analysis focusing exclusively on how the topic of work was addressed by people with diabetes who participated in DAWN2. The aim was to obtain insight into the range of experiences that PWD have in the context of work.

Materials and methods: The DAWN2 survey of adult PWD contained open-ended items about challenges of, successes with and wishes for improvement in living with diabetes. All responses to these questions provided by participants (1,368 with type 1 diabetes and 7,228 with type 2 diabetes) were reviewed by the first author and all direct and indirect references to work life were extracted for analysis. An emergent coding procedure was undertaken on the basis of the extracted data and all authors contributed to the development of the thematic framework. A coding manual was written describing the themes and sub-themes in detail. Validation of the thematic framework was performed by two independent coders. The coders worked with the text excerpts independently of one another, coding the data in accordance with the themes articulated in the coding manual. The coders' results were then systematically compared to determine the level of agreement between them. Cohen's Kappa statistic was applied as the interrater reliability correlation coefficient. The kappa score represents a measure of the extent to which a specific theme can be considered an accurate and robust expression of the data.

Results: In total, 343 PWD wrote about work, 95 (27.7%) with type 1 diabetes and 248 (72.3%) with type 2 diabetes, of whom 94 took insulin. The 343 PWD commenting on work included 204 men (59.5%) and 139 women (40.5%). Analysis of the data generated five primary themes (and twenty seven sub-themes): Restriction of Work Opportunity: Work Exclusion; Work downgrading; Work termination (voluntary & involuntary): Negative consequences of work restriction. Diabetes impacts upon Work: Hypo/hyperglycemia; Fatigue; Treatment demands; Distress; Complications. Work influences lifestyle/diabetes self-management: Work as cause of diabetes; Healthcare appointments; Medication & monitoring; Diet; Exercise; Glucose Control; Lack of support. Coping strategies and Benefits; Coping; Work as coping; Social support. Diabetes Awareness: Diabetes diagnosis at work; Compromised work ability; Diabetes specific social learning. Discrimination at work remains prevalent in our data e.g. in accounts of exclusion from work. More generally, specific challenges relating to the impact of diabetes upon work, and vice-versa, are clearly articulated. Finally, affirmative psychosocial experiences are identified in strategies for coping and in supportive social relations.

Conclusion: Recognizing the real limitations that diabetes, its treatment and its complications can have on PWD working, our analysis of the qualitative DAWN2 data highlights the importance of the physical and social environment in which work is undertaken. To the extent that these are supportive of the person with diabetes, the degree to which being at work is experienced as burdensome is likely to be diminished.

Disclosure: **B. Cleal:** None.

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Socioeconomic status and family status but not race/ethnicity are associated with glycaemic control in children, adolescents and young adults with type 1 diabetes

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Background and aims: Aim of the study was to examine associations between socioeconomic status, race/ethnicity, family status, and glycaemic control in children, adolescents and young adults with type 1 diabetes.
Materials and methods: The cross-sectional study included 222 subjects with type 1 diabetes up to the age of 22 years. Data about socioeconomic status, race/ethnicity, and family status were obtained by self-report questionnaires. Clinical data and HbA1c were assessed. Variables were analysed by Man-Whitney-U and Kruskal-Wallis tests, and by multiple regression.
Results: Out of all children, adolescents and young adults with type 1 diabetes (mean age 12.6+/-4.1 years, median HbA1c 8.1%, mean diabetes duration 5.8+/-3.3 years, socioeconomic status: 30% low, 37% moderate, 33% high) 21% lived in a single-parent family and 29% had non-German race/ethnicity. Children, adolescents and young adults from two-parent families had significantly lower HbA1c levels compared to subjects from single-parent families (median HbA1c 7.3% vs 8.0%, p=0.002). Multiple logistic regression analysis revealed that longer diabetes duration (p=0.01), lower socioeconomic status (p=0.006), and single-parent status (p=0.048) but not race/ethnicity (p=0.37) were significantly associated with higher HbA1c levels.
Conclusion: Diabetes duration, socioeconomic status, and family status but not race/ethnicity were associated with glycaemic control in children, adolescents and young adults with type 1 diabetes.
Disclosure: **A. Galler:** None.

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An informed shared decision making programme for patients with type 2 diabetes in primary care: cluster randomised controlled trial

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Background and aims: Informed shared decision making (ISDM) is not yet implemented in diabetes care. We have developed and evaluated an ISDM programme (ISDM-P) for patients with type 2 diabetes in a randomised controlled trial (RCT) at a single diabetes centre. Study results have been published. The aim of the present study was to translate the ISDM-P to primary care in Germany.
Materials and methods: We conducted a 6 months cluster RCT with 22 family practices that participated in the German Disease Management Programme (DMP) for type 2 diabetes and employed a medical assistant (MA) with special training in diabetes education. The ISDM-P comprises a 90 minutes group patient teaching session provided by MA, a decision aid booklet for patients, ISDM training modules for MAs and practitioners (GPs), and a patient-held documentation sheet with patient-defined treatment goals to be shared and discussed with the GP. The control group received standard care supplemented by an extract of the German National DMP Guideline for type 2 diabetes. Patients, 40 to 69 years, without diagnosis of ischemic heart disease or stroke were included. Primary endpoint was patients' adherence to antihypertensive or statin therapy by comparing prescriptions and patient-reported uptake after 6 months. Secondary endpoints included informed choice (risk knowledge and achievement of personal treatment goals), realistic expectations, and prioritised treatment goals. Interviews with MAs and GPs focused on the implementation process. The study was registered and a protocol has been published.
Results: Of 363 eligible patients 279 agreed to participate in the cluster RCT, and 268 completed the 6 months follow-up; 11 practices with 151 patients were randomised to ISDM-P (136 patients participated in a total of 35 group teaching sessions), and 11 practices with 128 patients to standard care. Baseline characteristics were comparable; 45% women, mean age 59 years, duration of diabetes 8 years, blood pressure 140/82mmHg, HbA1c 7.0%. At follow-up 218 patients were prescribed antihypertensive drugs and 107 statins. Adherence to antihypertensive drugs and statin intake was similar for both study groups (81.4% vs. 78.9% and 87.9% vs. 95.6%, resp.). More ISDM-P patients made informed choices regarding statin intake (60% vs. 8%; p<0.001), blood pressure control (65% vs. 7%; p<0.001), and glucose control (67% vs. 7%; p<0.001). Realistic expectations were 3/5 correct answers for ISDM-P and 1/5 for controls (mean difference 2 [95% CI 1.7 to 2.7]; p<0.001). Prioritisation of treatment goals differed significantly between groups. Among ISDM-P more patients prioritised blood pressure control rather than HbA1c targets. Matching of treatment goals between patients and GPs was higher for ISDM-P; prioritized goals matched for 89% vs. 57%; p<0.001. GPs stated that the patients were well prepared for decision making. MAs described workload for the ISDM-P as similar as for DMP patient education modules.
Conclusion: The ISDM-P was successfully implemented in the primary care setting. Patients were able to make informed choices. The patient-held documentation sheet ensures that patients and physicians pursue common treatment goals.
Clinical Trial Registration Number: ISRCTN77300204
Supported by: EFSD/AZ
Disclosure: **S. Buhse:** None.

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Theory based diabetes self-management education with pre-selection of participants: results of a randomised controlled trial with 2.5 years follow-up

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Background and aims: Many diabetes self-management programs could improve lifestyle and reduce cardiovascular risk, but changes tend to be small and short-lived and programs are not beneficial for all patients. The course Beyond Good Intentions in people with screen-detected type 2 diabetes showed sustained effectiveness after one year. It is a 12 weeks, group-based self-management course with four sessions focusing on sustained change of health behaviour by proactive coping. The aim of the current study was to evaluate the 2.5-year effectiveness of this course on clinical parameters for cardiovascular risk in a group of pre-selected patients with known type 2 diabetes.
Materials and methods: The Eindhoven Long-term Diabetes Education Study (ELDES) study is a parallel randomised controlled trial (1:1) conducted in Dutch primary care. Pre-selection of eligible participants who were most likely to benefit from a diabetes self-management education program was performed; patients were randomised to intervention group or control group. Changes in BMI, weight, systolic blood pressure, fasting glucose, HbA1c, and lipid profile between baseline and 2.5-year follow-up were analysed using ANCOVA, adjusted for baseline values.
Results: 108 participants were included in two separate periods (2015 and 2016) (56 intervention group, 52 control group); the first 48 were available for analysis. BMI and body weight decreased in both groups (intervention BMI from 30.0±5.2 kg/m^2 to 29.6±4.9 kg/m^2; decrease in percentage body weight -1.6%±5.5 and control BMI from 29.8±3.7 kg/m^2 to 28.8±3.4 kg/m^2 and decrease in percentage body weight -3.3%±4.6); no significant group effect (BMI F=1.729, p=0.195; body weight F=1.280, p=0.264). Although non-significant, systolic blood pressure decreased in the intervention group (from 133 ± 13.6 mmHg to 131 ± 16.9 mmHg) and increased in the control group (from 131 ± 12.9 mmHg to 134 ± 15.9 mmHg); no significant group effect (F=1.096, p=0.301). LDL-cholesterol showed a significant group effect in favour of the control group (F=4.869, p=0.032). Other values remained stable and on target over time (table).
Conclusion: Both groups were on target at baseline and during follow-up. Because patients from the first round were at baseline similar to patients from the second round, the results of current analysis are likely to hold true for the total study population. Since Dutch diabetes care is already efficient, no further beneficial effects can be expected from self management programs on biomedical factors. Perhaps the aim of self-management intervention should shift from further reducing cardiovascular risk to improving factors like quality of life and patient satisfaction.

Table – Baseline and 2.5-year follow-up scores

	Intervention group (n=23)		Control group (n=25)		Group effect	
	T0	T1	T0	T1	F	P-value
BMI (kg/m^2)	30.0 ± 5.2	29.6 ± 4.9	29.8 ± 3.7	28.8 ± 3.4†	1.729	0.195
Body weight (kg)	87.8 ± 16.4	86.2 ± 15.3	87.4 ± 17.3	84.2 ± 15.5†	1.673	0.203
Body weight (%)		-1.6 ± 5.5		-3.3 ± 4.6†	1.280	0.264
SBP (mmHg)	133 ± 13.6	131 ± 16.9	131 ± 12.9	134 ± 15.9	1.096	0.301
Venous fasting glucose (mmol/L)*	7.8 ± 2.1	7.9 ± 1.2	7.8 ± 2.4	7.2 ± 1.3	3.463	0.069
HbA1c (mmol/mol)*	48.0 ± 7	48.0 ± 8	50.0 ± 10	49.0 ± 7	0.005	0.941
Lipid profile						
Total cholesterol (mmol/L)	4.5 ± 1.0	4.5 ± 0.9	4.1 ± 0.9	4.1 ± 0.8	0.272	0.605
LDL-cholesterol (mmol/L)	2.6 ± 0.9	2.6 ± 1.0	2.3 ± 0.8	2.0 ± 0.7†	4.869	0.032‡
HDL-cholesterol (mmol/L)	1.3 ± 0.3	1.3 ± 0.4	1.2 ± 0.4	1.2 ± 0.5	0.193	0.663
Triglycerides (mmol/L)*	1.4 ± 0.9	1.4 ± 0.9	1.7 ± 0.8	1.6 ± 1.4	1.157	0.290

Data are means ± SD, or *medians ± IQR
† Significant change over time within group (p-value <0.05)
‡ Significant group effect (p-value <0.05)

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One off structured dietary education can improve diabetes management in Nepal

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Background and aims: The prevalence of diabetes is rising in urban Nepal. It is estimated that 20% of adult population have diabetes or pre-diabetes state. The treatment of diabetes is focused mainly on medication there. Diabetes education if at all provided is delivered by doctors in a busy clinic. There is no structured education programme. We wanted to see if one off structured education focusing on nutrition can improve control in newly diagnosed type 2 diabetes.
Materials and methods: Type 2 diabetes subjects (between the age of 30 to 60 years and within one year of diagnosis) who were attending one of the 5 urban clinics in Kathmandu were approached by the investigator. Out of 780 eligible subjects 150 gave written consent. Subjects were randomised to 'Education' or 'Control' group. Education group received one off 90 minutes structured education around dietary guidelines as recommended by WHO. Baseline and six month follow up data were collected from both groups.
Results: Sixty patients were randomised to 'Education' and 90 to 'Control; group. They were similar in age and gender ratio. Mean monthly expenditure was high in 'Education' group than in 'Control' group (€ 9.56 vs € 6.10) at baseline. At 6 months, there was a significant reduction in fasting glucose, 2 hour post prandial glucose and HbA1c in both groups in comparison to baseline. In 'Education' group this difference was significantly lower than the 'Control' group after 6 months. A trend in greater reduction was also seen in post prandial glucose and HbA1c in 'Education' group. There was no change in BMI or waist circumference between both groups at follow up. There was no difference in lipid profile however there was significant improvement in cooking style with Education group using more boiling but less frying. The mean numbers of fruit serving improved from 0.5 to 1.3 servings in the education group at post survey, whereas in control group, the mean declined from 0.4 to 0.3 serving.
Conclusion: This study shows that in resource limited countries, one off structured education can improve diabetes care in the form of low fasting blood glucose. There is a trend for the reduction in HbA1c. This short education programme changed dietary habit in a positive way and the effect lasted 6 months. Further larger scale studies are needed to validate this.

RESULTS

	Education		Control		P value
	Baseline	6 months	Baseline	6 months	
Fasting Glucose mmol	8.6 +/- 2.9	6.6 +/- 1.2	8.0 +/- 1.8	7.0 +/- 1.8	0.005
Post prandial Glucose	11.2 +/- 3.7	8.3 +/- 10.2	11.5 +/- 4.0	9.7 +/- 2.3	0. 06
HbA1c (%)	8.9 +/-2.1	7.8 +/- 1.5	8.7 +/-2.4	8.1 +/- 1.2	0.06
BMI	25.8	25.4	26.4	27	0.275

Disclosure: **J. Pradhan:** None.

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A diabetes education programme with supervised physical activities: results from 12 years and almost 20.000 attendances

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Background and aims: Diabetes education and physical exercise are important parts of the diabetes (DM) treatment. With this in mind, the Doce Desafio Program (DDP) was created in 2001 and with the data collected over the years it was possible to do a preliminary analysis that shows some relevant results.Aim: To describe the profile and the characteristics of those with DM that started with the program.
Materials and methods: DDP is a free diabetes education program in a public university in which classes of health education and supervised physical exercises are performed for people with DM. The meetings (120 min) occur 1-3 times a week. The regular routine is: 1) the capillary blood glucose (BG) and the blood pressure (BP) are measured at the beginning of each class. Diet, the medication and the overall well-being are also assessed; 2) there is a protocol for adjustments (with carbohydrates, insulin or exercise) when necessary. 3) 60 min of supervised physical activities; 4) a health educational activity (30min) taking taught by a multidisciplinary staff; 5) again measures (BG and BP). Data were collected from 2001-2012 (socio-demographic, clinical) from individual files.
Results: 786 people with DM participated with the following characteristics: Age= 4-90 (52+18) years; 63.1% female; 80.9% T2DM; time since DM diagnostic 0-50 (7+8) years; 36% in insulin therapy (IT); 26.5% uses public health system; education: fundamental 21.5%, high school 24.2%, superior 33.7%; attended 1-305 (24+40) classes. Regarding to the age, 7.5% are 4-17 years and 57.1% are >54 years old (23.2% in IT). At admission, 19.3% of T2DM were in insulin therapy (IT)[em1] . Most attendants with T2DM presented hypertension. Attendances frequency): 73.2% people had <25 classes (4076 attendances); 14.8% had 25-50 (4163), 10.1% had 51-170 (7438), and 1.9% (n=15) were present in >170 classes (4109 att. through >3 years). There were 19.782 attendances over 12 years with almost 40000 BG measures. In average, BG measure (in mg/dl) was 181+83 (56 to 579, median 163) before the classes and 144+68 (44 to 492, median 123) after this classes. After 12 classes the values were reduced consistently; in the 24th class the mean BG values were 124+34 (median 118).
Conclusion: Regular and continuous diabetes education and supervised physical activities are possible and can be a good way to the commitment.
Disclosure: **J. Dullius:** None.

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Assessment of the efficacy of intensive life style modification programme in overweight (obese) patients with type 2 diabetes

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Background and aims: To evaluate the efficacy of intensive life style modification program in overweight (obese) patients with Type 2 diabetes mellitus (T2DM) on clinical and metabolic parameters.
Materials and methods: 80 patients with T2DM were recruited (60 - intervention group, 20 - control group). Mean age (intervention group) was 54 years [46; 59.8]; diabetes duration 5 years [2; 10.8]; weight 96.7 kg [88.3; 103.2]; BMI 34.6 kg/m^2 [32; 36.5]; waist circumference 108.5 cm [105; 113]; fasting plasma glucose level 8.0 mmol/l [6.4; 9.9]; HbA_{1c} 6.9% [6.1; 8.2]. The control group was matched to the intervention group by the clinical and demographic characteristics. This program was adapted from Weight Achievement and Intensive Treatment (Why WAIT) a 12-week multidisciplinary program for weight reduction and intensive diabetes management designed and implemented at the Joslin Diabetes Center in Boston since 2005. The main components of the program were structured dietary intervention, individualized exercise intervention, cognitive behavioral intervention, group education and antidiabetic drug adjustment. During 1st phase (3 months) educational sessions were conducted once per week in small groups with team of specialists (dietician, diabetologist, clinical exercise physiologist and psychologist). During 2nd phase (9 months) monitoring was carried out monthly. Anthropometric, clinical and metabolic parameters were measured initially, after 3 and 12 months. Whenever applicable, diabetes medications were changed to ones that have little or no impact on body weight. Thus 21% of patients received metformin as the monotherapy; DPP-4 inhibitors were one of the antidiabetic medications in 55% of patients.
Results: Currently 47 patients of intervention group completed the study. Following parameters decreased: body weight from 97.0 kg [89.5; 103.3] to 88.2 [82.8; 95.1] after 3 months and to 87.0 [83.1; 98.5] after 12 months (p=0,000); BMI from 34.6 kg/m^2 [32.0; 37.0] to 32.1 [29.7; 34.9] and to 32.1 [28.4; 34.5] (p=0,000); waist circumference from 109 cm [105; 113] to 100 [96; 109] and 101 [97; 106] (p=0,000); fasting plasma glucose level from 8.3 mmol/l [6.5; 10.0] to 7.1 [6.2; 8.3] and to 6.4 [5,7; 8.3] (p=0,000); HbA_{1c} from 6.9 % [6.1; 8.1] to 6.2 [5.8; 7.3] and to 6.3 [6.0; 7.1] (p=0,000); triglycerides from 1.9 mmol/l [1.3; 2.7] to 1.6 [1.0; 2.0] and to 1.4 [0.9; 2.2] (p=0,000) after 3 and 12 months, respectively; C-reactive protein from 2.5 mg/L [1.6; 5.2] to 1.9 [0.8; 4.0] (p=0,047); sistolic BP from 130 mm Hg [120; 140] to 120 [120; 135] (p=0,010); diastolic BP from 85 mm Hg [80; 90] to 80 [75; 80] (p=0.000) after 12 months. There were no statistically significant changes in total cholesterol, LDL, HDL after 12 months. At the end of the study in 11 participants weight reduction was 5% and more and in 19 participants 10% and more. In patients on insulin therapy the daily dose of long-acting insulin was reduced from 10% to 50%. In patients on sulfonylurea drugs therapy was stopped in 58.3% while dose reduction by 50% was achieved in 16.7% of patients.
Conclusion: The program demonstrated significant weight and waist circumference reduction accompanied by improvement of metabolic parameters during 12 months as well as beneficial changes in diabetes medications.
Supported by: NOVARTIS
Disclosure: **S. Andreeva:** Grants; NOVARTIS.

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Glycaemic control changes after the programme of personalised support for the patients with type 2 diabetes on insulin therapy

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Background and aims: Patient education and long-term follow-up is regarded as the basis for improving glycemic control. The aim of the study was to assess glycemic control after the program of personalized support for the patients with type 2 diabetes on insulin therapy.
Materials and methods: Study group consisted of 305 patients with type 2 diabetes (68 men/237 women; mean age 61,3±8,4 years) from several regions of the country receiving different regimens of insulin therapy. All subjects were educated by a specially created structured program, which is a part of the project of personalized support of the patients realized by medical centre in cooperation with national public organization of diabetes patients. Unlike existing structured program in “Diabetes Schools” this program, besides 5 standard classes on main topics, includes 3 additional classes organized in 3, 6 and 9 months after the end of the basic course. Educational program included methodological guideline with the material discussed at the classes, “plates”-cards with the images of the food and educational posters. Before participation in the project endocrinologists were trained during a 2-days workshop. During the main course classes (one class a week in groups no more than 10 people, duration 2,5-3 hours) following topics were discussed: basic knowledge about

diabetes, diet, insulin therapy, blood glucose self-monitoring, diabetic complications prevention and treatment. During additional classes (duration 2,5 - 3 hours) individual dynamics of significant metabolic and clinical characteristics were analyzed. The achievement of individual target values was evaluated and the obstacles preventing from the effective control of the disease were discussed during those classes as well. Sustainment training of practical skills in insulin therapy and blood glucose control, insulin therapy regimens and doses of insulin or other medications - all this was done at the additional classes. HbA1c level was evaluated before the start of the educational program, and then in 3, 6 and 9 months after the end of the basic course.

Results: Before the beginning of the educational program the mean level of HbA1c was 8,4 [7,4; 9,3] %, in 3 months after the end of the main educational course the mean level of HbA1c was lowered to 8,0 [7,3; 9,2] % (p<0,001 comparing to the baseline level), in 6 months - to 7,9 [7,1; 9,1] % (p<0,001), in 9 months (around a year after the beginning of participation in the project) - to 7,7 [7,1; 8,6] % (p<0,001). Before the educational program most commonly recommended target level of HbA1c <7,0% was achieved only by 42 (13,8%) patients, and the amount of patients who had HbA1c level higher than maximum allowed target level according to the national guidelines (≥8,0%) was in 182 (59,7%) patients. At the end of the study HbA1c <7,0% was found in 47 (15,4%) patients (NS), and the amount of patients with HbA1c ≥8,0% was lowered to 130 (42,6%) patients (p<0,001).

Conclusion: The program of personalized support for the patients with type 2 diabetes on insulin therapy is the first educational program in the country with a long-term follow-up and personalized support of the patients. This program allows significantly decreasing the amount of patients not reaching the target level of glycemic control.

Supported by: Public organization "Russian Diabetes Association"

Disclosure: **A.Y. Mayorov:** Grants; Public organization Russian Diabetes Association.

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My diabetes my way: user experiences of an electronic personal health record for diabetes

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Background and aims: My Diabetes My Way (MDMW) is a website containing interactive resources for people with diabetes and their carers. It incorporates an electronic personal health record (ePHR) allowing online access to clinical results and assessments. We aimed to assess user experience and perceived benefits.

Materials and methods: In January 2015, an online evaluation survey was emailed to 3,979 active users of the MDMW ePHR. This questionnaire aimed to capture qualitative and quantitative data using a series or open and closed questions. Patients completed the questionnaire online using Survey Monkey and provided information detailing the impact the system had on their satisfaction and how it enhanced their ability to self-manage.

Results: 1,095 (27.5%) active users completed the survey. Key results: 92.1% - contained all expected features; 87% - serves as reminder of information discussed at consultations; 89.6% - helps make better use of consultation time; 85.2% - don't need to phone doctor for results; 89.1% - system was easy to use; 94.2% - supporting information helped to understand results better; 93.7% - helped find information tailored to own diabetes; 95.9% - graphs helped monitor changes over time; 96.7% - confident the system was secure; 88.2% - helped manage diabetes better; 90.3% - improved knowledge; 89.3% - improved motivation; 89.8% - helps set own goals; 96.4% - system will significantly improve diabetes self-care in Scotland. Feedback: *"Immensely satisfied with the system. Really amazing to see my results online. Real motivator"*;*"I don't have to make appointments to see GP or nurse which is good as I work and don't like taking time off work"*

Conclusion: MDMW is a highly-effective intervention in the pursuit of supported self-management. Patients report enhanced knowledge and understanding of diabetes and improved motivation to make positive changes. MDMW is a key resource to engage patients in order to achieve strategic aims for the diabetes population in Scotland. We are actively pursuing opportunities to extend the service into other parts of the UK, and beyond.

Supported by: Scottish Government eHealth and Scottish Diabetes Group

Disclosure: **S.G. Cunningham:** None.

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Improved treatment engagement among patients receiving insulin glargine 300 U/ml who enrolled and received live support through the COACH patient support programme

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Background and aims: The insulin glargine 300 U/ml (Gla-300) COACH Support Program provides support for patients with diabetes initiating Gla-300 by delivering tailored, product-focused educational materials and encouraging lifestyle changes for better glycaemic control. This analysis specifically evaluated the impact of COACH live phone support on treatment engagement.

Materials and methods: Patients who enrolled in COACH received a welcome call from a Guide to identify specific disease/therapy management needs. Ongoing support included contact with the Guide to reinforce healthcare provider recommendations and access to training sessions/digital tools. Patients who filled a first prescription for Gla-300 in April −December 2015 and completed the welcome call were matched to control patients receiving Gla-300 from the Symphony Health Solutions Integrated Dataverse™ prescription claims database, based on demographic attributes (age, gender, geographical location, prior use of insulin/other agents, diabetes type, insulin dose, co-pay, and payer type). Number of refills and days on therapy (days of Gla-300 supply summed for all patients divided by total number of patients) were determined at 6 and 9 months.

Results: The analysis population included 1,724 COACH patients and 1,724 matched controls. The COACH and control cohorts comprised: 52% men, 48% women; 22% aged 18−47 years, 23% 48−55 years, 27% 56−61 years, and 28% >61 years. A total of 99% of patients had used insulin in the 1-year period prior to the welcome call (45% insulin glargine 100 U/ml; 54% another insulin), and 71% had a co-pay of $15 on their first paid Gla-300 prescription claim. More COACH vs control patients had commercial health insurance (42% vs 34 %) and Medicare coverage (12% vs 5%); 43% and 58% COACH and control patients, respectively, received coupons/discount cards or payment aids ($P < 0.0001$ for all). After 6 months, COACH patients refilled their prescription on average 3.2 times vs 2.4 times for control patients; at 9 months, the average number of refills was 4.7 for COACH patients vs 3.6 for control patients ($P < 0.0001$ for both time points). The average number of days on therapy was 102.2 and 151.9 for COACH patients, and 81.5 and 121.6 for control patients, at 6 and 9 months of follow-up, respectively ($P < 0.0001$ for both time points). At 9 months, an increase of 32% and 25% in number of prescription refills and length of therapy, respectively, was observed for the COACH cohort vs control cohort.

Conclusion: Patients who received live phone support through the COACH Support Program were more likely to refill their prescriptions and stay on therapy. Enrolment in programmes with a phone-support component may contribute to improved insulin therapy.

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Disclosure: **J. Goldman:** Employment/Consultancy; consultant for Becton, Dickinson and Company. Other; member of the speaker's bureau for Sanofi US, Inc. and Novo Nordisk, Inc.

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The process of adaptation following a diagnosis of type 1 diabetes in adulthood

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Background and aims: Little consideration has been given to the experiences of patients following a diagnosis of type 1 diabetes in adulthood, despite the fact that more than half of all cases occur during this time. A diagnosis of type 1 diabetes is a major event and necessitates significant physical and psycho-social adaptation. The adaptive strategies adults engage in following their diagnosis may influence their future risk of diabetes complications and their psycho-social well-being. Therefore, to better understand and attend to the needs of adults at and following their diagnosis, the aims of the study were: Firstly, to explore the experience of being diagnosed with type 1 diabetes in adulthood and secondly, to explore the underlying bio-psycho-social phenomena that influence the process of adaptation when becoming a person with diabetes.
Materials and methods: Thirty participants were recruited from hospitals in Denmark and the UK, sixteen were men. At the first interview the median age of participants were 29 years (range 20-67); the median diabetes duration was 23.5 months (range 3-46) and median HbA1c was 48 mmol/mol (range 55 - 138 mmol/mol). Longitudinal semi-structured interviews were conducted face to face followed by a telephone interview six months later. A narrative approach detailing the themes emerging from the narratives was used for the analysis of the interviews.
Results: From the participants' narratives it was evident that a diagnosis of type 1 diabetes in adulthood is perceived as a major disruption to their daily life. Adapting to a life with diabetes was experienced as a multi-dimensional process occurring over a long period of time. The study identified the following bio-psycho-social phenomena as being influential in the adaptive process: their prior life experiences; their understanding of diabetes; their emotional responses to the diagnosis; the way diabetes is introduced to them and their ongoing interactions with healthcare professionals; the expectations placed on them and those they place upon themselves in relation to their diabetes control and behaviours; their experience of the physical elements of the disease; their perception of the impact diabetes has on their lives; their interactions with others (family, friends and the wider public); their perception of how others view them; and the extent to which they accept diabetes as part of themselves. The findings showed that the healthcare support adults receive following their diagnosis largely focuses on technical skills and disease specific knowledge. Less attention is given to support the accommodation of diabetes in their day-to-day lives.
Conclusion: Following their diagnosis adults engage in a lengthy and multi-faceted process of adapting to a life with type 1 diabetes. Our findings suggest that psycho-social support during this early 'fluid' phase of the disease may be important in preventing the development of harmful emotional responses and behaviours that can negatively impact their risk of complications and reduce their well-being short and long term. Such interventions need to be instigated early from the point of diagnosis, with a focus on developing positive adaptive strategies and thinking styles.
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Disclosure: **M. Due-Christensen:** Grants; Foundation of European Nurses in Diabetes.

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The influence of affective temperaments on the glycaemic control and glucose variability of patients with type 1 diabetes

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Background and aims: Increased glucose variability (GV) is an important clinical feature of type 1 diabetes mellitus (T1DM) and a dominant component of glycaemic control. Increased GV enhances the hypoglycaemia risk and worsens the quality of life. However, we have limited information about the possible causes of between-patient variation in GV. Affective temperaments (such as depressive, cyclothymic, hyperthymic, irritable and anxious) are genetically determined personality traits which remain unchanged through the life. They influence the emotional reactivity through affecting the level of activity, biorhythms, mood and thinking process. It's proven that affective temperaments show significant correlation with the compliance of patients having chronic diseases. The aim of our study was to examine the influence of affective temperaments on glycaemic control and GV in patients with T1DM.
Materials and methods: 186 T1DM patients on analogue insulin therapy (98 male, mean age 36,61±13,00 years, with 14,90±11,22 years of diabetes duration) completed the TEMPS-A questionnaire, which is a 110-item instrument to measure affective temperaments. The limit for dominant temperaments was determined by adding 2 standard deviations (SD) to the mean score of the population on the subscale. We compared the results with the pattern of affective temperaments of healthy Hungarian population. A one-way between-groups analysis of variance was conducted to explore the effect of affective temperaments on glycaemic control, as measured by actual HbA_{1c} level. Then a one-way between-groups multivariate analysis of variance was performed to investigate the association between temperaments and indicators of GV (MBG: Mean Blood Glucose, SD, MAGE: Mean Amplitude of Glucose Excursion, HBGI: High Blood Glucose Index and LBGI: Low Blood Glucose Index) calculated from a six-day-long continuous glucose monitoring (CGMS) of the patients. Clinical parameters (sex, age, diabetes duration, BMI, C-peptide and specific insulin dose) were used as covariates.
Results: Prevalence (14%) and distribution of dominant temperaments in the T1DM group fitted to the standard population ($p=0.5229$ and $p=0.481$, resp.). The higher score on the depressive and anxious subscale correlated significantly with higher HbA_{1C} values ($p=0.012$ and $p=0.041$, resp.) Statistically significant differences were found in patients with higher scores on the depressive, hyperthymic and anxious subscales on the combined indicators of GV ($p=0.013$; $p=0.031$ and $p<0.001$, resp.). When the results for certain indicators were considered separately, significant associations were perceived between the depressive scale and MBG and MAGE, the hyperthymic scale and LBGI, the anxious scale and MBG, SD and MAGE using a Bonferroni adjusted alpha level of 0.01. All results were independent from the clinical parameters.
Conclusion: To the best of our knowledge, this is the first report to show the direct and independent effect of affective temperaments on metabolic control and GV in patients with T1DM. Our study suggests that higher points on the depressive and irritable scales correlate with significantly worse metabolic state and higher glucose fluctuations, and hyperthym temperament correlates with increased risk for hypoglycaemia.
Disclosure: **S. Orbán:** None.

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Depression differed by midnight cortisol secretion, alexithymia and anxiety between diabetes types: a cross sectional comparison

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Background and aims: Increased prevalence of depression is found in both type 2 diabetes (T2D) and type 1 diabetes (T1D). Melancholia and atypical depression differ by cortisol secretion and clinical features. The aim was to compare between T1D and T2D patients the associations between self-reported depression, self-reported anxiety and alexithymia, midnight salivary cortisol (MSC), and obesity.

Materials and methods: Comparative cross-sectional design. The study was performed in one hospital diabetes outpatient clinic. Participants were T2D patients (n = 24; 31-59 years) and T1D patients (n = 148; 32-59 years). Self-reported depression, anxiety and alexithymia were assessed by Hospital Anxiety and Depression scale and Toronto Alexithymia Scale-20 items. MSC, HbA1c, anthropometrics and data from medical records were collected. Non-parametric tests and multiple logistic regression analysis were performed.

Results: Comparisons of prevalence between diabetes types showed for T2D/T1D: depression 25%/12% (P = 0.10); high MSC (≥9.3 nmol/L) 38%/22% (P = 0.13); alexithymia 25%/13% (P = 0.12); anxiety 38%/35% (P = 0.82). The prevalence of high MSC did not differ between depressed (17%) and non-depressed (44%) T2D patients (P = 0.35), but differed between depressed (53%) and non-depressed (18%) T1D patients (P = 0.003). The alexithymia prevalence differed between depressed (67%) and non-depressed (11%) T2D patients (P = 0.018), and between depressed (47%) and non-depressed (11%) T1D patients (P < 0.001). The anxiety prevalence did not differ between depressed (67%) and non-depressed (28%) T2D patients (P = 0.15), but differed between depressed (76%) and non-depressed T1D patients (30%) (P < 0.001). The obesity prevalence (BMI ≥30 kg/m^2) was for the depressed patients: T2D 80% and T1D 6%. In the T2D patients, depression was associated with alexithymia (AOR 15.0). In the T1D patients, depression was associated with anxiety (AOR 11.0), foot complications (AOR 8.5), HbA1C > 70 mmol/mol (AOR 6.4), and high MSC (≥9.3 nmol/L) (AOR 4.8).

Conclusion: The depressed T2D patients had traits of atypical depression, without associated high MSC (≥9.3 nmol/L) and anxiety, but the association with alexithymia was strong. The depressed T1D patients had traits of melancholia with associated high MSC and anxiety. The obesity prevalence was high in depressed T2D patients and low in depressed T1D patients.

Supported by: Department of Research and Development, Region Kronoberg, Växjö, Sweden

Disclosure: **E.O. Melin:** None.

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Validation of a shortened 11-item version of the Problem Areas in Diabetes scale to measure distress in adults with type 1 diabetes

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Background and aims: There is a need for a brief measure to assess diabetes-related emotional distress in people with type 1 diabetes (T1D) and also responsiveness following interventions aimed at reducing such distress. The 20-item Problem Areas in Diabetes (PAID-20) questionnaire is lengthy for routine use in clinical settings. We analysed PAID data to determine whether a shortened form could be derived without loss of reliability, validity and sensitivity to change in a large cohort of people with T1D.

Materials and methods: Information was extracted from a research database covering 10 UK centres, including PAID data collected before and one year after participants with T1D had attended a DAFNE (Dose Adjustment For Normal Eating) diabetes structured education course. Exploratory factor analysis (EFA) with a principal axis factoring method was conducted on the baseline (pre-intervention) data to determine whether a shorter PAID scale would retain the psychometric properties of the original scale. Confirmatory factor analysis (CFA) was conducted on 1-year post-intervention data to check the reliability of the initial factor solution.

Results: Of 2496 people taking the DAFNE course, baseline PAID-20 data were available for 1772, (mean age 48 (SD 14) years, duration of diabetes 24 (14) years and 51% female). One year follow-up data were complete in 1096 with HbA1c 8.67 (1.54)% (71.3 (16.9) mmol/mol) before and 8.37 (1.46)% (68.0 (15.1) mmol/mol) 12 months after DAFNE (P<0.001) and corresponding PAID-20 scores 27.9 (19.6) and 20.2 (17.4) (P<0.001). We successively removed from PAID-20: a) one question with a factor loading less than 0.50, b) 5 questions with mean scores below 1.0 (indicating floor effects) to create PAID-14 and finally, c) the 3 questions with the lowest reliability (convergence with both PAID-14 and PAID-20 < 0.65) to formulate an 11-item PAID. PAID-11 has high internal (Cronbach's α = .96) and test-retest (r = .61) reliability. For the post-DAFNE confirmatory analysis, the same 11 questions in PAID showed the highest factor loadings. The area under the curve analysis showed the optimal cut-off score for detecting significant diabetes related distress (equivalent to PAID-20 score of 33) was 16.5 in PAID-11. For the 1034 participants with complete, paired data, the PAID-11 score fell from 14.1 (10.1) at baseline to 11.0 (9.1) after DAFNE (P<0.001).

Conclusion: There is apparent redundancy in the PAID-20 questionnaire as applied to people with T1DM as an 11-item version of the questionnaire can be used without loss of sensitivity or specificity, and shows a similar responsiveness to change after an intervention. The PAID-11 questionnaire therefore appears suitable both to determine diabetes related distress in people with T1D and responsiveness to a relevant intervention. Its relative brevity may enhance its usefulness in everyday clinical practice.

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Vision related quality of life and locus of control in type 1 diabetes. A multicentre observational study

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Background and aims: Vision plays an important role in the ability of people to process information from their environment and to participate in everyday activities. Diabetic Retinopathy (DR) is considered to remain asymptomatic until it reaches its late stages. However, subtle changes in vision related quality of life (QoL) may occur even in mild DR and their perception may be related to a patient's locus of control (LoC) of his/her disease. To evaluate vision-related QoL and LoC in patients with Type 1 Diabetes and different stages of DR.

Materials and methods: The 25-item National Eye Institute Visual Functioning (NEI VFQ-25) and Locus of Control (LoC) questionnaires were self-administered to 258 patients between January 2014 and March 2017 in 9 DR screening centres. The NEI VFQ-25 explores 12 dimensions**:** General Health (GH), General Vision (GV), Ocular Pain (OP), Near Activities (NA), Distance Activities (DA), Visual Specific Social Functioning (VSSF), Mental Health (VSMH), Role Difficulties (VSRD), Dependency (VSD), Driving (D), Colour Vision (CV) and Peripheral Vision (PV). The LoC questionnaire includes 18 items assessing 3 areas: Internal Control of disease, the role of Chance and trust in Others (family members, health operators). Data on socio-anagraphic variables and presence of DR, cataract and previous laser treatment (LT) were collected.
Results: Patients included 124 women and 134 men aged 42.0±12.4 years and with 28.0±12.4 disease duration. DR was absent (n=74), mild-moderate (n=75), severe (n=96) and laser treated (n=13). DR severity was directly associated with disease duration (p<0.001) and measuring blood glucose less than 4 times a day (p=0.013). Smoking showed a trend to protect from DR. The patients with no DR had better scores for GH (p=0.0008), GV (p=0.0007), CV (p=0.045) and PV (p=0.0001) than those with mild and more severe DR. There were no differences in any of the LoC areas.
Conclusion: Even in mild asymptomatic stages, DR is associated with impaired perception of vision related quality of life, with specific reference to general health, general vision, colour vision and peripheral vision, independently of personality traits explored by the LoC tool. The possible protective effect of smoking confirms previous reports and requires further investigation
Disclosure: **M. Trento:** None.

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Non-disclosure of type 2 diabetes at work among Danish employees
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Background and aims: Increasing retirement age in many OECD countries, in addition to increasing prevalence of type 2 diabetes in younger people, is expected to result in a substantial increase of people with type 2 diabetes in the labour force. In many jobs, disclosure of diabetes at the work place is necessary for optimal self-management during work hours, whereas non-disclosure may lead to impaired self-management behaviours, such as adverse eating, inexpedient consumption of medication, or not taking necessary breaks. We explored which factors were associated with disclosing diabetes at work among Danish workers with type 2 diabetes.
Materials and methods: In total 705 people with type 2 diabetes successfully completed a questionnaire including work and diabetes related questions. Three logistic regressions were modelled to estimate the associations between the included factors and likelihood of disclosure to employer and colleagues. Associations were expressed by odds ratios and 95% confidence intervals. The models were adjusted for background characteristics, socio-economic factors, life-style factors, diabetes duration, diabetes treatment, and work-related factors.
Results: Almost a quarter of the participants (23%) had not disclosed their diabetes to employer and colleagues, whilst (13%) of the participants had not disclosed their diabetes to at least one colleague. In the adjusted logistic regression analysis, workers with, long diabetes duration, and use of diabetes medication, particular injections, were more likely to disclose their diabetes to their employer. People who reported receiving respect from their superiors were also more likely to disclose their diabetes. Likewise, participants who considered it an employer responsibility to provide flexible work conditions for people with chronic illness were more likely to disclose their diabetes. In contrast, people with the highest educational level were less likely to disclose their diabetes. Adjustment for covariates in the analyses slightly modified the estimates.
Conclusion: Workplace factors rather than individual factors seemed associated with disclosure. Future prospective studies should focus on reasons for concealing type 2 diabetes at work and impact of disclosure on diabetes self-management and work environment.

Table: Results from adjusted logistic regressions.
Associations between background characteristics and disclosure of type 2 diabetes. N = 705

Characteristic:	Model 1 Odds ratio (95 % CI)	Model 2 Odds ratio (95 % CI)
Age (year, continuous)	1.02 (0.99-1.04)	1.02 (0.99-1.05)
Gender (male)	0.85 (0.58-1.24)	0.76 (0.51-1.13)
Vocational training (ref: basic)	1.01 (0.58-1.75)	0.94 (0.53-1.66)
High Educational Level (ref: basic)	0.49 (0.25-0.98)	0.46 (0.23-0.94)
Diabetic complications (yes)	1.04 (0.61-1.78)	0.93 (0.53-1.62)
Treatment: Oral medication (ref: no medication)	2.01 (1.31-3.07)	2.02 (1.30-3.14)
Treatment: Injections (ref: no medication)	2.86 (1.69-4.85)	2.84 (1.66-4.86)
Diabetes Duration (year, continuous)	1.03 (1.00-1.07)	1.05 (1.01-1.09)
Well-being (WHO-5, continuous)	-	1.01 (1.00-1.02)
Perceive flexible work condition as an employer responsibility	-	1.21 (1.00-1.48)
Receive respect from employer	-	1.41 (1.09-1.82)

Disclosure: **O. Kasper:** None.

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People with type 1 diabetes and psychosis have a markedly increased risk of hospitalisation for acute diabetes complications
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Background and aims: The management of type 1 diabetes in people with psychosis is complex, and acute diabetes complications, such as ketoacidosis or hypoglycaemia, which require hospitalization, are probably more common than in people with type 1 diabetes without psychiatric diseases. However, little information is available on the effects of psychotic disorders on acute complications among patients with type 1 diabetes. The aim of our study was to examine the relationship between a history of hospitalization with a diagnosis of psychosis and acute diabetes complications for patients with type 1 diabetes.
Materials and methods: The principle of this population-based retrospective cohort study was to examine hospital data for all patients hospitalized in France for type 1 diabetes, from 2009 to 2012. In the medico-administrative database, people under 35 years with type 1 diabetes were identified from the codes E10 to E14 as the main, related or associated diagnoses. Then, two groups were formed: on the one hand, people with previous hospitalization with a diagnosis of psychosis (codes F2) over the two years before the index hospitalization; on the other hand, people with no hospitalization with a diagnosis of psychosis, over the two years before or after the index hospitalization, until 2015. Epidemiological follow-up was conducted at one and three years. It focused on hospitalization for acute diabetes complications (hypoglycaemia, hyperglycaemia, coma, ketoacidosis) and suicide attempts, but also on hospital mortality.
Results: From 2009 to 2012, 65,684 patients were hospitalized for type 1 diabetes complications. Among these, 350 had a previous hospitalization for psychosis: these patients were older (28.35 vs 20.99 years) and there were more men (67.71% vs 50.13%, p<.0001). At three years, patients with a history of psychosis were more frequently hospitalized for hypoglycaemia (p=0.002), hyperglycaemia (p=0.0003), coma (p=0.0003), ketoacidosis (p=0.02), all acute complications (p=0.001), or suicide attempts (p<.0001) and hospital mortality was higher (p=0.001). After adjustment for age and gender, the logistic regression showed that a history of hospitalization for psychosis for patients with type 1 diabetes was associated with an increased risk of all acute complications (hypoglycaemia, hyperglycaemia, ketoacidosis) (adjusted Odds-Ratio (aOR)=1.98 [1.47-2.66]), death (aOR=3.62 [1.92-6.82]), and suicide

attempts (aOR=12.38 [8.68-17.66]). Similar results were found for the follow-up at one year.

Conclusion: This nationwide population-based study showed that a history of hospitalization for psychosis in patients with type 1 diabetes was significantly associated with an increase in hospitalizations for acute diabetes complications and suicide attempts, and in in-hospital mortality. These results may be related to a lack of compliance with insulin therapy, self-aggressive behaviour, and social isolation. These results must encourage diabetologists to develop specific multidisciplinary health education actions for people with type 1 diabetes and psychiatric disorders in collaboration with psychiatric departments. A history of hospitalization for psychosis must be considered a warning sign for the risk of acute diabetes complications.

Disclosure: **J. Petit:** None.

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Endline assessment of patients' knowledge, attitudes, and practices on diabetes and tuberculosis comorbidities in South Africa

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Background and aims: Rates of DM are quickly increasing in high TB burden countries such as South Africa (SA). In most of these countries, service delivery for DM and TB are vertical, and therefore patients at risk of or with both diseases do not receive integrated care. In 2011, SA initiated the Integrated Chronic Disease Model to integrate clinical services and respond to the growing burden of chronic diseases in the country. This follow-up survey was administered to assess changes in patients' knowledge and behaviors regarding TB and DM services, since implementation of a series of educational, advocacy and capacity building interventions at patient, provider, and facility levels in three provinces in SA.

Materials and methods: A cross-sectional Knowledge, Attitudes, and Practices (KAP) survey was conducted among 395 and 420 patients attending TB and DM service sites respectively, in 22 public health facilities across three provinces in SA that were involved in the baseline (BL) survey (results reported elsewhere). Patients completed a structured questionnaire around patient's knowledge, beliefs, health-seeking behaviors and experience of TB and DM public health services in SA in December 2016.

Results: Survey participant characteristics and demographic profiles were similar at BL and endline (EL). Among patients with DM, 81% were female; while only 39% were women with TB. Similar to the BL results, most of the survey patients listed clinic as their main source of health information (over 80% of the study population). The survey revealed improvements in patient exposure to health education, counselling and screening for comorbidities (TB for DM patients and DM for TB patients). Compared to BL, TB testing among DM patients increased from 37% to 57% ($p<.01$) while DM testing among TB patients increased from 55% to 67% ($p<.01$). Counselling on comorbidities was also higher in both groups: counselling was higher by 41% for TB patients ($p<.01$) and by 59% for DM patients ($p<.00$). Exposure to health education doubled for DM patients from 22% at BL to 45% at EL ($p<.01$). Among TB patients, exposure to health education was higher by 71% ($p<.01$) from BL to EL. Patients with DM demonstrated good basic knowledge of how to maintain their health. At EL, the proportion of DM patients with knowledge on the importance of regular exercise, attending regular checkups, and taking medication as prescribed was higher compared to BL (+52%, +18%, and + 45%, respectively, $p<.01$; $<.01$; 0.02). However, the proportion of DM patients who understood the risk of developing complications if they did not regularly take medication as prescribed was lower, from 17% at BL to 9% at EL ($p<.01$). The proportion of DM patients knowing the link between DM and TB was also higher compared to BL. There was a high level of general knowledge about TB among both patient groups, but little understanding on the importance of treating co-morbidities.

Conclusion: While much research has been done in SA on patient KAP towards TB and HIV, and to a lesser extent towards DM, this is the first study of patient understanding of TB-DM comorbidity and experience of counselling, screening, and testing for both diseases at the facility level. Implementation of integrated care from educational, advocacy and capacity building interventions may contribute to provide overall quality care to patients with both diseases.

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PS 059 DPP4 inhibitors: utility update

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INITIAL combination therapy with vildagliptin/metformin in drug-naive Asian type 2 diabetes patients: influence of age, BMI and co-morbidities in a real world setting

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Background and aims: Individualising diabetes therapy is imperative to address the multi-factorial impact of the disease. Advancing age, overweight/obesity, hypertension, and elevated lipids are identified risk factors for cardiovascular (CV) disease and are useful considerations for making treatment decisions. We assessed these characteristics in the multinational INITIAL study.
Materials and methods: **I**nitial combi**N**ation therapy with vildagliptin/metformin **I**n drug-naïve **T**2DM patients **I**n a re**A**l **L**ife study was a 24-week, non-interventional, prospective study conducted across four Asian countries. Drug-naïve patients with documented HbA1c >7.5% (>8.0% in India) were prescribed vildagliptin/metformin (vilda/met) combination therapy within four weeks of study entry according to local label. The primary endpoint was change in HbA1c from baseline to week 24. Key secondary endpoints were safety, achievement of HbA1c ≤7.0%, and change in HbA1c by subgroups: age, BMI, associated co-morbidities.
Results: Out of 532 enrolled patients, 457 (85.9%) completed the study. Study population was relatively young with mean age 49.6±11.27 years (>90% patients were aged <65 years), mean HbA1c 9.3±1.57%, diabetes duration 0.8±2.47 years, and BMI 26.7±4.50 kg/m^2. At baseline, patients presented with hypertension (30.4%), dyslipidaemia (31.4%), obesity (17.9% or 38.7% using 30.0 or 27.5 kg/m^2 cut-off, respectively), and diabetes family history (28.4%). Overall, vilda/met therapy was associated with significant (p<0.001) reduction in HbA1c from baseline both at weeks 12 (−1.6 ±1.59%) and 24 (−1.9±1.70%) (Table). At week 24, body weight reduced by −1.1±2.62 kg and 39.6% patients achieved HbA1c ≤7.0%. Clinically meaningful and generally consistent HbA1c reductions were also seen across various sub-groups: age ≥65 years, −1.9% and <65 years, −1.8%; baseline BMI categories: obese vs. non-obese by Asian cut-off −1.7% vs. −2.0%; associated co-morbidities: no co-morbidity −1.6%, hypertension −1.8%, and dyslipidaemia −2.0%). The vilda/met initial combination was well tolerated in this population with adverse events reported in 48 (9.0%) patients.
Conclusion: We believe the INITIAL study complements the relatively limited evidence available assessing initial combination therapy in drug-naïve Asian patients with type 2 diabetes mellitus. Patients included in the study were relatively young, with high baseline HbA1c and often associated with CV risk factors. Vildagliptin/metformin was associated with early and statistically significant HbA1c reductions from baseline. Clinically meaningful and generally consistent HbA1c reductions were also observed, regardless of baseline age, BMI, or associated co-morbidity (i.e. hypertension, dyslipidaemia).

Table. Clinical parameters of the study population by visit: INITIAL study (full analysis set)

	Baseline (N=490)		Week 12 (N=490)		Week 24 (N=490)	
	n (%)	mean±SD	n (%)	mean±SD	n (%)	mean±SD
HbA1c, %	490 (100)	9.3±1.60	386 (78.8)	7.8±1.17	485 (99.0)	7.4±0.99
Change from baseline, %	NA	NA	NA	−1.6±1.59*	NA	−1.9±1.70*
HbA1c ≤7.0%, n (%)	NA	NA	386#	103 (26.7)§	399#	158 (39.6)§
Body weight (Kg)	488 (99.6)	70.2±12.51	422 (86.1)	70.1±12.13	443 (90.4)	69.0±11.92
HbA1c by age, %						
<65 years of age	443 (90.4)	9.3±1.57	345 (70.4)	7.9±1.19	439 (89.6)	7.5±1.00
≥65 years of age	47 (9.6)	9.1±1.80	41 (8.4)	7.6±0.98	46 (9.4)	7.4±0.96
HbA1c by conventional BMI cut-off, %						
<30 kg/m²	399 (81.4)	9.33±1.67	314 (64.1)	7.9±1.19	396 (80.8)	7.4±0.98
≥30 kg/m²	89 (18.2)	9.3±1.25	71 (14.5)	7.8±1.08	88 (18.0)	7.46±1.07
HbA1c by Asian BMI cut-off, %						
<27.5 kg/m²	297 (60.6)	9.4±1.74	232 (47.3)	7.9±1.19	295 (60.2)	7.4±0.91
≥27.5 kg/m²	191(39.0)	9.2±1.34	153 (31.2)	7.8±1.15	189 (38.6)	7.5±1.11
HbA1c by associated co-morbidity, %						
No co-morbidity	156 (31.8)	9.3±1.61	116 (23.7)	8.2±1.13	156 (31.8)	7.7±0.95
Hypertension	149 (30.4)	9.1±1.45	121 (24.7)	7.6±1.05	147 (30.0)	7.3±0.93
Dyslipidaemia	154 (31.4)	9.2±1.58	125 (25.5)	7.5±1.14	152 (31.0)	7.2±0.97

NA, not applicable; SD, standard deviation; *p<0.001; N, number of patients in the full analysis set; n, number of patients with observations; #number of patients evaluable (patients having HbA1c assessment at the specific time-point); §percentages are for the number of patients at the specific time-point (#).

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Clinically significant change in albumin creatinine ratio with saxagliptin: a post hoc analysis from the SAVOR-TIMI 53 trial

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Background and aims: A 30% decrease in Albumin Creatinine ratio (ACR) is considered a clinically significant improvement. It is associated with improved renal and possibly also cardiovascular outcomes. We tested whether saxagliptin is associated with ≥30% change in ACR.
Materials and methods: The SAVOR-TIMI 53 trial randomized 7,501 (45.5%) patients with ACR<15 mg/g, 2,197 (13.3%) patients with 15≤ACR<30 mg/g, 4,426 (26.8%) patients with micro-albuminuria (30≤ACR<300 mg/g) and 1,638 (9.9%) patients with macro-albuminuria (ACR ≥300 mg/g) to treatment with saxagliptin versus placebo. Median follow-up was 2.1 years and ACR was measured at baseline, 1-year, 2-year and end of the trial (EOT) time-points.
Results: At the EOT, there were more patients with ≥30% decrease in ACR in the saxagliptin arm versus placebo (38% vs. 33.8% respectively, p<0.001). Additionally, there were less patients with ≥30% increase in ACR in the saxagliptin arm versus placebo (39.5% vs. 44%, p<0.001). Both of these differences between the saxagliptin and placebo arms were also observed at different categories of ACR at baseline (table).
Conclusion: In a large population of patients with type 2 diabetes and increased cardiovascular risk, saxagliptin was associated with higher incidence of clinically significant improvement in ACR.

Table 1. Change in ACR, Baseline to EOT

	≥30% decrease			≥30% increase		
Baseline ACR (mg/g)	**Placebo**	**Saxagliptin**	**P-value**	**Placebo**	**Saxagliptin**	**P-value**
Total Population	**1823 (33.8%)**	**2125 (38.0%)**	**<0.001**	**2375 (44.0%)**	**2213 (39.5%)**	**<0.001**
ACR<15	698 (29.8%)	793 (32.2%)	0.081	1133 (48.4%)	1120 (45.4%)	0.037
15≤ACR<30	270 (31.5%)	328 (37.7%)	0.007	379 (44.2%)	333 (38.3%)	0.012
30≤ACR<300	613 (37.4%)	717 (42.6%)	0.002	671 (40.9%)	599 (35.5%)	0.001
ACR ≥300	242 (43.1%)	287 (49.8%)	0.024	192 (34.2%)	161 (28.0%)	0.022

Clinical Trial Registration Number: NCT01107886
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No major impact seen with sitagliptin on rates of cardiovascular death or hospitalisation for heart failure following myocardial infarction during TECOS

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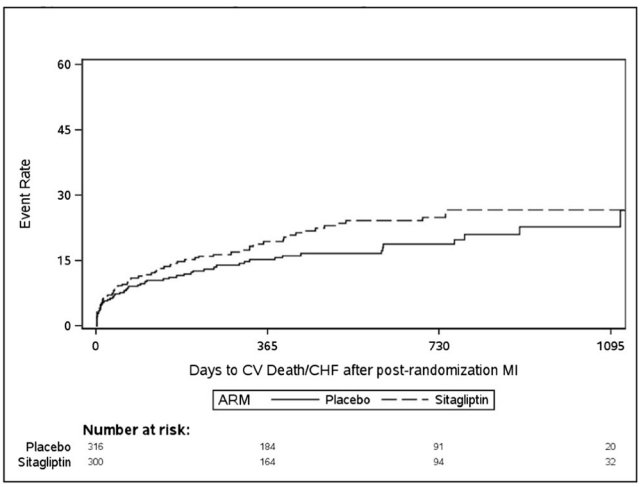
Background and aims: Dipeptidyl peptidase-4 inhibitors (DPP-4i) reduce myocardial infarction (MI) size in animal models, but the clinical relevance of these observations remains unknown.
Materials and methods: TECOS randomized 14,671 participants with type 2 diabetes (T2D) and atherosclerotic cardiovascular disease to sitagliptin or placebo therapy in addition to usual care. For all participants, time to a primary composite endpoint of cardiovascular death or hospitalisation for heart failure (hHF), and the two components separately, were analysed by Cox proportional hazards models left-censored at the time of the MI (without and with adjustment for potential confounders).
Results: During the trial 616 participants had an MI (300 and 316 allocated to sitagliptin and placebo, respectively); 102 (17%) subsequently died, including 81 (13%) with cardiovascular death; and 57 (9%) experienced hHF. At the time the MI occurred, 253 (41%) participants were taking a DPP-4i and 362 (59%) were not. Those with an MI during the trial generally had similar characteristics to those without but were more likely to be male (77.9% *vs.* 70.4%, p<0.0001), more often had pre-existing coronary artery disease (89.4% *vs.* 73.4%), previous MI (57.8% *vs.* 42.0%, both p<0.0001) or hHF (21.4% *vs.* 17.9%, p=0.024). Metformin treatment was reported less frequently (75. 5% *vs.* 81.8%), whilst insulin treatment was reported more frequently (32.5% *vs.* 22.8%, both p<0.0001). Drugs to treat cardiovascular conditions were used significantly more frequently in MI patients. Intention-to-treat analyses for the primary composite endpoint yielded non-significant hazard ratios of 1.17 (95% CI 0.82-1.67, p=0.38, unadjusted) and 1.22 (0.84-1.77, p=0.29, adjusted), with similar results for the two components (see Figure). As-treated analyses also yielded non-significant hazard ratios of 0.79 (0.55-1.14, p=0.21, unadjusted) and 0.82 (0.56-1.20, p=0.30 adjusted), mainly driven by a reduced hazard for cardiovascular death (0.65 [0.41-1.03], p=0.07 unadjusted; 0.61 [0.28-0.99], p=0.05 adjusted), with no obvious change in the risk of hHF.
Conclusion: These data do not provide conclusive evidence regarding the hypothesis that DPP-4i therapy ameliorates the consequences of MI in patients with T2D.



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Association between race and glycaemic response to sitagliptin: insights from TECOS

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Background and aims: Evidence from pooled small-scale efficacy studies suggests that Asians with type 2 diabetes have a greater glycaemic response to dipeptidyl-peptidase 4 inhibitors than other races. Asian patients may also be more reponsive to acarbose. We assessed the glycaemic impact of sitagliptin by race in the global cardiovascular safety study the Trial Evaluating Cardiovascular Outcomes with Sitagliptin (TECOS). We also evaluated whether there might be an additive incretin effect with sitagliptin-acarbose combination therapy in Asian patients.
Materials and methods: 14,671 participants with type 2 diabetes and established cardiovascular disease, of whom 3,265 (22.3%) were from Asian countries, were randomised to double-blind therapy with sitagliptin or placebo. Qualifying HbA_{1c} values were 51 to 64 mmol/mol (6.5% to 8.0%). The TECOS protocol required that background blood glucose-lowering therapies remain stable during four months after randomisation unless therapeutic intensification was considered essential, facilitating an assessment of whether glycaemic effects differed between self-identified Oriental (East) Asian, Other (South) Asian, White Caucasian, Hispanic, Black and Indigenous racial groups.
Results: Median baseline HbA_{1c} in the six racial groups ranged from 54 to 57 mmol/mol (7.1-7.4%). Mean placebo-adjusted relative HbA_{1c} reduction over four months (see Table) was greater in Oriental Asians than other groups (p<0.0001) except White Caucasians (p=0.08) after adjustment for weight, height, age, and use of statin, beta-blocker and thiazide therapies. After 4 months, Oriental and Other Asians were more likely to start additional oral therapy (metformin and sulfonylureas) than insulin compared with White Caucasians (p<0.0001). Utilisation of acarbose increased in the Asian patients but no glycaemic interaction with allocated therapy was observed (adjusted p=0.12).
Conclusion: In TECOS, Oriental Asians had the greatest initial HbA_{1c} reduction with sitagliptin. The relatively good baseline glycaemic control in TECOS participants may explain the smaller absolute between-group differences than seen in earlier Phase studies. No additive glycemic effect was seen when sitagliptin was given in conjunction with acarbose.

Table. Placebo-adjusted mean (95% CI) HbA_{1c} change from baseline to 4 months by race in TECOS.

	Number	mmol/mol	%
Caucasian	9,957	-3.3 (-3.7, -3.0)	-0.3 (-0.3, -0.3)
Asian (Other)	2,178	-3.8 (-4.4, -3.2)	-0.3 (-0.4, -0.3)
Asian (Oriental)	1,087	-5.0 (-5.9, -4.2)	-0.5 (-0.5, -0.4)
Hispanic	906	-4.1 (-5.0, -3.2)	-0.4 (-0.5, -0.3)
Black	447	-3.6 (-4.8, -2.3)	-0.3 (-0.4, -0.2)
Indigenous	96	-0.7 (-3.6, 2.1)	-0.1 (-0.3, 0.2)

Clinical Trial Registration Number: NCT00790205

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Disclosure: **T.M.E. Davis:** Employment/Consultancy; Member of Merck Advisory Boards. Grants; Participation as a local TECOS investigator and recipient of Merck-sponsored investigator initiated research. Lecture/other fees; Member of Merck speaker bureau.

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Resource use and UK-perspective costs in patients with type 2 diabetes during the Trial Evaluating Cardiovascular Outcomes with Sitagliptin (TECOS)

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Background and aims: We report data from TECOS, a cardiovascular (CV) safety trial in which 14,671 participants with type 2 diabetes and established CV disease from 38 countries were randomised to sitagliptin or placebo added to usual care, and were managed to achieve individualized good glycaemic control. Sitagliptin was non-inferior to placebo for the primary composite outcome of CV death, nonfatal myocardial infarction, nonfatal stroke, or hospitalisation for unstable angina. We consider secondary and tertiary comparisons of medical resource use and costs between treatment groups.
Materials and methods: Medical resource use data were collected from randomisation to study end. Here we report UK unit costs applied to all health care resource use. Hospitalisations and outpatient visits were costed from a UK perspective using Healthcare Resource Groups and national reference costs, study medication using list prices, and other antihyperglycaemic drugs using a 2015 weighted mix of generic and proprietary drugs. All costs beyond the first year were discounted at the UK recommended rate of 3.5% annually. Hierarchical generalized linear models (HGLM) were used to account for variable practice patterns across participating countries.
Results: Mean and median follow-up was 3.0 years in both groups. Resource use and costs were similar for both treatments (Table). Sitagliptin-treated patients had 5 fewer hospitalisations per 100 patients (p=0.16). Total costs, exclusive of sitagliptin, were £6,058 vs £6,219 for sitagliptin vs placebo. Mean sitagliptin costs were £1,072 per patient. After adding study medication costs, mean total costs for the sitagliptin group were £7,130, and the mean (95% CI) cost difference between arms was £911 (£627 to £1201).
Conclusion: This large randomised trial, designed to assess CV outcomes in the setting of glycaemic equipoise between treatment groups, suggests that within a UK perspective, lower costs from small reductions in hospitalisation rates with sitagliptin slightly offset the additional treatment costs.

Table.

	Sitagliptin (n=7332)	Placebo (n=7339)	Difference (95% CI with bootstrap method)	P-value
Resource use over full follow-up period, mean (SD)				
Hospitalizations	0.66 (1.29)	0.70 (1.43)	-0.049 (-0.095 to -0.008)	0.16
Inpatient days	5.50 (16.38)	5.74 (16.54)	-0.243 (-0.768 to 0.241)	0.99
All outpatient visits	19.42 (17.36)	19.43 (17.35)	-0.008 (-0.520 to 0.583)	0.86
*Costs over full follow-up period, mean (SD)***				
Inpatient care	£2,629 (7960)	£2,760 (8262)	-131 (-406 to 116)	*
Outpatient care	£2,648 (2287)	£2,654 (2294)	-6 (-73 to 73)	0.93
Diabetes medications	£781 (870)	£805 (868)	-24 (-50 to 5)	0.81
Total, excluding sitagliptin	£6,058 (8769)	£6,219 (9061)	-162 (-445 to 119)	0.60
Study medication	£1,072 (485)	0 (0)	-	-
Total costs	£7,130 (8822)	£6,219 (9061)	911 (627 to 1201)	<0.0001

*HGLM did not converge ** discounted at 3.5% per annum

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Disclosure: **A.M. Gray:** Grants; Research funding from Merck to University of Oxford and Duke University.

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CARMELINA® trial baseline characteristics: a cardiovascular and renal microvascular outcome trial with linagliptin in patients with type 2 diabetes at high vascular risk

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Background and aims: Recent cardiovascular (CV) outcome trials in type 2 diabetes (T2D) focused on populations at high risk for macrovascular complications. Generally, patients with moderate-to-severe microvascular burden, such as advanced kidney disease, were relatively underrepresented. Thus, the impact of glucose-lowering therapies on combined cardio-renal outcomes in T2D remains unclear.
Materials and methods: CARMELINA® is an ongoing, global, randomized, double-blind, placebo-controlled clinical trial designed to assess the impact of the DPP-4 inhibitor linagliptin 5 mg daily on CV and renal outcomes in a population enriched for both macrovascular and renal microvascular complications. The primary objective is to establish CV safety of linagliptin versus standard of care assessed by a composite outcome of 3-point MACE (CV death, nonfatal myocardial infarction, or nonfatal stroke). A composite renal outcome (renal death, end stage renal disease, or sustained ≥40% decrease in eGFR from baseline) is a prespecified secondary endpoint and will be tested after non-inferiority for MACE is established. All CV and renal outcome events will be confirmed by central adjudication.
Results: Baseline characteristics of the treated study population (n=6978) randomized between July 2013 and August 2016 include: (mean±SD) age 65.8±9.1 yrs, 62.9% male, BMI 31.3±5.3 kg/m², HbA1c 7.9±1.0 %. Most patients had long-standing T2D (64.7% >10 yrs) and 57.8% were treated with insulin. As defined by study inclusion criteria, established CV disease (n=3990 [57.2%]), overt CKD (i.e. GFR <60ml/min/1.73m^2; n=4348 [62.3%]) or presence of micro- (n=2896 [41.5%]) or macroalbuminuria (n=2691 [38.6%]) were frequent at baseline.
Conclusion: CARMELINA® will explore both CV and renal outcomes in patients with T2D. In addition, it will add evidence to the long-term clinical safety profile of linagliptin by including patients at advanced stages of kidney disease.
Clinical Trial Registration Number: NCT01897532
Supported by: Boehringer Ingelheim and Eli Lilly
Disclosure: **V. Perkovic:** Employment/Consultancy; Boehringer Ingelheim. Grants; Boehringer Ingelheim. Lecture/other fees; Boehringer Ingelheim.

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A long-term, open-label extension study to investigate the long-term safety of alogliptin in subjects with type 2 diabetes

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Background and aims: Alogliptin is a potent, highly selective, orally available inhibitor of the dipeptidyl peptidase 4 enzyme. This study evaluated the long-term safety of alogliptin when used as monotherapy or in combination with other therapies in subjects with T2DM who completed 1 of 7 phase 3 studies.

Materials and methods: In this international, multicenter, open-label extension study, T2DM subjects who had completed 1 of 7 phase 3 alogliptin studies were randomized (1:1) to receive alogliptin 12.5 mg (12.5 mg completed group) or 25 mg (25 mg completed group) once a day (QD) for up to 4 years. Subjects previously rescued from 1 of the 7 phase 3 studies due to hyperglycemia received alogliptin 25 mg (25 mg rescued group). Subjects previously receiving add-on therapy continued this treatment. Investigators were free to modify or add add-on therapy per clinical need. Primary endpoints included study drug exposure and adverse event (AE) rates, including hypoglycemia. Secondary endpoints included HbA_{1c} levels, fasting plasma glucose (FPG) levels, and incidence of marked hyperglycemia (FPG ≥200 mg/dL).
Results: A total of 3323 subjects were enrolled; 3320 (99.9%) received ≥1 dose of study drug (safety set), and 1996 (60.1%) completed the 4-year treatment period. Median duration of exposure was 191.7 weeks (62.5% exposed for ≥180 weeks) in the 12.5 mg completed group (n=1394), 191.9 weeks (64.9% exposed for ≥180 weeks) in the 25 mg completed group (n=1399), and 162.0 weeks (49.0% exposed for ≥180 weeks) in the 25 mg rescued group (n=527). In the safety set, 2880 (86.7%) subjects experienced treatment-emergent AEs (TEAEs), and 806 (24.3%) had study drug-related TEAEs. Rates of TEAEs and treatment-emergent serious AEs were similar across the 3 groups (Table). Hypoglycemic episodes were self-reported by 13.1% of subjects in the safety set. Marked hyperglycemia (defined as FPG ≥200 mg/dL) was reported by 55.7% of subjects in the safety set. However, only 1 subject withdrew as a result of hyperglycemia. HbA_{1c} increased slightly then remained generally steady in the 12.5 mg and 25 mg completed groups. An immediate and sustained HbA_{1c} decrease was observed in the 25 mg rescued group.
Conclusion: Alogliptin administered as either 12 mg or 25 mg QD is well tolerated and can be used to maintain adequate glycemic control for up to 4 years in subjects with T2DM.

Table. Overview of treatment-emergent adverse events (Safety Set)

	Open-Label Treatment Group			
	Completed Phase 3		Rescued From Phase 3 Alogliptin 25 mg (n=527)	Total Alogliptin 25 mg (N=1926)
	Alogliptin 12.5 mg (n=1394)	Alogliptin 25 mg (n=1399)		
Subjects with any TEAE, n (%)	1215 (87.2)	1219 (87.1)	446 (84.6)	1665 (86.4)
Subjects with any study drug–related TEAE, n (%)	357 (25.6)	318 (22.7)	131 (24.9)	449 (23.3)
Subjects with any TEAE leading to treatment discontinuation/study termination, n (%)	98 (7.0)	86 (6.1)	40 (7.6)	126 (6.5)
Subjects with any treatment-emergent SAE, n (%)	233 (16.7)	228 (16.3)	83 (15.7)	311 (16.1)
Subjects with any study drug–related treatment-emergent SAE, n (%)	36 (2.6)	29 (2.1)	10 (1.9)	39 (2.0)
Treatment-emergent deaths, n (%)	20 (1.4)	14 (1.0)	5 (0.9)	19 (1.0)

TEAE, treatment-emergent adverse event; SAE, serious adverse event.

Clinical Trial Registration Number: NCT00306384
Supported by: Takeda Pharmaceutical Company Limited
Disclosure: **N. Smith:** Employment/Consultancy; Takeda.

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Impact of sitagliptin on hypoglyceamia in elderly subjects with type 2 diabetes treated with insulin

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Background and aims: Hypoglycemia (HYPO) is uncommon with sitagliptin (SITA) treatment but occurs more frequently when SITA is added to insulin (INS) due to insulin's ability to cause hypoglycemia. HYPO is of special concern in elderly INS-treated patients.
Materials and methods: To assess the effect of SITA on glycemic control and event rates (ERs) of HYPO in the elderly treated with INS compared to younger (<65 years) subjects, data from two 24-week studies that compared the addition of SITA with PBO in subjects with T2DM on INS ± metformin were pooled (N=1299, 320 elderly; INS dose held stable in one, intensively titrated in the other) and treatment effect and event rates of HYPO were analyzed. We also examined ERs of HYPO by subgroups characterized by selected baseline characteristics.
Results: Reductions in A1C were similar in both age groups (Table). In general, elderly subjects had similar or lower ERs of HYPO compared to younger subjects. ERs of symptomatic HYPO and nocturnal HYPO were lower with SITA compared with PBO (Table) in both younger and elderly. In subgroups evaluated by baseline A1C, eGFR and duration of T2DM, ERs of HYPO were lower with SITA compared with PBO in both age groups.
Conclusion: In this pooled analysis of SITA vs PBO added to INS, SITA provided similar improvement in glycemic control and reductions in event rates of HYPO in both elderly and younger patients.

Parameter	<65 years N = 979		≥65 years N = 320	
A1C (%)	**Sitagliptin** n = 487	**Placebo** n = 492	**Sitagliptin** n = 164	**Placebo** n = 156
Baseline Week 24	8.76 (0.96) 7.77 (1.17)	8.78 (1.00) 8.28 (1.28)	8.48 (0.85) 7.57 (0.95)	8.56 (0.97) 8.16 (1.10)
Change from baseline[1]	-0.98 (-1.08, -0.89)	-0.51 (-0.60, -0.41)	-0.91 (-1.05, -0.78)	-0.36 (-0.50, -0.22)
Sita vs. placebo[2]	-0.48 (-0.61, -0.34) p<0.001	---- ----	-0.55 (-0.74, 0.36) p<0.001	---- ----
Event rate of hypoglycemia (events/subject year)	**Sitagliptin** n = 487	**Placebo** n = 492	**Sitagliptin** n = 164	**Placebo** n = 156
Symptomatic[3] Severe[4] Nocturnal (CT)[5]	1.32 0.05 0.66	2.18 0.10 0.89	1.50 0.04 0.38	1.65 0.01 0.90

Values are mean ± standard deviation unless otherwise noted.
[1] Least squares (LS) mean (95% CI). [2] Difference in LS mean changes from baseline (95% CI). [3] Symptoms consistent with hypoglycemia. [4] Hypoglycemia requiring assistance, or with marked loss of consciousness or seizures. [5] Hypoglycemia occurring between 11 PM and 6 AM.

Clinical Trial Registration Number: NCT00395343/NCT01462266
Supported by: Merck
Disclosure: **R.R. Shankar:** Employment/Consultancy; Merck. Stock/Shareholding; Merck.

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Angiogenic T lymphocytes are decreased in type 2 diabetes and are recruited by dipeptidyl peptidase-4 inhibitor linagliptin

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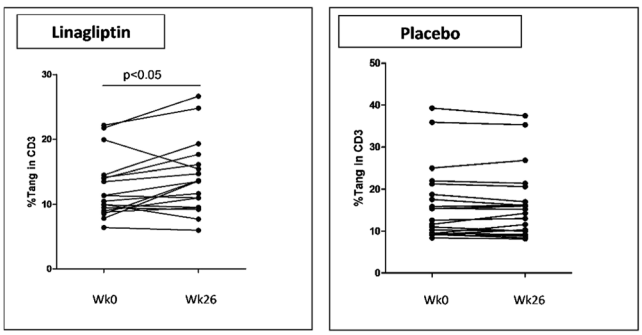
Background and aims: Endothelial progenitor cells (EPCs) are involved in vascular repair and decreased in type 2 diabetes (T2D). Recently, angiogenic T lymphocytes (Tang) were identified, which facilitate EPCs in vascular repair through stromal cell-derived factor 1 (SDF-1α) mediated secretion of proangiogenic cytokines. Their role in T2D has not been investigated. Since dipeptidyl peptidase-4 (DPP4) inhibition may increase EPC levels in T2D though SDF-1α, we aimed to study whether Tang are decreased in T2D and whether their production is stimulated by treatment with the DPP4-inhibitor linagliptin.
Materials and methods: A total of 45 treatment naive T2D patients (age 63 (IQR 54-66) years, 61% male, HbA1c 6.3±0.4 (%)) without cardiovascular disease were randomized to linagliptin 5 mg/day or placebo for 26 weeks in a double-blind fashion. Additionally, 19 healthy age/gender matched controls were included (age 56 (IQR 44-67] years, 63% male). Peripheral blood mononuclear cells were isolated from venous whole blood in EDTA tubes, at baseline and 26 weeks, and in controls. Fluorescence-activated cell sorting was used to identify EPCs (CD34+ CD133+KDR+) and Tang in total CD3 (CD3+CD31+CXCR4+) and

CD4/CD8 subsets. Plasma levels of SDF-1α, interleukin 8 (IL8), and vascular endothelial growth factor (VEGF) were measured by ELISA.
Results: Total Tang number (CD3: 11.50% vs 20.39% ; p<0.0001) and in CD4 (7.65% vs 13.18%; p<0.01) and CD8 (26.68% vs 46.5%; p<0.001) subsets, were significantly lower in T2D at baseline compared with controls. While EPCs in T2D correlated negatively with IL-8 (r=-0.41; p=0.008), VEGF (r=-0.41; p=0.008) and CRP (r=-0.32; p=0.042) at baseline, no association with Tang was observed. After 26 weeks of linagliptin treatment, a clear rise in SDF-1α levels was observed (delta 166 (122) vs -7.4(28.8); p<0.0001), while IL-8 and VEGF remained stable. Furthermore, Tang increased in linagliptin treated patients and remained stable in placebo (figure; delta 1.4(2.62) vs 0.066(1.52), p=0.033 (paired), p=0.055 (for change)). The change in Tang correlated negatively with change in HbA1c (r=0.39; p=0.020). No changes in EPCs were observed.
Conclusion: This is the first study to demonstrate that the recently defined subset of angiogenic T lymphocytes are decreased in T2D. Interestingly, this placebo controlled trial suggests that linagliptin enhances their recruitment, through which DPP4-inhibitors may exert beneficial effects on vascular repair.



Clinical Trial Registration Number: NCT02015299
Supported by: Boehringer Ingelheim
Disclosure: **D.J. Mulder:** None.

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Treatment failure with sitagliptin compared to sulphonylureas for type 2 diabetes inadequately controlled on metformin

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Background and aims: The risk of treatment failure with adding either sitagliptin or sulphonylureas to metformin for type 2 diabetes mellitus has not been comparatively evaluated in UK "real world" clinical practice. In this study, we aim to evaluate treatment failure with sitagliptin against sulphonylureas as add-on to metformin across 2 domains. The first treatment failure domain examines a failure to achieve desirable glycaemic targets while the second focuses on the requirement for augmentation of the antidiabetic regimen (intensification or switching to another anti-diabetic).
Materials and methods: We undertook a retrospective cohort study by identifying all individuals aged ≥ 18 with type 2 diabetes between 2007-2013 who had either sitagliptin or sulphonylureas added to metformin from The Health Improvement Network UK primary care database. We followed them for 30 months, till they left the practice, died or end of 2014. We used multivariable Cox regression to analyse the time before individuals on sitagliptin and sulphonylurea 1) recorded an undesirable HbA1c level of ≥ 58 mmol/mol (7.5%) and 2) underwent treatment-augmentation with another anti-diabetic as identified through issue of a prescription for an alternate anti-diabetic. The first 6 months after add-on initiation with sitagliptin/sulphonylureas were excluded from analysis to allow time for treatments to have effect. We adjusted for age, sex, baseline hbA1c, weight, duration of diabetes, smoking status, history of hypoglycaemias as well as an extensive range of comorbidities and prescribed medications at baseline.
Results: We identified 23,809 individuals; 19,564 prescribed sulphonylureas and 4,245 prescribed sitagliptin as add-on to metformin. We found that more individuals prescribed sitagliptin than sulphonylurea recorded an undesirable HbA1c level of ≥ 58 mmol/mol (7.5%) at 18 months (1.09 Hazard Ratio 95%CI 1.03 to 1.14) however, not at 36 months (0.95 HR 95%CI 0.85 to 1.06). When this analysis was restricted only to those individuals who were prescribed continuous prescriptions for 18 or 30 months of either add-on treatment and metformin (with no greater than 60 day gaps between successive issued prescriptions), no significant difference was found in the number of individuals recording a hbA1c of ≥ 58 mmol/mol (7.5%). More sitagliptin patients had their treatment augmented compared to sulphonylureas at 18 months, (3.67 HR 95%CI 3.40 to 3.96) and 30 months (2.65 HR 95%CI 2.35 to 3.00). When this analysis was restricted to those on continuous treatment, estimates remained similar for treatment augmentation.
Conclusion: Though sitagliptin users were only 9% more likely to have a recording of a undesirable HbA1c level of ≥ 58 mmol/mol (7.5%) 18 months after initiation, treatment augmentation i.e. replacement or intensification was over 3.5 times more likely with sitagliptin at 18 months and 2.6 times more likely at 30 months. This may indicate an underlying treatment inertia for "real world" patients prescribed sulphonylureas compared to those prescribed sitagliptin. This could be due to physician inertia to augment treatment due to the higher propensity of sulphonylureas to cause hypoglycaemia particularly when added to other anti-diabetic treatments. Or alternatively, the requirement for gradual dose titration with sulphonylureas (unlike sitagliptin) which may delay clinicians and patients in achieving optimal dosing and in turn augmenting treatment.
Supported by: a grant from Novo Nordisk A/S
Disclosure: **M. Sharma:** Grants; Novo Nordisk A/S.

PS 060 Incretin: alternative formulations

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ITCA 650: a novel therapeutic approach to treating patients with type 2 diabetes

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Background and aims: ITCA 650 is a novel drug/device combination product containing the GLP-1 receptor agonist, exenatide, in the Medici delivery system™. ITCA 650 has the potential to continuously deliver exenatide subcutaneously (SC) for up to 12 months after sub-dermal placement of the small, 44 mm titanium osmotic mini-pump. The Medici delivery system maintains consistent therapeutic exenatide plasma levels and virtually ensures treatment adherence when the device is in situ. In clinical trials of up to 33 months which tested 3 and 6-month ITCA 650 mini-pumps, significant reductions in HbA1c and body weight were seen and treatment was well tolerated in patients with T2D who were uncontrolled on anti-diabetes medications. We present the experience with placement and removal procedures for the ITCA 650 from the FREEDOM Phase 3 program.
Materials and methods: Placement and removal of ITCA 650 is performed by trained healthcare professionals in a simple, brief, office procedure. The sterile mini-pump is placed in the sub-dermis of the abdominal wall using a placement tool and is removed or replaced through a small incision (~5 mm) and closed with Steri-Strips. Investigative site personnel were provided with a kit containing all supplies necessary to conduct the procedure and were trained and certified via a standardized online and hands-on training program prior to the initiation of any study procedures.
Results: As of 26 July 2016, 20,701 procedures to place, replace, and remove the ITCA 650 were performed in 5,134 patients by MDs, NPs, and PAs at 493 clinical sites in 28 countries. Approximately 1% of scheduled procedures to remove the device were initially unsuccessful due to user errors on the initial placement depth of the mini-pump (i.e. deep placements) and required the assistance of a surgeon or radiologist to complete (assisted removal). Since the introduction of a placement aid (depth guide) into the development program, no assisted removals have been reported. To date, only 27 (0.5%) patients permanently discontinued study treatment due to a procedure AE. Most of the discontinuations were the result of difficulty with removal of the device, and 12 events (0.2%) were for difficulty/failure with device placement. The most common (incidence by patient) application site adverse events (AEs): application site pain (1.8%), bleeding (1.7%), bruise (1.4%), vesicles (1.3%), and pruritus (1.1 %) were generally mild, transient, and reflected the normal healing process. No serious AEs due to the placement or removal procedures were observed.
Conclusion: Once or twice-yearly dosing of ITCA 650 has the potential to improve therapeutic outcomes. Over 20,000 procedures to place, replace, and remove the mini-pumps have been completed. Procedures are simple, well tolerated, easy to teach, and have been performed safely by a wide variety of healthcare practitioners.
Supported by: Intarcia Therapeutics, Inc.
Disclosure: **A. Whitson:** Employment/Consultancy; Intarcia Therapeutics, Inc.

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ITCA 650 improves glycaemic control and reduces the need to advance antidiabetic therapy

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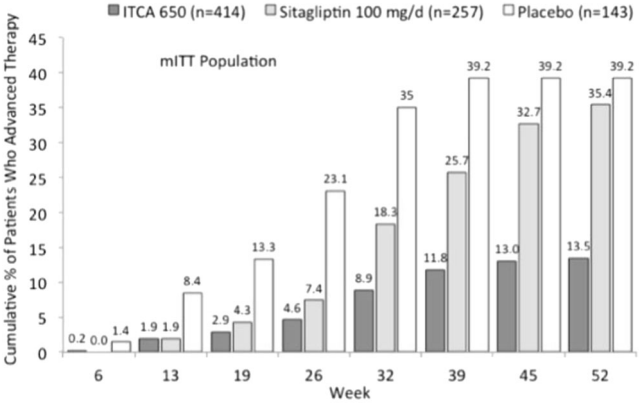
Background and aims: ITCA 650, an osmotic mini-pump in development for T2D, continuously delivers exenatide SC for up to 6 months after subdermal placement. Significant reductions in HbA1c and weight occurred in 2 clinical trials: FREEDOM-1, a 39-week, placebo-controlled trial in pts on OADs and FREEDOM-2, a 52-week, study of ITCA 650 vs sitagliptin (SITA) in pts uncontrolled on metformin. Advancement of antidiabetes therapy (AT), a meaningful measure of the effectiveness and sustainability of antidiabetes therapy was evaluated for the pooled populations of these trials.
Materials and methods: Combined data from 814 uncontrolled T2D pts on OADS (414 on ITCA 650, 257 on sitagliptin, 143 on placebo) was analyzed for AT. Mean baseline HbA1c was 8.6%. Data for ITCA 20 mcg/day for 13 weeks, then 60 mcg/day were pooled (n=413). AT, the addition of or increase of therapy from baseline, was protocol mandated based on predefined criteria. AT was required after Week 26 for HbA1c >8%.
Results: The majority of ITCA 650 treated pts achieved and maintained glycemic control below the threshold for AT (Fig. 1). An increase in AT occurred in all groups at Week 26 but 88% of ITCA 650 pts remained on assigned therapy at Week 39. In contrast, there was a progressive increase in the need for AT in the placebo and SITA groups after Week 26.
Conclusion: In uncontrolled T2D, ITCA 650 results in significantly improved and stable glycemic control without AT in the majority of patients.

Figure 1 Percentage of Patients who Advanced Antidiabetes Therapy on or Before Certain Weeks



ITCA 650 included FREEDOM1 and FREEDOM2; Sitagliptin was from FREEDOM2 and Placebo was from FREEDOM1.

Supported by: Intarcia Therapeutics, Inc.
Disclosure: **R. Henry:** Employment/Consultancy; Intarcia Therapeutics, Inc. Grants; Intarcia Therapeutics, Inc.

786

SNAC-mediated absorption mechanism of action in an oral formulation of semaglutide

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Background and aims: Oral delivery of peptides is typically hindered by the limited permeability of the gastrointestinal tract and rapid enzymatic and pH-induced degradation in the stomach. Clinically, co-formulation in a tablet of the human glucagon-like peptide-1 analogue, semaglutide, with the absorption enhancer, sodium N-(8-[2-hydroxybenzoyl] amino) caprylate (SNAC) gives rise to therapeutically relevant exposures of semaglutide upon oral administration. In vivo data from humans and dogs illustrate that absorption takes place in the stomach in an area immediately below the tablet. These studies investigated the mechanism of absorption mediated by SNAC.
Materials and methods: The transepithelial transport of semaglutide was examined in cell monolayers of gastric epithelium (NCI-N87) upon

exposure to SNAC and compared to that in unexposed cell monolayers. The amount of semaglutide transported from the donor chamber (apical side) to the acceptor chamber (basolateral side) was measured every 15 min over 60 min and thereafter the apparent permeability coefficient (P_{app}) was calculated. To evaluate the time-course profile of the absorption-enhancing effect of SNAC, segments of rat gastric mucosa were mounted in Ussing chambers and exposed to SNAC (30 mM) for 10 min. The trans-epithelial electrical resistance (TEER) was continuously monitored prior, during and following SNAC exposure. Complementary studies examined the window of absorption for semaglutide elicited by SNAC. The P_{app} of semaglutide across rat gastric mucosa segments was measured after exposure to SNAC and upon addition of semaglutide either immediately post-SNAC exposure or following an interval of 30 or 60 min. Experimental values were expressed as fold increase in P_{app} relative to semaglutide alone. Finally, to elucidate the route via which SNAC mediates semaglutide absorption, the intracellular uptake of semaglutide was examined in NCI-N87 cell monolayers after exposure to SNAC (80 mM) or ethylenediaminetetraacetic acid (EDTA) (15-75 mM), a modulator of tight junction function, and subsequent addition of semaglutide. Cells were lysed upon termination of the experiment (60 min after semaglutide addition) and the amount of semaglutide accumulated within the intracellular fraction was quantified.

Results: The absorption enhancing action of SNAC on semaglutide was found to require concentrations in the mM range as reflected by a significant increase in the P_{app} of semaglutide across NCI-N87 cell monolayers (1×10^{-8} cm/s [control] vs 7×10^{-8} cm/s [80 mM]; $p<0.05$). Exposure of segments of rat gastric mucosa mounted in Ussing chambers to SNAC resulted in a 25% decline ($p=0.05$) in TEER, which was restored within 60 min. Emphasising its relatively short window of action, the fold change in P_{app} of semaglutide elicited by SNAC was reduced from 2.06 ±0.53 (10 min post-exposure) ($p=0.05$) to 1.30±0.27 (30 min post-exposure) ($p=0.22$) following the removal of SNAC. Intracellular accumulation of semaglutide measured in NCI-N87 gastric epithelial cells revealed a substantial increase upon exposure to SNAC compared to control cells (89.9 pmol vs 4.3 pmol, respectively; $p<0.001$). In contrast, EDTA had no significant effect. Together, these divergent patterns show that SNAC mediates absorption via the transcellular route.

Conclusion: Collectively, these studies reveal that SNAC promotes absorption of semaglutide across gastric mucosa in a concentration-dependent manner via effects on transcellular pathways, which is both transient and fully reversible.

Supported by: Novo Nordisk

Disclosure: **S.T. Buckley:** Employment/Consultancy; Novo Nordisk A/S. Stock/Shareholding; Novo Nordisk A/S.

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A pharmacoscintigraphic study of the relationship between tablet erosion and pharmacokinetics of oral semaglutide

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Background and aims: Semaglutide, a glucagon-like peptide-1 (GLP-1) analogue, has been co-formulated in a tablet with the absorption enhancer, sodium N-(8-[2-hydroxybenzoyl] amino) caprylate (SNAC), to allow for oral administration. This trial investigated the anatomical site of tablet erosion, the rate of tablet erosion and the gastrointestinal transit of oral semaglutide, and correlated these parameters to the pharmacokinetic properties of oral semaglutide in order to test if tablet erosion kinetics may influence the extent of absorption of oral semaglutide.

Materials and methods: In a randomised, open-label, crossover trial design, 26 healthy males (mean±SD age 38±11 years, body weight 83.4 ±11.0 kg, body mass index 25.9±2.3 kg/m^2) in the fasted state received single doses of oral semaglutide (10 mg with 300 mg SNAC) administered with 50 mL and 240 mL of water, in two different treatment periods, each followed by 4 hours post-dose fasting. Tablet erosion and gastrointestinal transit were assessed by gamma scintigraphy. Oral semaglutide tablets contained ^{111}In labelled ion-exchange resin, and the water used for tablet administration was labelled with ^{99m}Tc (to provide an outline of the stomach). Dynamic imaging was performed during the first minute after dosing, while subjects were sitting. Subsequently, static images were recorded frequently until 4 hours post-dose, while subjects were standing (and allowed to sit or remain moderately active in between static imaging time points). Semaglutide plasma concentrations were measured frequently until 24 hours after administration.

Results: Complete tablet erosion (CTE) occurred in the stomach irrespective of water volume administered with the tablet. CTE appeared to occur approximately 50% later after dosing with 50 mL vs 240 mL water, although this was not statistically significant (estimated mean time to CTE 85 vs 57 min; ratio 50/240 mL [95% confidence interval] 1.51 [0.96;2.37], $p=0.072$). Semaglutide plasma exposure ($AUC_{0\text{-}24h}$) and maximum concentration (C_{max}) were approximately twice as high when dosed with 50 mL vs 240 mL water. Slower tablet erosion and slower gastric emptying were significantly correlated with higher semaglutide exposure ($AUC_{0\text{-}24h}$ and C_{max}; all $p<0.001$). These results suggest that the higher semaglutide exposure levels observed with the lower water volume may be mediated through slower rate of tablet erosion and slower delivery to the small intestine. Overall, the safety profile was as expected for the GLP-1 receptor agonist drug class. The most frequently reported adverse events were headache, nausea and vomiting.

Conclusion: Semaglutide tablet erosion occurs in the stomach irrespective of water volume. A slower rate of tablet erosion in the stomach, as seen when administering the tablet with 50 mL vs 240 mL of water, and a delayed delivery to the small intestine result in higher semaglutide plasma exposure.

Clinical Trial Registraiton Number: NCT01619345
Supported by: Novo Nordisk

Disclosure: **A. Connor:** Grants; Novo Nordisk A/S.

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Pharmacokinetics and tolerability of oral semaglutide in subjects with renal impairment

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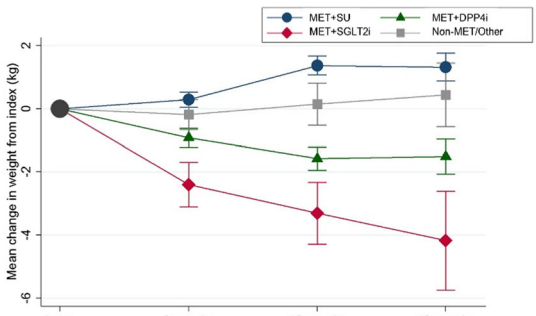
Background and aims: Semaglutide, a human glucagon-like peptide-1 (GLP-1) analogue, has been formulated in a tablet with the absorption enhancer, sodium N-(8-[2-hydroxybenzoyl] amino) caprylate (SNAC). This study investigated the pharmacokinetics and tolerability of oral semaglutide in subjects with renal impairment or normal renal function.

Materials and methods: Based on creatinine clearance (CL_{Cr}) estimated using the Cockcroft-Gault formula, subjects were classified into 4 groups: normal renal function (CL_{Cr} ≥90 mL/min; N=24), or mild (CL_{Cr} 60-89 mL/min; N=12), moderate (CL_{Cr} 30-59 mL/min; N=12) or severe (CL_{Cr} 15-29 mL/min; N=12) renal impairment. A group of subjects with end-stage renal disease (ESRD) requiring haemodialysis was also included (N=11). Subjects received once-daily oral semaglutide (5 mg for 5 days followed by 10 mg for 5 days) in the fasting state with 120 mL water and with 30 minutes post-dose fasting. Semaglutide plasma concentrations after the 10th daily dose were compared between subjects with impaired renal function and subjects with normal renal function.

Results: There was no consistent pattern of increase or decrease in semaglutide exposure ($AUC_{0\text{-}24h,Day\ 10}$) and maximum concentration ($C_{max,Day\ 10}$) on Day 10 across renal function groups (Table). Time to maximum concentration ($t_{max,Day\ 10}$) was comparable for all groups (median of 1.0, 1.0, 1.0, 1.5 and 1.0 hour for normal, mild, moderate, severe and ESRD groups, respectively). Half-life ($t_{½,Day\ 10}$) appeared to increase slightly from normal renal function to severe renal impairment (geometric

mean of 152, 159, 163, 165 and 153 hours, respectively). Except for 1 subject in the ESRD group, semaglutide was not detected in urine. Haemodialysis did not affect semaglutide pharmacokinetics. The safety profile was as expected for the GLP-1 receptor agonist drug class with no trend towards a greater proportion of subjects with adverse events (AEs) with increasing degree of renal impairment. The most frequently reported AEs were gastrointestinal disorders.
Conclusion: There was no apparent effect of renal impairment on the pharmacokinetics and tolerability of oral semaglutide. Based on this study, renal impairment should not affect dose recommendations for oral semaglutide.

Figure. Baseline-adjusted mean (SE) change in body weight from index in those who initiated, and remained on, second-line therapy after metformin monotherapy



N	Index	6 months	12 months	18 months
Metformin+SU	4606	1550	1092	567
Metformin+DPP4i	2880	993	714	359
Metformin+SGLT2i	440	185	103	46
Non-metformin/ other combination	1091	403	220	109

BMI, body mass index; DPP4i, dipeptidyl peptidase-4 inhibitors; MET, metformin; SGLT2i, sodium-glucose cotransporter 2 inhibitors; SU, sulfonylurea

Clinical Trial Registration Number: NCT02014259
Supported by: Novo Nordisk
Disclosure: **T.W. Anderson:** Employment/Consultancy; Novo Nordisk A/S. Stock/Shareholding; Novo Nordisk A/S.

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Pharmacokinetics and tolerability of oral semaglutide in subjects with hepatic impairment

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Background and aims: Oral semaglutide is a novel tablet formulation of semaglutide, a human glucagon-like peptide-1 (GLP-1) analogue, with the absorption enhancer sodium N-(8-[2-hydroxybenzoyl] amino) caprylate (SNAC). This study investigated the pharmacokinetics and tolerability of oral semaglutide in subjects with impaired hepatic function compared to subjects with normal hepatic function.
Materials and methods: Subjects were classified into groups of normal hepatic function (N=24), and mild (Child-Pugh Grade A; N=12), moderate (Child-Pugh Grade B; N=12) or severe (Child-Pugh Grade C; N=8) hepatic impairment, and received once-daily oral semaglutide (5 mg for 5 days followed by 10 mg for 5 days) in the fasting state with 120 mL water and with 30 minutes post-dose fasting. Semaglutide plasma concentrations after the 10th daily dose were compared between subjects with impaired hepatic function and subjects with normal hepatic function.
Results: Semaglutide exposure ($AUC_{0\text{-}24h,Day\ 10}$) and maximum concentration ($C_{max,Day\ 10}$) on Day 10 appeared similar across hepatic function groups (Table). There was no apparent effect of hepatic impairment on time to maximum concentration ($t_{max,Day\ 10}$; median of 1.0, 1.0, 1.0 and 1.5 hour for normal, mild, moderate and severe groups, respectively) or half-life ($t_{½,Day\ 10}$; geometric mean of 156, 142, 147 and 154 hours, respectively). The safety profile was as expected for the GLP-1 receptor agonist drug class independent of the degree of hepatic impairment. The most frequently reported adverse events were gastrointestinal disorders.
Conclusion: There was no apparent effect of hepatic impairment on the pharmacokinetics and tolerability of oral semaglutide. The results of this study suggest that dose adjustment of oral semaglutide is not necessary in subjects with hepatic impairment.

Semaglutide exposure in subjects with hepatic impairment versus subjects with normal hepatic function

	$AUC_{0\text{-}24h,Day\ 10}$ Ratio [90% CI]	$C_{max,Day\ 10}$ Ratio [90% CI]
Mild vs. Normal	0.91 [0.60;1.40]	0.92 [0.60;1.40]
Moderate vs. Normal	0.87 [0.57;1.31]	0.85 [0.55;1.30]
Severe vs. Normal	0.90 [0.61;1.32]	0.88 [0.61;1.28]

$AUC_{0\text{-}24h,Day\ 10}$: Area under the semaglutide plasma concentration-time curve from 0 to 24 hours after the 10th daily dose
$C_{max,Day\ 10}$: Maximum semaglutide plasma concentration after the 10th daily dose

Clinical Trial Registration Number: NCT02016911
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Disclosure: **F.L. Søndergaard:** Employment/Consultancy; Novo Nordisk A/S. Stock/Shareholding; Novo Nordisk A/S.

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Safety, tolerability and pharmacokinetics of multiple once-daily dosing of oral semaglutide in healthy males and in males with type 2 diabetes

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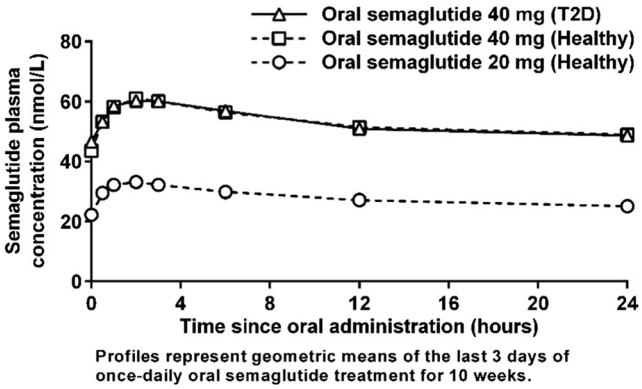
Background and aims: Semaglutide, a glucagon-like peptide-1 (GLP-1) analogue, has been co-formulated with the absorption enhancer, sodium N-(8-[2-hydroxybenzoyl] amino) caprylate (SNAC), in a tablet for oral administration. This trial investigated the safety, tolerability and pharmacokinetics of multiple once-daily (OD) dosing of oral semaglutide in healthy males and in males with type 2 diabetes (T2D).
Materials and methods: In this randomised, double-blind, placebo-controlled trial, 84 healthy males (mean±SD age 45±12 years, body weight 83.5±10.2 kg, body mass index 25.9±2.4 kg/m^2) and 23 males with T2D (treated with diet and exercise and/or stable doses of metformin, age 55±8 years, body weight 94.9±13.9 kg, body mass index 29.4 ±3.2 kg/m^2, HbA_{1c} 7.5±0.7%, duration of diabetes 5.6±3.2 years) received OD oral semaglutide, placebo or placebo with SNAC for 10 weeks (data not shown for placebo with SNAC). The healthy males received oral semaglutide 20 mg (N=16), 40 mg (N=32), placebo (N=18) or placebo with SNAC (N=18). The males with T2D received oral semaglutide 40 mg (N=11), placebo (N=6) or placebo with SNAC (N=6). Subjects were dose escalated starting with 5 mg OD during week 1 and 10 mg OD during week 2, increasing to 20 mg OD from week 3 and to 40 mg OD from week 5 (in the 40 mg treatment groups). Trial products were administered in the morning after an overnight fast, with 50 mL water and with 2 hours post-dose fasting.
Results: Overall, the safety profile was as expected for the GLP-1 receptor agonist drug class. A similar proportion of subjects reported adverse events (AEs) across treatment groups. The majority of AEs were mild, however the severity of AEs increased with increasing dose. The proportions of subjects with gastrointestinal disorders were 50%, 84% and 28% for oral semaglutide 20 mg, 40 mg and placebo in healthy males, and 73% and 50% for oral semaglutide 40 mg and placebo in males with T2D. Body weight reduction was greater for oral semaglutide vs placebo in healthy males (treatment difference in change from baseline, oral semaglutide - placebo [95% CI]: -4.3 kg [-6.3;-2.3] for 20 mg and -7.2 kg [-8.9;-5.4] for 40 mg) and in males with T2D (-5.4 kg [-8.5;-2.3]). In males with T2D, HbA_{1c} was reduced with oral semaglutide vs

placebo (-1.5% [-1.8;-1.3]). Total exposure ($AUC_{0\text{-}24h,SS}$) and maximum concentration ($C_{max,SS}$) of semaglutide increased with increasing dose in healthy males (estimated ratios 40/20 mg [95% CI]: 1.89 [1.23;2.92] and 1.83 [1.20;2.79]), and were similar in healthy males vs males with T2D (1.00 [0.60;1.66] and 1.00 [0.61;1.62]) (Figure). Half-life ($t_{½,SS}$) was comparable between groups (geometric means of 153, 161 and 158 hours in healthy 20 mg, healthy 40 mg and males with T2D 40 mg, respectively).

Conclusion: 10 weeks of OD oral semaglutide up to 40 mg was safe and tolerable in healthy males and in males with T2D, and the pharmacokinetic properties support that oral semaglutide is suitable for OD dosing.



Clinical Trial Registration Number: NCT01686945
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Disclosure: **C. Granhall:** Employment/Consultancy; Novo Nordisk A/S. Stock/Shareholding; Novo Nordisk A/S.

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A study of drug-drug interactions of oral semaglutide with metformin and digoxin

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Background and aims: Oral semaglutide is a novel tablet in development for treatment of type 2 diabetes (T2D), containing the human glucagon-like peptide-1 (GLP-1) analogue, semaglutide, and the absorption enhancer, sodium N-(8-[2-hydroxybenzoyl] amino) caprylate (SNAC; 300 mg). Due to absorption enhancement by SNAC and/or possible delay of gastric emptying by semaglutide, oral semaglutide may alter the absorption of concomitantly administered oral drugs. This trial investigated the effect of oral semaglutide on the pharmacokinetic properties of metformin and digoxin. Metformin is a Biopharmaceutics Classification System (BCS) class III drug (high solubility/low permeability) and was chosen for the present trial mainly because it is the most commonly used drug in patients with T2D. Digoxin has low solubility with an incomplete absorption and was chosen for the present trial mainly due to its narrow therapeutic index and because it is used in patients with T2D having cardiovascular diseases.

Materials and methods: On separate visits, in a one-sequence, crossover trial design, healthy subjects (mean±SD age 59±12 years, body weight 76.0±11.1 kg, body mass index 25.4±2.1 kg/m^2) received metformin (850 mg twice daily for 4 days) or digoxin (500 μg single dose) alone, and subsequently together with SNAC (300 mg single dose) and, on a separate occasion, together with oral semaglutide (20 mg once daily at steady state). Pharmacokinetic blood sampling was performed until 30 hours after the last dose for metformin and until 120 hours after the single dose for digoxin. Lack of effect of oral semaglutide on exposure of victim drug was concluded if the 90% CI for the ratio of Co-administration/Alone was within the pre-defined interval of (0.80;1.25).

Results: The area under the curve (AUC) of metformin was increased by oral semaglutide co-administration, while maximum concentration (C_{max}) of metformin and AUC and C_{max} of digoxin were unaffected (Table). SNAC alone did not affect AUC and C_{max} of metformin (ratio [90% CI] metformin+SNAC/metformin alone: 1.05 [0.99;1.12] and 1.06 [0.98;1.14], respectively), indicating that the increase in metformin exposure by oral semaglutide co-administration was not due to the absorption enhancing effect of SNAC. There were no apparent effects of oral semaglutide on the half-life ($t_{½}$) of metformin (geometric means of 16 and 13 hours for metformin alone and co-administered with oral semaglutide, respectively) or digoxin (42 and 43 hours for digoxin alone and co-administered with oral semaglutide, respectively). Based on the wide therapeutic index of metformin, the higher AUC of metformin when co-administered with oral semaglutide was not considered clinically relevant. The safety profile was as expected for the GLP-1 receptor agonist drug class with gastrointestinal events being the most frequent adverse events.

Conclusion: Oral semaglutide has no clinically relevant effect on the exposure of metformin and digoxin.

Exposure of victim drug when co-administered with oral semaglutide vs. when administered alone

	AUC Ratio [90% CI] Co-administration/Alone	C_{max} Ratio [90% CI] Co-administration/Alone
Metformin	1.32 [1.23;1.43]	0.98 [0.90;1.06]
Digoxin	1.03 [0.96;1.11]	0.98 [0.89;1.09]

Number of subjects contributing to the analyses: 31
CI: Confidence interval
AUC: Area under the concentration-time curve from 0 to 12 hrs at steady state for metformin, and from 0 to infinity after a single dose for digoxin
C_{max}: Maximum concentration between 0 and 12 hrs after dosing for metformin, and after a single dose for digoxin

Clinical Trial Registration Number: NCT02249910
Supported by: Novo Nordisk
Disclosure: **M. Thomsen:** Employment/Consultancy; Novo Nordisk A/S.

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Evaluation of the effects of water volume with dosing and post-dose fasting period on pharmacokinetics of oral semaglutide

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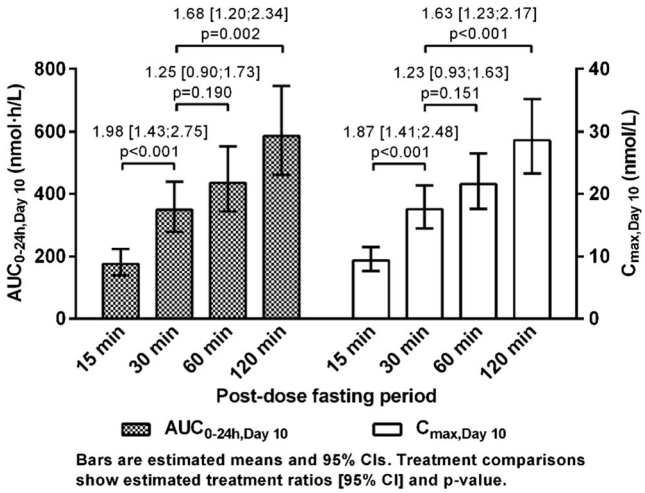
Background and aims: Semaglutide has been co-formulated in a tablet with the absorption enhancer, sodium N-(8-[2-hydroxybenzoyl] amino) caprylate (SNAC), to provide the first glucagon-like peptide-1 receptor agonist (GLP-1 RA) for oral administration. Absorption of oral semaglutide when co-formulated with an absorption enhancer, like SNAC, may be sensitive to dosing conditions, such as the duration of post-dose fasting and the volume of water used for administration. Therefore, this trial evaluated the administration of oral semaglutide using different dosing conditions in order to elucidate the minimum dosing requirements needed to ensure a clinically relevant semaglutide exposure level and at the same time an acceptable safety profile.

Materials and methods: In an open-label, randomised, parallel-group trial design, 158 healthy male subjects (mean±SD age 40.5±9.7 years, body weight 82.1±9.9 kg, body mass index 25.2±2.4 kg/m^2) received oral semaglutide (10 mg formulated with 300 mg SNAC) each morning (after having fasted overnight) for 10 days. Before the first dose, subjects were randomised into 8 different treatment arms, in which oral semaglutide was administered with either 50 mL or 120 mL water, and the duration of post-dose fasting was either 15, 30, 60 or 120 minutes. Semaglutide

concentrations were measured in plasma for up to 504 hours after administration on Day 10.

Results: There was no statistically significant interaction between the effects of water volume and post-dose fasting period for semaglutide exposure ($AUC_{0\text{-}24h,Day\ 10}$) and maximum concentration ($C_{max,Day\ 10}$) on Day 10 (p=0.53 and p=0.39, respectively). $AUC_{0\text{-}24h,Day\ 10}$ and $C_{max,Day\ 10}$ were unaffected by water volume (p=0.54 and p=0.68, respectively). In a post-hoc statistical analysis conducted with post-dose fasting period as the only fixed effect (i.e., the two water volumes were combined), $AUC_{0\text{-}24h,Day\ 10}$ and $C_{max,Day\ 10}$ increased statistically significantly with longer post-dose fasting (Figure). The time to maximum semaglutide plasma concentration ($t_{max,Day\ 10}$) did not change with water volume, but increased with longer post-dose fasting (median of 0.5, 1.0, 1.5 and 2.0 hours for 15, 30, 60 and 120 minutes post-dose fasting, respectively, when the 2 groups with different water volumes were combined). Water volume or post-dose fasting period had no apparent effect on half-life ($t_{½,Day\ 10}$; geometric mean range 150-159 hours). The safety profile was as expected for the GLP-1 RA drug class. Gastrointestinal disorders were the most frequent adverse events (reported in 61% of subjects).

Conclusion: Administration of oral semaglutide in the fasting state with up to 120 mL water and a post-dose fasting period of at least 30 minutes results in clinically relevant semaglutide exposure. These dosing conditions are expected to be acceptable to patients.



Clinical Trial Registration Number: NCT01572753
Supported by: Novo Nordisk
Disclosure: **M. Donsmark:** Employment/Consultancy; Novo Nordisk A/S. Stock/Shareholding; Novo Nordisk A/S.

PS 061 Incretins: new formulations

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BioChaperone technology enables the development of glargine GLP1-RA (liraglutide and dulaglutide) formulations

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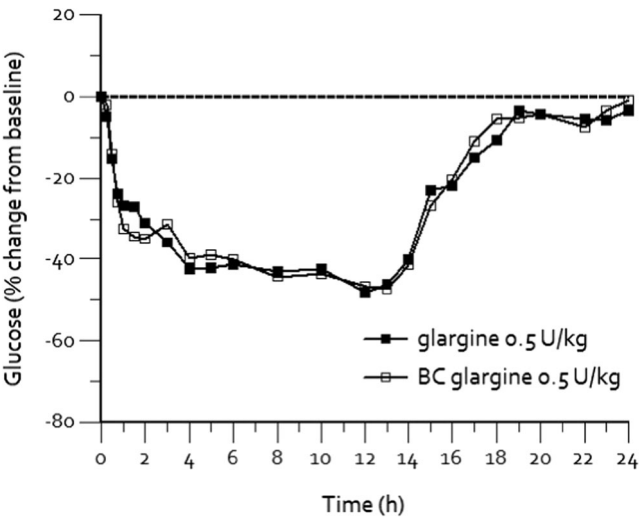
Background and aims: Basal insulin-GLP1 RA combinations provide remarkable medical benefits compared to basal insulin monotherapy and lead to less GLP1-related adverse events with a simple regimen. However, leading GLP1 RA liraglutide and once a week GLP1 RA dulaglutide cannot be combined with the most used basal insulin, insulin glargine, due to formulation pH incompatibility. BioChaperone (BC) technology enables the solubilisation of glargine at neutral pH with a similar efficacy. This makes the combination of glargine with liraglutide or dulaglutide possible.

Materials and methods: BC glargine dulaglutide and BC glargine liraglutide have been evaluated for physical stability by visual imaging, flow imaging microscopy (MFI) and light scattering. Chemical stability has been followed by RP-HPLC. Effect of BC glargine alone was evaluated in a cohort of 10 fasted beagle dogs by measuring blood glucose after an single SC injection of 0.5 U/kg of BC glargine or glargine in two distinct periods.

Results: At 30°C, physically stable BC glargine dulaglutide (BC GlaD) and BC glargine liraglutide (BC GlaLira) formulations have been obtained for at least 9 weeks and 6 weeks respectively. The chemical stability of BC GlaD formulation shows a similar protein recovery to the one of commercial glargine and dulaglutide formulations with only 3% of protein related degradation products found by RP-HPLC analysis after 9 weeks at 30 °C. Furthermore, the long acting profile of glargine is maintained upon injection of BC glargine compared to commercial glargine in an in vivo dog model (figure).

Conclusion: In summary, BioChaperone technology enables the development of glargine-GLP1 RA formulations based on liraglutide and dulaglutide affording new options towards more compliant treatments for T2D patients.



Disclosure: **R. Soula:** Employment/Consultancy; ADOCIA. Stock/Shareholding; ADOCIA.

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Low incidence of gastrointestinal adverse events over time with a fixed-ratio combination of insulin glargine and lixisenatide vs lixisenatide alone

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Background and aims: iGlarLixi, a titratable fixed-ratio combination of insulin glargine (iGlar) 100 U/ml and the glucagon-like peptide-1 (GLP-1) receptor agonist lixisenatide (lixi), was associated with low rates of nausea, vomiting, and diarrhoea (≤ 10% each) in the LixiLan phase 3 clinical programme, leading to very low discontinuation rates (≤ 1% each). This post hoc analysis assessed frequency and timing of gastrointestinal adverse events in 2 trials evaluating iGlarLixi in patients with type 2 diabetes (T2D) uncontrolled with oral antidiabetes agents and/or basal insulin vs iGlar alone or lixi alone.
Materials and methods: Pertinent data on nausea, vomiting, and diarrhoea from the randomised, open-label LixiLan-O and LixiLan-L trials were used in this analysis. The incidence of these gastrointestinal adverse events was calculated with descriptive statistics; results were summarised by treatment group.
Results: Within the first 60 days of treatment initiation, 11.7% and 9.6% of patients treated with iGlarLixi experienced gastrointestinal adverse events in the LixiLan-O and -L trials, respectively, compared with 27.5% of patients on lixi in LixiLan-O, and 4.7% and 1.4% of patients on iGlar in LixiLan-O and -L. The percentage of patients with onset of gastrointestinal adverse events from Day 60 to study end (~210 days) was 7.7% and 4.1% for iGlarLixi in LixiLan-O and -L, respectively, 6.9% for lixi in LixiLan-O, and 3.2% and 2.2% for iGlar in LixiLan-O and -L. After 80 days, incidences of new nausea, vomiting, or diarrhoea events were 3.4%, 1.1%, and 3.0%, respectively, for iGlarLixi, compared with 2.6%, 1.3%, and 2.6% for lixi in LixiLan-O. Most gastrointestinal adverse events reported for iGlarLixi were mild (53-78%) to moderate (22-45%) in severity, with only one reported severe nausea event in LixiLan-L and no severe gastrointestinal adverse events in LixiLan-O. The median durations of nausea, vomiting, and diarrhoea were 5.0, 1.0, and 3.5 days, respectively, with iGlarLixi vs 7.5, 3.0, and 3.0 days with lixi, and 2.0 days for each gastrointestinal adverse event with iGlar in LixiLan-O. In LixiLan-L, the median durations were 6.0, 2.0, and 2.5 days with iGlarLixi vs 3.0, 1.0, and 2.0 days with iGlar. The occurrence of gastrointestinal adverse events is expected with the use of GLP-1 receptor agonists. Nausea, vomiting, and diarrhoea events associated with iGlarLixi treatment were predominantly transient, mainly occurring during the initial 8-week titration period in a small proportion of patients; by week 12 they were rare. As expected, the overall proportion of patients with gastrointestinal adverse events was higher with iGlarLixi than with iGlar, but lower than with lixi; this may be due to the slow increase in lixi dose in the fixed-ratio iGlarLixi combination.
Conclusion: Patients with T2D treated with iGlarLixi show a low rate of gastrointestinal adverse events compared to lixi, and these adverse events tend to occur early and to be transient and mild, rarely requiring discontinuation.
Clinical Trial Registration Number: NCT02058147, NCT02058160
Supported by: Study funding and editorial support provided by Sanofi US, Inc.
Disclosure: **J. Trujillo:** Other; member of the advisory panel for Sanofi US, Inc.

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Simultaneous vs sequential combination of insulin glargine and lixisenatide in type 2 diabetes uncontrolled on metformin

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Background and aims: A single daily injection of basal insulin and a glucagon-like peptide-1 receptor agonist (GLP-1 RA) combined, as in iGlarLixi (a titratable, fixed-ratio combination of insulin glargine 100 U [iGlar] + lixisenatide [Lixi] recently approved for clinical use), is potentially more effective, less complex and with better tolerance than multiple injections of each component separately for the treatment of type 2 diabetes (T2DM). In this exploratory analysis, we determined whether iGlarLixi, from the LixiLan-O trial, is more effective and better tolerated than multiple separate injections of each component given sequentially, in the GetGoal Duo-1 trial, in insulin-naïve patients with T2DM, uncontrolled on metformin ± other oral antidiabetic drugs (OADs).
Materials and methods: Propensity score matching based on baseline covariates (age, race, BMI, HbA_{1c}, fasting plasma glucose [FPG], diabetes duration and OAD/metformin use) to minimize confounding factors was used to indirectly compare the treatment strategy of simultaneous administration with iGlarLixi in LixiLan-O (*N*=469) vs sequential initial iGlar therapy for 12 weeks, followed by addition of Lixi if iGlar was insufficient, in GetGoal Duo-1 (*N*=223).
Results: iGlarLixi showed significant treatment benefits vs sequential administration of iGlar and Lixi in 87 carefully matched pairs, including a greater proportion of patients reaching HbA_{1c} <7% and greater reductions in HbA_{1c} and FPG. In addition, weight loss was achieved in the iGlarLixi group but not with sequential treatment (Table). The proportion of patients reporting symptomatic documented hypoglycaemia (hypo; ≤3.9 mmol/L) and gastrointestinal (GI) adverse events was lower with iGlarLixi vs sequential treatment (hypo: 20.7% vs 25.3%; nausea: 9.2% vs 20.7%; vomiting: 1.1% vs 10.3%, respectively). Of note, the end-of-treatment insulin dose was lower in the iGlarLixi group compared with the sequentialtreatment group (mean ± SD: 31 ± 14 U vs 56 ± 26 U).
Conclusion: This indirect comparison suggests that early treatment with a simultaneous combination of basal insulin and a GLP-1 RA such as iGlarLixi may be more effective, with weight loss and less hypoglycaemia, and have better GI tolerability compared with a sequential approach of adding a GLP-1 RA when basal insulin therapy needs intensification in T2DM uncontrolled on OADs.

Table. Efficacy of simultaneous vs sequential treatment with insulin glargine and lixisenatide in propensity score-matched participants

	iGlarLixi (LixiLan-O)* Wk 24/30 LOCF (*N*=87)		iGlar + Lixi (GetGoal Duo-1)† Wk 24 LOCF (*N*=87)		LS mean difference (95% CI)	*p*-value
Efficacy	**Baseline**	**Change/Wk 30**	**Baseline**	**Change/Wk 30**		
HbA_{1c}, %	7.7 ± 0.7	−1.3 ± 0.8	7.7 ± 0.5	−0.8 ± 0.8	−0.6 (−0.8, −0.4)	**<0.0001**
HbA_{1c} <7.0% (*n*)		79.3% (69)		50.6% (44)		**<0.0001**
2-hr PPG, mmol/L	13.0 ± 3.2	−4.4 ± 3.7	13.3 ± 3.5	−3.5 ± 5.1	−0.3 (−1.7, 1.0)	0.639
FPG, mmol/L	7.7 ± 1.6	−1.6 ± 1.9	7.6 ± 1.8	−0.9 ± 2.4	−0.6 (−1.1, −0.2)	**0.0094**
Weight, kg	89.5 ± 17.1	−1.3 ± 3.4	89.2 ± 19.8	0.1 ± 3.0	−1.3 (−2.3, −0.3)	**0.0102**

*LixiLan-O patients were randomized after a 4-week run-in if: HbA_{1c} ≥7% and ≤10%, and FPG ≤13.9 mmol/L. Metformin use was allowed during the study.
†GetGoal Duo-1 patients were randomized after a 12-week run-in if: HbA_{1c} ≥7% and ≤9%, and fasting SMPG for the past 7 days averaged 7.0 mmol/L early in the trial or ≤7.8 mmol/L after a protocol amendment. Metformin ± TZD use was allowed during the study.
Data are mean ± SD unless indicated otherwise. FPG, fasting plasma glucose; LOCF, last observation carried forward; LS, least squares; PPG, postprandial plasma glucose; SMPG, self-monitored plasma glucose; Wk, week; TZD, thiazolidinedione.

Clinical Trial Registration Number: LixiLan-O, NCT02058147; GetGoal Duo-1, NCT00975286
Supported by: Sanofi
Disclosure: **J. Rosenstock:** Employment/Consultancy; AstraZeneca, Boehringer Ingelheim, Daiichi-Sankyo, Eli Lilly, Intarcia, Janssen, Merck, Novo Nordisk, Sanofi. Lecture/other fees; AstraZeneca, Boehringer Ingelheim, Daiichi-Sankyo, Eli Lilly, Intarcia, Janssen, Merck, Novartis, Novo Nordisk, Sanofi. Non-financial support; AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Daiichi-Sankyo, Eli Lilly, GlaxoSmithKline, Intarcia, Janssen, Lexicon, Merck, Novartis, Novo Nordisk, Pfizer, Sanofi, Hanmi.

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Efficacy and safety of insulin degludec/liraglutide (IDegLira) vs basal-bolus therapy in patients with type 2 diabetes: DUAL VII trial

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Background and aims: Basal-bolus (BB) insulin therapy is considered the gold standard regimen for treating patients with type 2 diabetes (T2D) who cannot achieve glycaemic control with basal insulin. DUAL VII therefore aimed to assess whether glycaemic control could be attained using IDegLira, with lower hypoglycaemic rates and reduced weight gain compared with BB.
Materials and methods: In a 26-week, open-label, treat-to-target trial, 506 patients with T2D uncontrolled on metformin and 20-50 U insulin glargine 100 U/mL (IGlar U100) were randomized 1:1 to IDegLira or BB therapy (IGlar U100 + insulin aspart up to 4 times a day).
Results: Mean HbA_{1c} decreased from 8.2% at baseline to 6.7% at end of trial in both arms; non-inferiority (by <0.3%) for IDegLira was confirmed (p<0.0001; Table). A similar proportion of patients achieved HbA_{1c} targets with IDegLira vs BB (66.0% vs 67.0% for <7% and 49.6% vs 44.6% for ≤6.5%, respectively). Total daily insulin dose was lower for IDegLira (40.4 U) vs BB (84.1 U) (p<0.0001). Body weight decreased with IDegLira and increased with BB (p<0.0001); the rate of hypoglycaemic episodes (HEs) was lower with IDegLira vs BB (p<0.0001). More patients achieved a triple composite endpoint (HbA_{1c} <7% with no HE in the last 12 weeks and no weight gain) with IDegLira vs BB (34.9% vs 4.7%; odds ratio 12.56 [95% CI 6.46; 24.45] p<0.0001). Mean pre- to postprandial plasma glucose increment decreased more with BB vs IDegLira (p=0.0032). Short Form Health Survey 36 Version 2 (SF-36) (mental component summary) and Treatment Related Impact Measure - Diabetes (TRIM-D) (total scores) improved more with IDegLira vs BB (p=0.0074 and p<0.0001 respectively). Adverse event rates were similar.
Conclusion: In patients with HbA_{1c} >7% on metformin and IGlar U100, IDegLira vs BB resulted in similar HbA_{1c} reductions, lower insulin dose, weight loss and lower risk of HEs.

Table: Change in HbA_{1c} and body weight, and week-26 FPG and hypoglycaemic episodes at end of trial. DUAL VII trial: NCT02420262

	IDegLira + met (n=252)	IGlar U100 + IAsp + met (n=254)	Treatment contrast [95% CI]	P-value
Change in HbA_{1c}, %*	−1.49 (0.93)	−1.48 (0.91)	ETD −0.02 [−0.16; 0.12]†	<0.0001‡
Week 26 FPG, mmol/L*	6.09 (1.77)	6.39 (2.20)	ETD -0.31 [-0.67; 0.05]†	0.0936
Change in body weight, kg*	−0.92 (3.23)	2.64 (3.72)	ETD −3.57 [−4.19; −2.95]†	<0.0001
Overall hypoglycaemic episodes,§ episodes/PYE	1.07	8.17	ERR 0.11 [0.08; 0.17]¶	<0.0001
Nocturnal hypoglycaemic episodes, episodes/PYE	0.13	1.66	ERR 0.08 [0.04; 0.17]¶	<0.0001

*Data are mean (SD). †MMRM analysis values based on on-treatment observed data at planned scheduled visits. The model includes treatment, region and visit as fixed factors, and baseline response as covariate. Interactions between visit and all factors and covariates were also included in the model. ‡Two-sided p-value test for non-inferiority with non-inferiority margin 0.3%. §Hypoglycaemia defined as severe (requiring the assistance of another person) or BG-confirmed (<56 mg/dL) hypoglycaemic episodes with symptoms consistent with hypoglycaemia. Severe or BG-confirmed symptomatic hypoglycaemic episodes with onset between 00:01 and 05:59 h (inclusive) were classified as nocturnal. Efficacy endpoints are based on FAS and hypoglycaemia data summaries are SAS, hypoglycaemia statistical analyses are based on FAS. ¶Negative binomial regression model with log link and log exposure + 7 days as offset. The model includes region and treatment as factors.
HbA_{1c}, glycated haemoglobin; BG, blood glucose; CI, confidence interval; ERR, estimated rate ratio; ETD, estimated treatment difference; FAS, full analysis set; FPG, fasting plasma glucose; IAsp, insulin aspart; IDegLira, insulin degludec/liraglutide; IGlar U100, insulin glargine 100 units/mL; met, metformin; MMRM, mixed-effect model for repeated measurement; PYE, patient-year of exposure; SAS, safety analysis set; SD, standard deviation

Clinical Trial Registration Number: NCT02420262
Supported by: Novo Nordisk A/S
Disclosure: **L.K. Billings:** Honorarium; Novo Nordisk.

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ITCA 650 exenatide vs sitagliptin added-on to metformin: estimates of the need for further diabetes therapy

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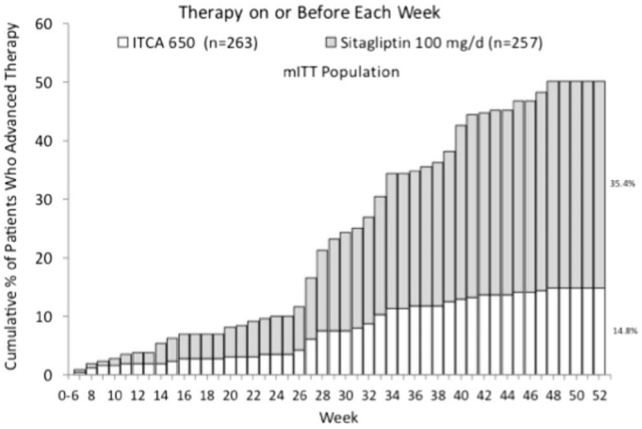
Background and aims: The need for advancing antidiabetes therapy is a valid indicator of the effectiveness and sustainability of an antidiabetes agent. ITCA 650 is an osmotic mini-pump in development for type 2 diabetes (T2D) that continuously delivers exenatide SC for up to 6 months with subdermal placement. ITCA 650 20 mcg/day for 13 weeks followed by a maintenance dose of 60 mcg/day every 6 months was tested vs sitagliptin 100 mg (SITA) in the FREEDOM-2 study. ITCA 650 demonstrated greater reductions in HbA1c (1.5 % vs 0.8%, p<0.001) and body weight (4 kg vs 1.3 kg, p<0.001).
Materials and methods: This exploratory analysis from FREEDOM-2 assessed the need for further therapy in addition to ITCA 650 or SITA added to metformin in 530 uncontrolled T2D pts (mean baseline HbA1c 8.6%). Further therapy was protocol mandated based on predefined criteria that became more stringent over time including any HbA1c >8% after Week 26.
Results: More SITA pts advanced therapy compared to ITCA 650; the incidence increased significantly and progressively with SITA after Week 26 (Figure). In contrast, most ITCA 650 treated pts achieved and maintained glycemic control. At 52 weeks, 85.2% of pts on ITCA 650 remained on two therapies (Met + ITCA 650) compared to 64.6% on SITA.
Conclusion: In conclusion, addition of ITCA 650 for 52 weeks led to better sustained glycemic control in uncontrolled T2D on metformin, significantly reducing the need for further therapy compared to addition of SITA.

Figure 1 Percentage of Patients who Advanced Antidiabetes Therapy on or Before Each Week



Clinical Trial Registration Number: NCT01455870
Supported by: Intarcia Therapeutics, Inc.
Disclosure: **M. Baron:** Employment/Consultancy; Intarcia Therapeutics, Inc.

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Efficacy and safety of fixed-dose combination therapy of gemigliptin/rosuvastatin compared to each mono therapy in type 2 diabetes patients with dyslipidaemia: BALANCE study

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Background and aims: Diabetes is highly likely to be accomapanied with dyslipidemia, which is one of the major risk factors for cardiovascular disease in type 2 diabetes mellitus (T2DM) patients. This study was to evaluate the efficacy and safety of the fixed-dose combination (FDC) therapy of gemigliptin, a potent and selective DPP-4 inhibitor and rosuvastatin, a potent HMG-CoA reductase inhibitor compared to each mono-therapy in T2DM patients with dyslipidemia.
Materials and methods: In this randomized, placebo-controlled, double-blind, phase III trial, a total of 290 T2DM patients with dyslipidemia who couldn't achieve adequate glycemic control with stable metformin mono therapy (≥1000mg/day) were randomized (1 : 1 : 1) to gemigliptin/rosuvastatin FDC (n=96), gemigliptin (n=97) or rosuvastatin (n=97). Subjects received gemigliptin 50mg and rosuvastatin with forced up-titration from 5mg to 20mg at 8-week interval, while maintained metformin dose during the study period. The primary endpoints were changes from baseline in HbA1c and low-density lipoprotein cholesterol (LDL-C) after 24 weeks.
Results: Baseline demographics and clinical characteristics were well balanced among treatment groups (mean HbA1c 7.79 ± 0.78 %; LDL-C 136.3 ± 27.8 mg/dL; age 55.9 ± 10.1 years; BMI 25.5 ± 3.2 kg/m2, duration of T2DM 6.6 ± 5.7 years). At 24 weeks, the LSM (least square mean+SE) difference of change of HbA1c (%) [(gemigliptin/rosuvastatin FDC)-(rosuvastatin)] was -0.81 ± 0.11% [95% CI(-1.04, -0.59)] and the LSM difference of % change of LDL-C (mg/dL) [(gemigliptin/rosuvastatin FDC)-(gemigliptin)] was -51.9 ± 2.7 % [95% CI (-57.3, -46.5)]. The differences in proportions achieving an HbA1c < 7% or LDL-C < 100 mg/dL were also statistically significant (p<0.001) between the FDC and the mono therapy group, respectively. There were no significant differences in adverse event among gemigliptin/rosuvastatin FDC, gemigliptin and rosuvastatin group (45.8%, 30.9% and 39.2% respectively).
Conclusion: Gemigliptin/rosuvastatin FDC was well tolerated and produced significant improvement in HbA1c and LDL-C levels in T2DM patients with dyslipidemia.
Clinical Trial Registration Number: NCT02126358
Supported by: LG Chem
Disclosure: **J. Bae:** None.

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The effect of ideglira on glycaemic control and cardiometabolic risk factors compared to the non fixed administration of degludec and liraglutide

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Background and aims: IDegLira is a novel, once-daily, titratable, fixed-ratio combination of insulin degludec and the glucagon-like peptide-1 receptor agonist (GLP-1RA) liraglutide that has been developed for the treatment of people with type 2 diabetes. The efficacy and safety of IDegLira and the benefits of its complementary mode of action have been examined in the large global DUAL clinical trial program. The aim of this study is to examine the effect of the IDegLira on the glycemic control and the cardiometabolic risk factors compared to the non fixed adiministration of degludec and liraglutide.
Materials and methods: 347 patients were included in the study. 124 uncontrolled patients on oral antidiabetic drugs switched to a therapeutic regime with OADs and IDegLira (Group A), 114 patients who were under treatment with OADs and liraglutide switched to a therapeutic regime with degludec added to the existing therapeutic regime (Group B) and 109 patients under treatment with degludec and OADs switched to a therapeutic regime with liraglutide added to the existing treatment (Group C). All patients also received metformin while none of them received sulfonylurea. Follow-up was 6 months and changes in HbA1c, fasting plasma glucose, waist circumference, weight, body mass index (BMI), hypoglycemic episodes, treatment adherence (with the 8-item Morisky Medication Adherence Scale (MMAS-8)) were recorded and insulin dose in study's groups.
Results: There was no statistically significant difference at baseline between the three Groups as to age (mean age 60.53±9.47 yers, p=0.224), diabetes duration (mean value 13.56±7.72, p=0.322), HbA1c (mean HbA1c: 8.56±1.12, p=0.116) and BMI (32.14±3.56, p=0.142). The mean HbA1c after 6 months was 6.98±0.85, 7.11±1.27, 7.28±1.33 (p=0.037) for Groups A, B and C respectively. More patients achieved the therapeutic target for HbA1c in Group A (78% compared to 71% and 68% for Groups B and C (p=0.048). The weight change was -2.8±1.2 Kg for Group A, 1.1±1.4 Kg for Group B and -3.9±2.1Kg for Group C (p<0.001). Waist circumference improved in Group A (-3.1±2.2 cm) and Group C (-3.9±1.9 cm) but not in Group B (1.9±) (p=0.001). There was no difference in the hypoglycemic episodes between the three Groups (p=0.336). Greater adherence was achieved in Group A compared to Group B and C (7.55±1.23, 6.33±1.35 and 6.45±1.25 (p=0.033) respectively). Total insulin dose was lower for Group A compared to Group B and C (35.66±8.93, 38.72 ±11.25, 42±9.78 (p=0.040) respectively).
Conclusion: Uncontrolled patients on oral antidiabetic drugs when switched to a therapeutic regime with IDegLira achieved a signifficant improvement in their glycemic control along with a decrease in their body weight in most of them and with a sighifficant adherence to their therapeutic regime. The use of degludec and liraglutide in a nonfixed combination improves glycemic control and cardiomaetabolic risk factor but not as impressively as the use of IDegLira while the adherence to treatment is not signifficant enough.
Disclosure: **A. Sotiropoulos:** None.

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Impact of lixisenatide (LIXI) dose range on glycaemic outcomes with fixed-ratio combination iGlarLixi in patients with type 2 diabetes

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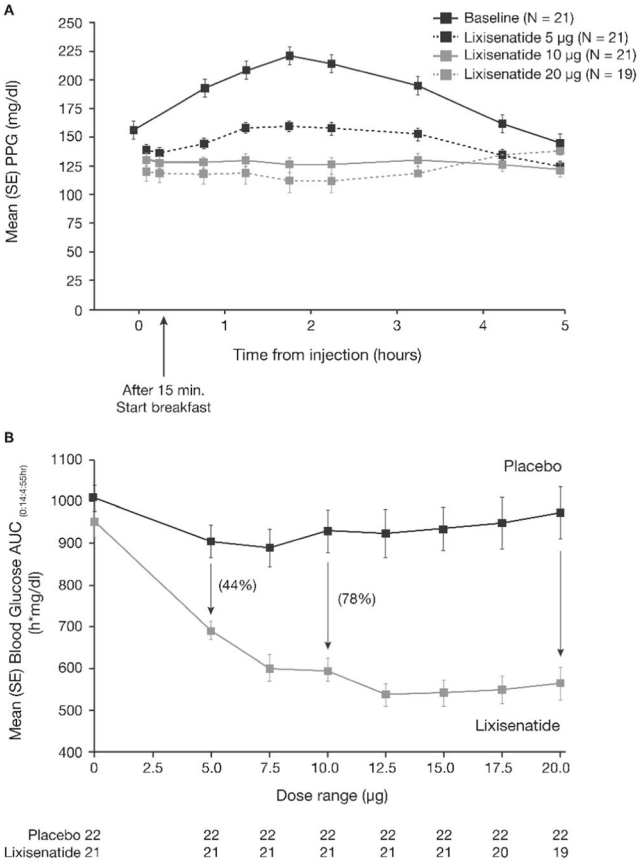
Background and aims: iGlarLixi is a novel, titratable fixed-ratio combination of insulin glargine 100U/ml (iGlar) and LIXI (a glucagon-like peptide-1 receptor agonist that selectively attenuates postprandial glucose [PPG]) intended for patients with T2D not achieving glycemic targets with basal insulin or LIXI. iGlar/LIXI doses in iGlarLixi range from 15U/5 μg to 60U/20 μg. We evaluated the impact of LIXI (5-20 μg) on PPG in patients with T2D receiving LIXI alone or as part of iGlarLixi.
Materials and methods: In a 28-day study (ACT6011) in patients on oral antidiabetes drugs, PPG was suppressed by LIXI 5-20 μg (Fig. A). LIXI 5 μg reduced PPG area under the curve to a value that was 44% of that observed with LIXI 20 μg (Fig. B). In a 13-week dose-ranging trial (DRI6012), LIXI 5 μg reduced PPG by −38.2 mg/dl and HbA_{1c} by −0.47% from baseline; 47% of patients reached target HbA_{1c} < 7.0% (published data), also LIXI 5-20 μg dose was reported safe and tolerable.
Results: In a 30-week, phase 3 study (LixiLan-O) in patients uncontrolled on oral antidiabetes drugs, patients on iGlarLixi doses containing LIXI 5-10, 10-15 or 15-20 μg had clinically meaningful changes from baseline in PPG excursions −29.1, −36.0, or −52.4 mg/dl. HbA_{1c} reduced more with iGlarLixi vs iGlar and LIXI (−0.29% and −0.78% difference), with both components contributing to an overall treatment effect (published data).

Conclusion: The treatment effect on HbA_{1c} across LIXI dose categories is consistent with the overall effect, suggesting a contribution of LIXI across the entire iGlarLixi dose range (5-20 μg), even at 5 μg.

Figure. Lixisenatide suppression (A) and reduction (B) of PPG over dose range of 5–20 μg



AUC, area under the curve; SE, standard error; min, minute.

Clinical Trial Registration Number: ACT6011, DRI6012 and LixiLan-O: NCT02058147

Supported by: Study funding and editorial support provided by Sanofi US, Inc.

Disclosure: **M. Roberts:** Employment/Consultancy; Sanofi US.

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The association between iGlarLixi and patient satisfaction with their treatment's ability to control type 2 diabetes is mediated by reduced glycaemic variability (GV)

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Background and aims: The aim of this post hoc analysis was to evaluate the association between iGlarLixi, GV, and patients' satisfaction with their treatment's ability to control their diabetes (measured by the Diabetes Management [DM] score of Treatment-Related Impact Measure for Diabetes [TRIM-D].)

Materials and methods: LixiLan-L is a phase 3, 30-week randomized controlled trial in patients with T2D previously on basal insulin ± oral antidiabetes drugs treated with insulin glargine 100U/ml (iGlar) or iGlarLixi, a fixed-ratio combination of iGlar and the glucagon-like peptide-1 receptor agonist lixisenatide. The DM domain of TRIM-D contains questions on patients' satisfaction with diabetes treatment effects. DM scores were recorded at baseline and Week 30, together with 7-point self-measured plasma glucose (SMPG) profiles used to calculate several GV metrics. A DM score increase ≥ 8.2 is a clinically meaningful difference; patients with DM increase ≥ 8.2 were considered DM responders.

Results: At Week 30, 39.3% of iGlarLixi- vs 32.6% of iGlar-treated patients were DM responders ($P = 0.0577$). Among DM responders, iGlarLixi (vs iGlar) resulted in significantly greater reductions in GV metrics such as mean amplitude of glucose excursions (24% vs 5%; $P < 0.0001$) and area under the SMPG curve (19.1% vs 5.6%; $P < 0.0001$) indicating reduced GV. Most prominent was the reduction in the magnitude of postprandial SMPG excursions (measured by the High Blood Glucose Index) 56% for iGlarLixi vs 17% for iGlar, $P < 0.0001$. High Blood Glucose Index reduction was correlated with a greater change in DM score, particularly for iGlarLixi vs iGlar ($r = -0.30$; $P = 0.0019$ vs $r = -0.19$; $P = 0.0822$).

Conclusion: In patients with T2D, iGlarLixi reduces GV more than iGlar alone (published data). We conclude that this differential effect is preserved in the subpopulation of patients who reported more satisfaction with their treatment's ability to control their diabetes; this correlation is larger with iGlarLixi, and the self-reported effect of treatment is correlated with a reduction in the magnitude of postprandial glucose excursions.

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PS 062 Fixed ratio injectable: two in one

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Is double-blinding achievable in a placebo-controlled trial with the glucagon-like-peptide-1 receptor agonist liraglutide?

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Background and aims: Double-blinding is fundamental for bias-reduction in clinical trials. In case of interventions with substantial effect and/or side-effects, successful blinding may be difficult to obtain. Perception of the intervention may affect psychological and physical responses of the participants as well as investigators' assessment of outcomes. The aim of this study was to assess successful double-blinding in a randomised, double-blinded, placebo-controlled trial evaluating the effect of the glucagon-like peptide-1 receptor agonist, liraglutide, on glycaemic control in women with prior gestational diabetes mellitus (pGDM).
Materials and methods: Overweight, non-diabetic women with pGDM were randomised to liraglutide 1.8 mg once-daily (n=49; age: 38±5 (mean±SD) years; BMI: 32±6 kg/m^2) or placebo (n=55; age: 38±5 years; BMI: 31±3 kg/m^2) for one year. Immediately before un-blinding at one-year follow up, all participants and two principal investigators answered a five-option questionnaire whether they were "Certain" or "Thought it likely" that the participant had received active treatment, if they were "Unsure" of treatment, or if they were "Certain" or "Thought it likely" that the participant had received placebo. Blinding was defined as successful if the answer was either of the two incorrect answers or "Unsure". Successful double-blinding was defined as cases were both participants and investigators answered incorrect or "Unsure".
Results: Eighty-nine women completed one-year treatment and questionnaire. Successful blinding of participants was achieved in 32% (n=14 of 44) of liraglutide-treated cases and 27% (n=12 of 45) of placebo-treated (29% in total) with no difference between groups (p=0.593). Successful blinding of investigators was achieved in 38% (n=33 of 88) for liraglutide and 36% (n=32 of 90) of cases for placebo (37% in total). Successful double-blinding was achieved in 9% (n=4 of 45) of cases in the placebo group and in 14% (n=6 of 44) of cases in the liraglutide group with no difference between groups (p=0.478).
Conclusion: In conclusion, successful double-blinding seems low when comparing liraglutide (9%) and placebo (14%) treatment in women with pGDM. Nevertheless, successful blinding may be achieved in 29% of women with pGDM treated with liraglutide/placebo and investigators may be blind to 37% of cases regardless of allocation to active or placebo treatment. Opinions on how successful blinding can be expected remain different from study to study according to design and obvious effects of the intervention.
Clinical Trial Registration Number: 2012-001371-37-DK
Disclosure: **S. Foghsgaard:** Grants; Unrestricted grant from Novo Nordisk.

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Shorter time to glycaemic control with fixed-ratio combination of insulin glargine and lixisenatide compared with insulin glargine treatment alone

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Background and aims: Simultaneous treatment with insulin glargine (iGlar) + lixisenatide (Lixi) as a titratable, fixed-ratio combination (iGlarLixi) is a new therapeutic option in type 2 diabetes mellitus (T2DM) in patients uncontrolled on metformin ± a second oral antidiabetic (OAD) or basal insulin. A post hoc analysis was used to evaluate time to glycaemic control with iGlarLixi vs iGlar alone in patients with T2DM uncontrolled on OADs (LixiLan-O trial) or basal insulin (LixiLan-L trial).
Materials and methods: The Kaplan-Meier method was used to estimate time to control, defined as time (days) to first achieve HbA_{1c} <7% or fasting plasma glucose (FPG) ≤7.2 mmol/L.
Results: In LixiLan-O and LixiLan-L, 60% and 46% of patients, respectively, reached target HbA_{1c} with iGlarLixi at 12 weeks (Table). In LixiLan-O, 50% of patients achieved HbA_{1c} <7% in approximately half the time with iGlarLixi vs iGlar (median, 85.0 days vs 166.0 days; HR: 1.5; p<0.0001). In LixiLan-L, the HbA_{1c} target was achieved by 50% of patients in a median time of 153.0 days with iGlarLixi, but target HbA_{1c} was never reached by 50% of patients with iGlar (HR: 2.0; p<0.0001). In contrast, when time to glycaemic control was analysed for FPG, results were comparable vs iGlar in both studies (Table).
Conclusion: In addition to lowering FPG via iGlar, iGlarLixi lowers postprandial plasma glucose, likely contributing to its greater efficacy in achieving glycaemic control vs iGlar alone. In conclusion, in patients with T2DM uncontrolled on OADs or basal insulin, iGlarLixi induced glycaemic control (HbA_{1c} <7%) earlier and in more patients vs iGlar alone.

Table. Achievement of glycaemic control targets in the LixiLan-O and LixiLan-L trials (safety population)

	iGlarLixi	iGlar
LixiLan-O*	***N*=469**	***N*=467**
HbA_{1c} <7%		
Patients achieving target, *n* (%)†		
n	468	466
At 8 weeks	186 (39.7)	128 (27.5)
At 12 weeks	279 (59.6)	209 (44.8)
Days to first HbA_{1c} <7%, median‡	85.0	166.0
HR (95% CI)§	1.5 (1.3, 1.7)	
p-value¶	<0.0001	
FPG ≤7.2 mmol/L		
Days to first FPG ≤7.2 mmol/L, median‡	56.0	57.0
HR (95% CI)§	1.1 (1.0, 1.2)	
p-value¶	0.2134	
LixiLan-L*	***N*=365**	***N*=365**
HbA_{1c} <7%		
Patients achieving target, *n* (%)†		
n	366	365
At 8 weeks	116 (31.7)	73 (20.0)
At 12 weeks	168 (45.9)	87 (23.8)
Days to first HbA1c <7%, median‡	153.0	NR
HR (95% CI)§	2.0 (1.7, 2.5)	
p-value¶	<0.0001	
FPG ≤7.2 mmol/L		
Days to first FPG ≤7.2 mmol/L, median‡	1.0	1.0
HR (95% CI)§	0.9 (0.8, 1.1)	
p-value¶	0.2137	

*In both LixiLan-O and LixiLan-L, drug titrations were based on the same algorithm.
†Responder analysis based on the mITT population: estimated by proportion of patients achieving targets at Weeks 8 and 12. ‡Median time to control: defined as 50% of patients reaching target as estimated by the Kaplan–Meier method. §Estimated using Cox regression model with treatment as the only factor. ¶Calculated using the log-rank test.
FPG, fasting plasma glucose; mITT, modified intent-to-treat; NR, not reached (i.e. target not reached by 50% of patients).

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Patient-reported outcomes with insulin degludec/liraglutide (IDegLira) vs basal-bolus therapy in patients with type 2 diabetes: DUAL VII trial

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Background and aims: Basal-bolus (BB) insulin therapy is considered the gold standard regimen for treating patients with type 2 diabetes (T2D) who do not achieve glycaemic control with basal insulin, but fear of hypoglycaemia and weight gain are recognised barriers to therapy intensification. The aim of this analysis was to see whether insulin degludec/liraglutide combination (IDegLira) could provide an alternative intensification option that was more acceptable to patients.
Materials and methods: In this 26-week open-label trial, 506 adult patients with T2D, HbA_{1c} 7-10% on metformin (met) and 20-50 units insulin glargine 100 units/mL (IGlar U100) were randomised 1:1 to receive once-daily IDegLira or BB insulin (once daily IGlar U100 + insulin aspart ≤4 times a day). Patients' perceived health status and treatment experiences were quantified using patient-reported outcomes (PROs) questionnaires, adding the patient perspective to treatment evaluation.
Results: Patients on IDegLira had an equal reduction in HbA_{1c}, lower burden of hypoglycaemia, fewer injections/day, and weight loss versus BB. Treatment-Related Impact Measure-Diabetes (TRIM-D) showed greater improvements in favour of IDegLira vs. BB in all domains (Table) and Total Score (estimated treatment difference [ETD] 6.50 [95% CI 4.44; 8.57] $p<0.0001$). The greatest improvements were in diabetes management (likely driven by items on avoiding hypoglycaemia/weight gain), treatment burden and compliance. Short Form Health Survey 36 v2 (SF-36) ETD was in favour of IDegLira vs. BB for the mental component summary (1.83 [95% CI 0.26; 3.40] $p=0.023$), driven by an improvement in mental health (ETD 2.29 [95% CI 0.62; 3.96] $p=0.0074$). Other SF-36 ETDs were not significant. In a motivation survey 26 weeks after randomisation, 84.5% of IDegLira patients were willing to stay on study therapy vs. 68.1% of BB patients (odds ratio 2.54 [95% CI 1.63; 3.98] $p<0.0001$) 16.8% of IDegLira patients preferred pre-trial therapy vs. 28.0% BB (odds ratio [OR] 0.52 [95% CI 0.33; 0.81] $p=0.004$).
Conclusion: IDegLira induced greater improvements in PROs, mainly in diabetes management and treatment burden, whilst achieving similar glycaemic control vs. BB in patients with HbA_{1c} 7-10% switched from met and IGlar U100.

Table: Change from baseline to week 26 in TRIM-D domain scores. DUAL VII trial

	IDegLira + met Observed mean change* (n=252)	IGlar U100 + IAsp + met Observed mean change* (n=254)	Estimated treatment difference (ETD) [95% CI]	*p*-value
Diabetes management	16.7	6.8	10.76 [7.62; 13.90]	<0.0001
Treatment burden	12.4	4.3	10.50 [7.34; 13.67]	<0.0001
Compliance	9.1	3.9	6.25 [3.82; 8.69]	<0.0001
Daily life	3.5	−0.4	4.23 [1.09; 7.37]	0.0083
Psychological health	5.7	3.0	2.77 [0.32; 5.21]	0.0268

Observed data; MMRM with treatment, region and visit as fixed factors, and baseline value as covariate. Interactions between visit and all other factors and covariate are included. TRIM-D questions available at: https://eprovide.mapi-trust.org/instruments/treatment-related-impact-measure-for-diabetes.* Positive number denotes improvement in parameter being measured.

CI, confidence interval; IAsp, insulin aspart; IDegLira, insulin degludec/liraglutide; IGlar U100, insulin glargine 100 units/mL; met, metformin; MMRM, mixed-model repeat measurement; TRIM-D, Treatment-Related Impact Measure–Diabetes.

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Disclosure: **E. Jodar:** Lecture/other fees; Janssen, Lilly, MSD, Novo Nordisk, Sanofi.

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iGlarLixi reduces HbA_{1c} to a greater extent than basal insulin therapy regardless of HbA_{1c} levels at screening

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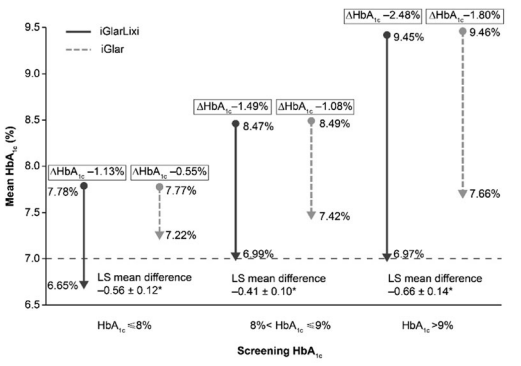
Background and aims: LixiLan-L was a 30-week trial in patients with type 2 diabetes uncontrolled (7.5-10% HbA_{1c} at screening) on basal insulin ± oral agents. After a 6-week run-in with insulin glargine (iGlar), patients were treated with either a fixed-ratio combination of iGlar and lixisenatide (iGlarLixi) ± metformin or iGlar ± metformin (30-week completers: n=325 and n=331, respectively). Mean HbA_{1c} (8.5%) was reduced more from screening to study end with iGlarLixi than iGlar (−1.7% vs −1.1%; $p<0.0001$), and iGlarLixi-treated patients achieved a mean HbA_{1c} within ADA recommendations (mean study end HbA_{1c}: 6.9% vs 7.4% for iGlar). A post hoc analysis was performed to examine differences in efficacy according to pre-study levels of glycaemic control.
Materials and methods: Patients were split into three categories by HbA_{1c} level at screening: $HbA_{1c} \leq 8\%$, $8\% < HbA_{1c} \leq 9\%$, $HbA_{1c} > 9\%$. Change from screening to study end (30 weeks post-randomization) was determined by ANOVA of 30-week completers in the modified intent-to-treat population.
Results: Reductions in HbA_{1c} were greater for iGlarLixi than iGlar in all categories (−1.1%, −1.4%, −2.4% vs −0.5%, −1.0%, −1.8%, respectively; $p<0.0001$ for all). The respective mean observed HbA_{1c} levels at study end were all ≤7.0% for iGlarLixi (6.7%, 7.0%, 7.0%), but >7.0% for iGlar (7.2%, 7.4%, 7.7%; Figure).
Conclusion: Although patients with higher initial HbA_{1c} had the greatest effects for both treatment strategies, iGlarLixi was more effective in controlling HbA_{1c} irrespective of initial levels.

Figure. HbA_{1c} change in LixiLan-L 30-week completers based on screening HbA_{1c} value



30-week completers are patients who have completed the 30-week treatment period without rescue therapy.
*$p<0.0001$ for difference in LS mean ± SE from screening to Week 30 for iGlarLixi vs iGlar.
LS, least squares; SE, standard error.

Clinical Trial Registration Number: NCT02058160
Supported by: Sanofi
Disclosure: **E. Niemoeller:** Employment/Consultancy; Sanofi. Stock/Shareholding; Sanofi.

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Characteristics and outcomes of type 2 diabetes patients titrated to 60U/day with insulin glargine/lixisenatide fixed-ratio combination vs insulin in LixiLan-L

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Background and aims: iGlarLixi is a titratable fixed-ratio combination of insulin glargine 100U/ml (iGlar) and lixisenatide (LIXI), with a maximum daily dosage of 60U iGlar/20 μg LIXI. In LixiLan-L, patients with type 2 diabetes (T2D) uncontrolled on basal insulin ± oral antidiabetes drugs were randomized to iGlarLixi (n = 366) or iGlar (n = 365) for 30 weeks, after 6 weeks of iGlar optimization.
Materials and methods: This post hoc analysis explored characteristics and outcomes in patients on the maximal dose of iGlarLixi or iGlar allowed in the trial (60U/day) at study end.
Results: In the iGlarLixi and iGlar arms, 27.0% and 30.7% of patients, respectively, reached 60U at 30 weeks. Regardless of dose, more patients on iGlarLixi achieved HbA_{1c} < 7.0% (60U: 43%; < 60U: 66%) vs iGlar (60U: 24%; < 60U: 34%). In both arms, patients reaching 60U were younger [mean age, iGlarLixi: 57.0 years (60U) vs 60.6 years (< 60U), $P = 0.0004$; iGlar: 58.2 years (60U) vs 61.3 years (< 60U), $P = 0.0015$], had higher baseline weight [mean weight, iGlarLixi: 95.7 kg (60U) vs 84.9 kg (< 60U), $P < 0.0001$; iGlar: 91.9 kg (60U) vs 84.8 kg (< 60U), $P < 0.0001$], fasting plasma glucose (FPG) [mean FPG, iGlarLixi: 140.0 mg/dl (60U) vs 129.1 mg/dl (< 60U), $P = 0.0080$; iGlar: 140.5 mg/dl (60U) vs 128.2 mg/dl (< 60U), $P = 0.0021$], and insulin dose vs patients using < 60U (**Table**). Higher weight and insulin dose at baseline may have contributed to the need to titrate to 60U. Regardless of final dose, patients in the iGlarLixi arm had numerically greater mean HbA_{1c} reductions [iGlarLixi: −1.0% (60U) vs −1.2% (< 60U), $P = 0.1233$; iGlar: −0.5% (60U) vs −0.6% (< 60U), $P = 0.0935$] and greater postprandial glucose (PPG) reductions from baseline. Maximal dose users in both arms had lower incidence of symptomatic hypoglycaemia, but similar FPG and PPG changes from baseline vs patients using < 60U.
Conclusion: Despite similar weight-adjusted insulin doses in both arms, HbA_{1c} reduction and percentage of patients achieving glycaemic goal was greater with iGlarLixi vs iGlar in patients reaching 60U. The characteristics of 60U users may assist in guiding clinical decisions.

Characteristics and outcomes of patients in LixiLan-L by dose at Week 30 (all analyses based on modified intent-to-treat population).	iGlarLixi < 60U (n = 267, 73.0%)	iGlarLixi = 60U (n = 99, 27.0%)	*P* Value	iGlar < 60U (n = 253, 69.3%)	iGlar = 60U (n = 112, 30.7%)	*P* Value
T2D duration in years, mean (SD)	12.3 (6.7)	11.4 (6.4)	0.2837	12.7 (6.9)	10.8 (6.7)	0.0143
Weight change from baseline to study end in kg, mean (SD)	−0.8 (3.2)	−0.2 (3.0)	0.1489	0.5 (2.6)	1.2 (2.5)	0.0254
HbA_{1c} at baseline, % mean (SD)	8.0 (0.6)	8.2 (0.8)	0.0373	8.0 (0.7)	8.1 (0.8)	0.3037
FPG change from baseline to study end in mg/dl, mean (SD)	−9.6 (45.5)	−10.3 (43.9)	0.8854	−13.7 (47.1)	−3.6 (42.6)	0.0524
2-hour PPG at baseline mg/dl, mean (SD)	266.5 (68.5)	272.1 (70.5)	0.5045	270.3 (65.9)	265.6 (62.3)	0.5415
2-hour PPG change from baseline to study end in mg/dl, mean (SD)	−93.2 (77.3)	−90.2 (80.7)	0.7565	−31.8 (73.8)	−23.8 (64.6)	0.3419
Insulin dose at baseline in U, mean (SD)	32.4 (8.2)	41.7 (8.2)	< 0.0001	32.9 (7.9)	40.6 (7.8)	< 0.0001
Insulin dose at study end in U, mean (SD)	40.3 (11.0)	60.0 (0.2)	< 0.0001	40.8 (10.6)	60.1 (0.8)	< 0.0001
Insulin dose/kg at baseline in U/kg, mean (SD)	0.4 (0.1)	0.4 (0.1)	< 0.0001	0.4 (0.1)	0.5 (0.1)	< 0.0001
Insulin dose/kg at study end in U/kg, mean (SD)	0.5 (0.1)	0.6 (0.1)	< 0.0001	0.5 (0.1)	0.7 (0.1)	< 0.0001
Incidence of symptomatic hypoglycaemia accompanied by a measured plasma glucose concentration of ≤ 70 mg/dl at study end, n/N (%)	118/267 (44.2)	28/99 (28.3)	0.0058	122/253 (48.2)	33/112 (29.5)	0.0008

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Insulin degludec/liraglutide (IDegLira) provides clinical benefits across all end of trial dose ranges: analyses from DUAL I and V

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Background and aims: The DUAL clinical trial program investigated the efficacy and safety of the fixed ratio combination IDegLira in various type 2 diabetes populations. IDegLira (1 dose step/unit [U] = 1 U insulin degludec [IDeg] + 0.036 mg liraglutide; max dose 50 U) lowered glycaemia more effectively than IDeg (DUAL I) or insulin glargine U100 (IGlar U100; DUAL V); with no max dose of basal insulin in either trial. This *post hoc* analysis aimed to evaluate the efficacy, hypoglycaemia risk and weight change across comparable end of trial (EOT) daily insulin dose groups upon treat to target titration, thereby assessing the effect of varying liraglutide doses within IDegLira.
Materials and methods: Patients who participated in the DUAL I and V clinical trials were grouped by their EOT insulin dose (DUAL I: <20, ≥20-<30, ≥30-<40, ≥40 U; DUAL V: <30, ≥30-<40, ≥40 U) and HbA_{1c}, weight change, hypoglycaemia rate and dose were assessed.
Results: Baseline HbA_{1c} ranged from 8.0-8.4% and HbA_{1c} reductions were greater with IDegLira (-1.5 to -2.1%) vs IDeg (-1.1 to -1.9%) or IGlar U100 (-1.2 to -1.6%) for all dose groups (Table). The percent of patients achieving an EOT HbA_{1c} <7% was higher with IDegLira vs IDeg or IGlar U100 for all dose groups, except IDegLira vs. IDeg in the ≥30-<40 U group. In DUAL V, with IDegLira, change in HbA_{1c} (both -1.8%) and the proportion of patients with HbA_{1c} <7% (68.4 vs 73.8%) were similar for EOT dose of 50 U vs. <50 U. IDegLira resulted in weight loss across dose ranges while both of the basal insulins resulted in weight gain, except in the IGlar U100 in the ≥30-<40 U group. Hypoglycaemia rates (events/patient year of exposure [PYE]) were lower for IDegLira vs. IDeg and IGlar U100, except in the DUAL I <20 U group. In DUAL I, mean EOT insulin doses for <20 U were IDegLira: 14.0 U, IDeg: 14.9 U; ≥20-<30 U IDegLira: 24.2 U, IDeg: 24.6 U; ≥30-<40 U IDegLira: 34.2 U, IDeg: 34.5 U; and ≥40 U IDegLira: 48.4 U, IDeg: 69.2 U. In DUAL V, mean EOT insulin doses for <30 U were IDegLira: 23.3 U, IGlar U100: 24.7 U; ≥30-<40 U IDegLira: 34.6 U, IGlar U100: 34.9 U; and ≥40 U IDegLira: 48.3 U, IGlar U100: 73.6 U.
Conclusion: Overall, the glucose-lowering effect, lower risk of hypoglycaemia and change in weight with IDegLira appeared consistent, supporting an effect of the liraglutide component across the dose range.

Table	DUAL I: IDegLira vs. IDeg					DUAL V: IDegLira vs. IGlar U100			
Dose group	Overall	<20 U/day	≥20–<30 U/day	≥30–<40 U/day	≥40 U/day	Overall	<30 U/day	≥30–<40 U/day	≥40 U/day
IDegLira, N	833	99	115	155	447	278	53	53	172
ΔHbA$_{1c}$, % (SD)	-1.9 (1.1)	-1.5 (1.0)	-1.8 (1.1)	-2.1 (1.0)	-2.0 (1.1)	-1.8 (1.1)	-1.5 (1.1)	-2.0 (1.1)	-1.9 (1.1)
HbA$_{1c}$ <7% at EOT, %	80.6	79.8	85.2	88.4	79.0	71.6	62.3	83.0	70.9
Δbody weight, kg (SD)	-0.5 (3.5)	-0.3 (3.7)	-0.7 (3.2)	-0.6 (3.3)	-0.5 (3.6)	-1.4 (3.5)	-1.8 (3.0)	-1.7 (4.0)	-1.1 (3.5)
Hypo rate, events/PYE	1.8	3.4	2.3	2.4	1.2	2.2	6.4	2.8	1.0
Basal insulin, N	**413**	**42**	**63**	**44**	**259**	**279**	**17**	**31**	**231**
ΔHbA$_{1c}$, % (SD)	-1.4 (1.0)	-1.1 (1.0)	-1.3 (0.8)	-1.9 (1.2)	-1.5 (1.0)	-1.1 (1.0)	-0.6 (1.0)	-1.0 (0.7)	-1.2 (1.0)
HbA$_{1c}$ <7% at EOT, %	65.1	50.0	76.2	88.6	61.8	47.0	17.6	45.2	49.4
Δbody weight, kg (SD)	1.6 (4.0)	0.9 (2.5)	0.6 (3.5)	0.9 (4.7)	2.2 (4.1)	1.8 (3.6)	1.2 (1.7)	-0.2 (2.2)	2.1 (3.7)
Hypo rate, events/PYE	2.6	3.2	3.1	3.6	2.2	5.1	12.8	5.3	4.5

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Titratable fixed-ratio vs sequential combination of insulin glargine and lixisenatide in type 2 diabetes uncontrolled on basal insulin

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Background and aims: Treatment of type 2 diabetes mellitus (T2DM) uncontrolled on basal insulin glargine (iGlar) may be advanced by adding lixisenatide (Lixi), a glucagon-like peptide-1 receptor agonist (GLP-1 RA), as a separate injection or switched to a titratable fixed-ratio combination of iGlar and Lixi (iGlarLixi). In this exploratory analysis, we determined if iGlarLixi, from the LixiLan-L trial, was more effective and better tolerated than sequential injections of iGlar and Lixi, from the GetGoal Duo-2 trial, in patients with long-standing iGlar-treated uncontrolled T2DM.
Materials and methods: The treatment strategy of simultaneous administration of iGlar with Lixi by switching from basal insulin to fixed-ratio combination (iGlarLixi) in the LixiLan-L trial (n=367) was compared indirectly with sequentially adding Lixi to basal insulin in the GetGoal Duo-2 trial (n=298) using propensity score-matching according to baseline covariates to minimize confounding. Baseline covariates included age, race, BMI, HbA_{1c}, fasting plasma glucose, diabetes duration and oral antidiabetic drug/metformin use.
Results: In 241 well-matched pairs comprising ~80% of participants, iGlarLixi provided significant treatment benefits vs sequential administration of iGlar and Lixi, including greater reductions in HbA_{1c}, greater proportions of patients attaining HbA_{1c} <7% and numerically greater decreases in postprandial plasma glucose excursions (Table). The proportion of patients reporting symptomatic documented hypoglycaemia (≤3.9 mmol/L; 36.8% vs 35.7%) was similar between treatments despite better glycaemic control with iGlarLixi. Fewer patients reported gastrointestinal (GI) adverse events with iGlarLixi vs sequential treatment (nausea: 10.0% vs 27.0%; vomiting: 3.3% vs 8.7%, respectively). At end-of-treatment, the insulin dose was lower with iGlarLixi vs the sequential treatment group (mean ± SD: 44 ± 12 U vs 66 ± 36 U).
Conclusion: In basal insulin-treated T2DM in need of insulin intensification, this indirect comparison suggests that switching to a titratable fixed-ratio combination of basal insulin and a GLP-1 RA, as demonstrated in this analysis with iGlarLixi, may provide better efficacy and GI tolerability over combining insulin and a GLP-1 RA in two separate injections.

Table. Efficacy of simultaneous vs sequential treatment with iGlar and Lixi in propensity score-matched participants

	iGlarLixi (LixiLan-L)* Week 24/26/30 LOCF (*N*=241)		iGlar + Lixi (GetGoal Duo-2)† Week 26 LOCF (*N*=241)		LS mean difference (95% CI)	*p*-value
Efficacy	**Baseline**	**Change/Wk 30**	**Baseline**	**Change/Wk 26**		
HbA_{1c}, %	7.9 ± 0.6	−1.0 ± 0.8	7.9 ± 0.5	−0.6 ± 0.8	−0.4 (−0.6, −0.3)	**<0.0001**
HbA_{1c} <7.0%, (n)		62% (148)		33% (79)		**<0.0001**
PPG excursion‡, mmol/L	7.1 ± 3.4	−4.3 ± 4.1	7.3 ± 3.2	−3.3 ± 4.0	−0.4 (−1.5, 0.7)	0.488
FPG, mmol/L	7.0 ± 1.8	−0.2 ± 2.2	6.8 ± 1.8	−0.1 ± 2.1	−0.04 (−0.4, 0.3)	0.840

*LixiLan-L patients were randomized after a 6-week run-in if: HbA_{1c} ≥7% and ≤10% and mean fasting SMPG ≤7.8 mmol/L. Metformin use was allowed during the study.
†GetGoal Duo-2 patients were randomized after a 12-week run-in if: HbA_{1c} ≥7% and ≤9% and mean FPG ≤7.8 mmol/L. Metformin use was allowed during the study.
‡PPG excursion was calculated by subtracting the plasma glucose value from the 2-h post-meal value 30 min before standardized breakfast and before study drug injection; data only from patients who had a standardized meal test.
Data are mean ± SD unless indicated otherwise. *N* indicates number of propensity score-matched pairs.
FPG, fasting plasma glucose; LOCF, last observation carried forward; LS, least squares; PPG, postprandial plasma glucose; SMPG, self-monitored plasma glucose; Wk, week.

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Insulin degludec/liraglutide (IDegLira) is efficacious and safe in patients with type 2 diabetes with normal, mild or moderate renal impairment: analyses from phase 3 trials

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Background and aims: The DUAL I-V clinical trials investigated the efficacy and safety of IDegLira vs. different comparators; basal insulin, glucagon-like peptide-1 receptor agonist (GLP-1 RA) and placebo. This *post hoc* analysis aimed to evaluate the effects of IDegLira vs. comparators in patients with type 2 diabetes (T2D) as a function of baseline renal function.
Materials and methods: Patients were grouped by their baseline renal function (normal, mild or moderate impairment, with estimated glomerular filtration rates [eGFR] of ≥90, ≥60-<90 and ≥30-<60 mL/min/1.73m^2, respectively).
Results: HbA_{1c} reductions from baseline to end of trial were significantly greater with IDegLira vs. comparators in all baseline renal function groups (Figure). Across renal function groups, hypoglycaemia rates were lower with IDegLira vs. basal insulin but higher vs. GLP-1 RA and placebo, and eGFR was unchanged at the end of trial for all treatments. Adverse event rates (per patient-year of exposure) were similar for patients with normal, mild and moderate renal impairment, respectively (IDegLira [4.1, 3.8 and 4.6]; basal insulin [3.6, 3.6 and 3.5]; GLP-1 RA [4.8, 4.9 and 4.5]; placebo [3.0, 4.1 and 4.6]).
Conclusion: In conclusion, IDegLira is safe and more efficacious than comparators in patients with T2D with mild or moderate renal impairment with lower hypoglycaemia rates when compared with basal insulin. The results resemble those observed in patients with normal renal function.

Table: Estimated treatment differences (ETD) [95% CI] in HbA_{1c} reduction (%)

Study – IDegLira vs comparator	ETD [95% CI] (Normal renal function)	ETD [95% CI] (Mild renal impairment)	ETD [95% CI] (Moderate renal impairment)
DUAL I: IDegLira vs. IDeg	-0.48% [-0.63; -0.33]	-0.40% [-0.58; -0.22]	-0.83% [-1.31; -0.35]
DUAL I: IDegLira vs. Lira	-0.65% [-0.80; -0.50]	-0.56% [-0.73; -0.39]	-0.81% [-1.28; -0.34]
DUAL II: IDegLira vs. IDeg (max dose 50 U)	-1.23% [-1.52; -0.94]	-0.78% [-1.09; -0.47]	-1.30% [-2.09; -0.51]
DUAL III: IDegLira vs. unchanged GLP-1RA	-1.06% [-1.29; -0.82]	-0.84% [-1.09; -0.59]	-0.72% [-1.36; -0.07]
DUAL IV: IDegLira vs. placebo	-0.96% [-1.20; -0.72]	-1.02% [-1.25; -0.79]	-1.33% [-1.84; -0.83]
DUAL V: IDegLira vs. IGlar U100 uptitration	-0.51% [-0.71; -0.31]	-0.59% [-0.81; -0.37]	-1.44% [-2.02; -0.85]

Based on the full analysis set with last observation carried forward. Analysed using an ANOVA with treatment, baseline renal function, interaction between treatment and baseline renal function, region, baseline HbA_{1c} stratum (DUAL I), sub-study (DUAL I), concomitant diabetes treatment (DUAL I) and pre-trial medication (DUAL II, III and IV), as fixed factors and baseline HbA_{1c} as covariate. GLP-1RA, glucagon like peptide-1 receptor agonist; IDeg, insulin degludec; IDegLira, insulin degludec/liraglutide; Lira, liraglutide.

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The effect on lipid profiles of iGlarLixi versus iGlar in the LixiLan-L trial

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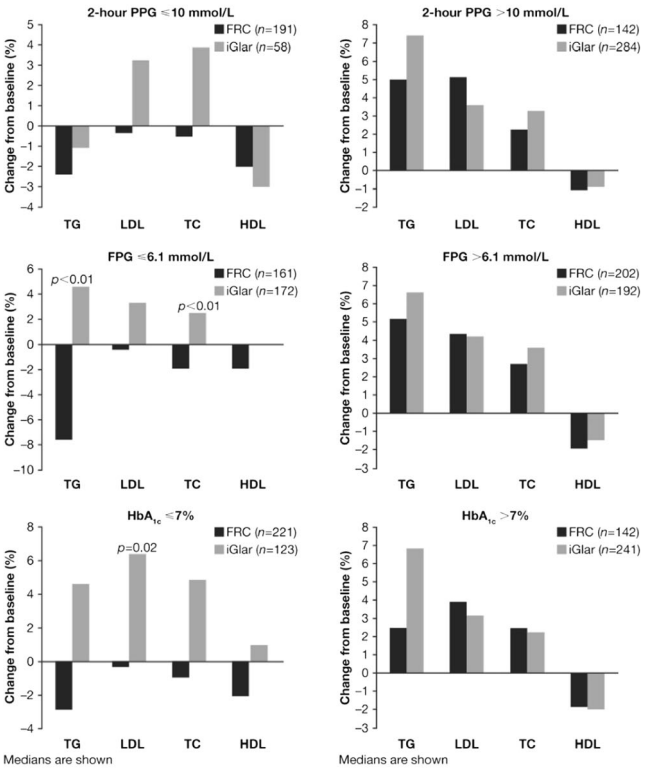
Background and aims: A number of studies have reported that short-acting glucagon-like peptide-1 receptor agonists (GLP-1 RAs) such as exenatide and lixisenatide (Lixi) improve postprandial proatherogenic lipids and improve vascular endothelial dysfunction. In addition, long-term use of short-acting GLP-1 RAs has been associated with favourable changes in the lipid profiles of patients with type 2 diabetes mellitus (T2DM). In this post hoc analysis, we determined whether iGlarLixi, a titratable fixed-ratio combination of insulin glargine (iGlar) and the short-acting GLP-1 RA Lixi, improved lipid levels versus iGlar alone in the 30-week LixiLan-L trial in patients with T2DM inadequately controlled on basal insulin. Additionally, we assessed whether achievement of glycaemic targets impacted the results.
Materials and methods: LixiLan-L had a 6-week run-in when iGlar was introduced or optimized; patients were then randomized to iGlarLixi or iGlar. Post hoc analyses were performed to assess the percentage change in lipid levels from baseline to Week 30. Changes in lipid levels were also assessed within subgroups according to achievement of glycaemic targets: HbA_{1c} ≤7, >7%; fasting plasma glucose ≤6.1, >6.1 mmol/L; 2-hour postprandial plasma glucose ≤10, >10 mmol/L.
Results: A total of 666 patients (iGlarLixi, *n*=335; iGlar, *n*=331) had lipid values at baseline and Week 30. Baseline lipid values and proportion of people using lipid-lowering drugs were similar between treatment groups (54% vs 55%). After 30 weeks of treatment, median percent change (25th: 75th percentiles) in fasting triglycerides showed nearly no increase (0.30% [-19.8: 24.2]) in the iGlarLixi group versus an increase of 6.50% (-15.8: 33.5) in the iGlar group; the difference was statistically significant (p=0.035). Total cholesterol did not change with iGlarLixi but increased in the iGlar group (0% [-7.3: 10.1] vs 3.4% [-5.6: 12.0], showing a trend of better cholesterol levels with iGlarLixi (p=0.059 for the difference between the two groups). Except for HDL, in subgroups of patients who had met glycaemic targets, there was an improvement in the lipid profile with iGlarLixi versus iGlar; this difference was not observed in patients who had not met glycaemic targets (Figure).
Conclusion: In patients with T2DM uncontrolled on basal insulin, achieving glycaemic control was associated with improvement in the lipid profile with iGlarLixi compared with iGlar alone. Previous studies have shown that in patients with T2DM improvements in lipid levels contribute to cardiovascular benefits.

Figure. Changes in fasting lipid levels according to achievement of glycaemic targets



FRC, fixed-ratio combination; FPG, fasting plasma glucose; iGlar, insulin glargine; PPG, postprandial plasma glucose; TC, total cholesterol; TG, triglycerides.

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Using machine learning algorithms to identify predictive factors of clinical outcomes with iGlarLixi or iGlar in the LixiLan-L trial

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Background and aims: Identification of predictive factors of adequate glycaemic control and hypoglycaemia occurrence in patients (pts) with type 2 diabetes mellitus (T2DM) treated with basal insulin (BI) or a fixed-ratio combination (FRC) of BI and a glucagon-like peptide-1 receptor agonist may lead to a personalized therapy approach and optimized treatment allocation. The phase III LixiLan-L (LLL) trial studied the efficacy and safety of iGlarLixi (FRC of lixisenatide + insulin glargine [iGlar]) vs iGlar alone in pts inadequately controlled with BI. The study randomized 736 pts and demonstrated the superiority of iGlarLixi vs iGlar in improving HbA_{1c}.
Materials and methods: A post hoc exploratory analysis of LLL was conducted using a sub-group discovery algorithm (Q-Finder), which tests combinations of predictors to identify those not recognized using traditional methods. A total of 600 variables including pt baseline (BL) characteristics (demography, vital signs, glycaemia, medical history, previous medications, biology, quality of life) and the evolution of key parameters in the first weeks of treatment (dose, glycaemia, other biological parameters) were used. The evaluated end of study (EoS) outcomes were glycaemic control (HbA_{1c} < 7%), change from BL (Δ) in HbA_{1c},

Δweight, and occurrence of hypoglycaemic and gastrointestinal (GI) events. To avoid bias in hypoglycaemia events triggered by the titration phase, only events occurring after 3 months were considered. All results reaching statistical significance (adjusted for multiple testing) were replicated in three independent data sets from Phase 3 studies involving a combination of lixisenatide and iGlar.

Results: HbA1c level at BL was the most relevant predictive factor for EoS glycaemic control (HbA1c < 7%) and improvement of HbA1c: a low HbA1c at BL (HbA1c ≤ 7.5%) was highly predictive of reaching HbA1c < 7% at EoS (67% probability, RR = 1.5; $p < 5\times10-14$); in contrast, a high HbA1c at BL (HbA1c ≥ 8.4%) was highly predictive of a large HbA1c improvement (ΔHbA1c = −1.21 vs ΔHbA1c = −0.65 in patients with BL HbA1c < 8.4%; $p < 5\times10-8$). In pts with high BL HbA1c, a relatively short history of BI treatment (≤ 1.6 years) was associated with a larger HbA1c decrease. Pts who titrated their insulin dose less during the first month of treatment had a higher probability (RR = 1.74; $p < 2\times10-6$) of achieving HbA1c improvement > 0.8% and no weight gain; this was highly specific to the iGlarLixi arm (45.1%) vs iGlar (16.7%). No BL characteristics were associated with a higher risk of GI AE occurrence. For pts with a low BMI, a higher frequency of hypoglycaemia (RR = 1.6, $p < 5\times10-6$; iGlarLixi: RR = 1.4, p = 0.02; iGlar: RR = 1.8, $p = 5\times10-5$) was observed. Those results were successfully replicated in the three test data sets.

Conclusion: The major role of BL HbA1c was confirmed as a predictive factor of treatment response in pts with TD2M inadequately controlled with BI at screening. The relationship between "less titration" at the beginning of treatment and HbA1c improvement is currently under investigation.

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PS 063 Semaglutide sustaining the effect of GLP-1

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Semaglutide reduces HbA_{1c} and body weight across multiple background OAD treatment categories

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Background and aims: Recommended HbA_{1c} targets can be a challenge to achieve for many patients with type 2 diabetes (T2D) despite treatment with oral antidiabetic drugs (OADs). Semaglutide, a glucagon-like peptide 1 (GLP-1) analogue in development for once-weekly treatment of T2D, demonstrated superior reductions in HbA_{1c} and body weight (BW) vs comparators in the SUSTAIN 1-5 clinical trials. This post-hoc analysis evaluated the efficacy and safety of semaglutide s.c. across background OAD treatments in SUSTAIN 2-4, where semaglutide was tested in combination with 1-2 OADs.

Materials and methods: In these 3 phase 3a trials, 3133 subjects with T2D (HbA_{1c} 7.0-10.0/10.5%) were randomised to once-weekly semaglutide s.c. 0.5 or 1.0 mg vs once-daily sitagliptin 100 mg for 56 weeks (n=1231; background, metformin [MET], thiazolidinediones [TZD] or MET+TZD; SUSTAIN 2); once-weekly exenatide extended release (ER) 2.0 mg for 56 weeks (n=813; background, 1-2 OADs, MET, TZD, sulphonylurea [SU]; SUSTAIN 3; semaglutide 1.0 mg only); or once-daily titrated insulin glargine (IGlar) for 30 weeks (n=1089; background, MET or MET+SU; SUSTAIN 4). The following background therapy groups were defined for analysis: MET; MET+SU; or other OADs (TZD, MET+TZD or SU+TZD).

Results: In subjects on background MET, treatment with both doses of semaglutide significantly reduced HbA_{1c} vs comparators (**Table**). Furthermore, in subjects on background MET+SU, both doses of semaglutide significantly reduced HbA_{1c} vs comparators (**Table**). In subjects on other OAD background therapies, HbA_{1c} was significantly reduced with semaglutide 1.0 mg vs sitagliptin, with non-significant reductions observed with semaglutide 0.5 mg vs sitagliptin and semaglutide 1.0 mg vs exenatide ER (**Table**). In all background OAD subgroups, mean BW reduction was significantly greater with semaglutide 1.0 mg vs sitagliptin, exenatide ER and IGlar (all p<0.05). In MET and MET+SU-treated subjects, mean BW reduction with semaglutide 0.5 mg was significantly greater vs comparators (all p<0.0001); the reduction in the other background OAD group did not reach statistical significance. The rate of severe or blood glucose-confirmed symptomatic hypoglycaemia with both semaglutide doses was similar, or lower than, comparators irrespective of background OAD treatment.

Conclusion: Once-weekly semaglutide s.c. consistently improved HbA_{1c} and reduced BW vs comparators in subjects with T2D, with a low rate of hypoglycaemia, regardless of background OAD treatments investigated in the SUSTAIN 2, 3 and 4 trials.

Table. Change from baseline to end-of-treatment in HbA_{1c} by background OAD therapy

	Number of subjects (n)	Semaglutide 0.5 mg		Semaglutide 1.0 mg		Comparator
		Change from baseline (%)	ETD [95% CI]	Change from baseline (%)	ETD [95% CI]	Change from baseline (%)
SUSTAIN 2: semaglutide vs sitagliptin 100 mg (56 weeks)						
MET background therapy	1159	−1.3	−0.79 [−0.95; −0.64]	−1.6	−1.07 [−1.22; −0.92]	−0.5
Other background therapy	69	−1.3	−0.59 [−1.23; 0.06]	−1.7	−0.95 [−1.62; −0.28]	−0.8
SUSTAIN 3: semaglutide vs exenatide ER 2.0 mg (56 weeks)						
MET background therapy	400			−1.3	−0.37 [−0.63; −0.12]	−1.0
MET+SU background therapy	367			−1.8	−0.90 [−1.16; −0.65]	−0.9
Other background therapy	45			−1.8	−0.47 [−1.25; 0.31]	−1.3
SUSTAIN 4: semaglutide vs IGlar (30 weeks)						
MET background therapy	527	−1.2	−0.45 [−0.66; −0.25]	−1.6	−0.89 [−1.09; −0.68]	−0.7
MET+SU background therapy	558	−1.2	−0.31 [−0.51; −0.11]	−1.7	−0.77 [−0.97; −0.57]	−0.9

'Number of subjects' refers to those randomised and in receipt of the specific background medication. Values are estimated means and ETDs with 95% CIs using a mixed model for repeated measurements analysis with treatment and background diabetes medication as fixed factors, interaction between treatment and background diabetes medication, and baseline HbA_{1c} as covariate, all nested within visit. CI, confidence interval; ETD, estimated treatment difference vs comparator; exenatide ER, exenatide extended release; IGlar, insulin glargine.

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Semaglutide reduces HbA_{1c} and body weight across baseline HbA_{1c} subgroups in the SUSTAIN 1-5 clinical trials

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Background and aims: Semaglutide, a glucagon-like peptide 1 (GLP-1) analogue in development for once-weekly treatment of type 2 diabetes (T2D), demonstrated superior reductions in HbA_{1c} and body weight vs comparators in the SUSTAIN 1-5 clinical trials. Here the efficacy of semaglutide by baseline HbA_{1c} subgroup vs placebo (as monotherapy and as add-on to insulin) and active comparators, was evaluated in a post-hoc analysis of the SUSTAIN 1-5 clinical trials.
Materials and methods: In these phase 3a trials, 3918 subjects with T2D (HbA_{1c} 7.0-10.0/10.5%) were randomised to once-weekly s.c. semaglutide 0.5 or 1.0 mg vs placebo for 30 weeks (n=388; SUSTAIN 1); once-daily sitagliptin 100 mg for 56 weeks (n=1231; SUSTAIN 2); once-weekly exenatide extended release 2.0 mg for 56 weeks (n=813; SUSTAIN 3; semaglutide 1.0 mg only); once-daily titrated insulin glargine for 30 weeks (n=1089; SUSTAIN 4); or placebo for 30 weeks (n=397; SUSTAIN 5). Subjects in SUSTAIN 2-4 were on 1-2 oral antidiabetic drugs (metformin, sulphonylureas, thiazolidinediones), while subjects in SUSTAIN 5 were on basal insulin ± metformin. Change in HbA_{1c} and body weight by baseline HbA_{1c} (≤7.5%, >7.5 to 8.0%, >8.0 to 8.5%, >8.5 to 9.0% and >9%) were analysed.
Results: Semaglutide reduced mean HbA_{1c} from baseline to end of treatment in all subgroups vs all comparators. Mean HbA_{1c} decreased by 0.7-2.5% with semaglutide 0.5 mg and 0.9-2.8% with semaglutide 1.0 mg. With comparators, change from baseline in mean HbA_{1c} ranged from a decrease of 1.8% to an increase of 0.6%. (**Table**). Across trials the reduction in HbA_{1c} was consistently greater with higher baseline HbA_{1c}. In subjects with the highest baseline HbA_{1c} (>9%), the HbA_{1c} target of <7% was achieved in 33-47% and 40-61% of subjects treated with semaglutide 0.5 and 1.0 mg, respectively, vs 3-21% with comparators; while 61-79% and 71-94% vs 7-60% achieved an HbA_{1c} level of <8%. Greater weight reduction with semaglutide vs comparators was observed across all baseline HbA_{1c} subgroups: mean body weight decreased by 2.8-5.1 kg with semaglutide 0.5 mg, 2.5-9.2 kg with semaglutide 1.0 mg, and by 2.9 kg to an increase of 2.6 kg with comparators. There was no relationship between the magnitude of weight reduction and baseline HbA_{1c} level. No new safety or tolerability issues were observed with semaglutide in the SUSTAIN 1-5 trials.
Conclusion: Semaglutide treatment consistently showed greater efficacy in lowering HbA_{1c} and body weight vs comparators, regardless of baseline HbA_{1c}. The reduction of HbA_{1c} was consistently greater with higher baseline HbA_{1c}; there was no relationship with the magnitude of weight reduction and baseline HbA_{1c}.

Table. Change in HbA_{1c} from baseline across the SUSTAIN 1–5 trials

	Number of subjects (n)	Semaglutide 0.5 mg (%)	Semaglutide 1.0 mg (%)	Comparator (%)
SUSTAIN 1: semaglutide vs placebo (30 weeks)				
All subjects	**387**	**−1.5**	**−1.6**	**0.0**
≤7.5%	132	−0.9	−1.1	−0.1
>7.5–8.0%	78	−1.2	−1.1	0.6
>8.0–8.5%	57	−1.6	−1.7	−0.4
>8.5–9.0%	68	−2.1	−1.8	−0.1
>9.0%	52	−2.3	−2.8	0.0
SUSTAIN 2: semaglutide vs sitagliptin 100 mg (56 weeks)				
All subjects	**1225**	**−1.3**	**−1.6**	**−0.5**
≤7.5%	426	−0.8	−1.1	−0.3
>7.5–8.0%	276	−1.1	−1.3	−0.4
>8.0–8.5%	190	−1.4	−1.9	−0.6
>8.5–9.0%	128	−1.6	−2.0	−0.9
>9.0%	205	−2.5	−2.6	−1.1
SUSTAIN 3: semaglutide vs exenatide ER 2.0 mg (56 weeks)				
All subjects	**809**	N/A	**−1.5**	**−0.9**
≤7.5%	188		−0.9	−0.5
>7.5–8.0%	182		−1.2	−0.6
>8.0–8.5%	130		−1.4[a]	−1.1
>8.5–9.0%	108		−1.7	−1.0
>9.0%	201		−2.5	−1.4
SUSTAIN 4: semaglutide vs IGlar (30 weeks)				
All subjects	**1082**	**−1.2**	**−1.6**	**−0.8**
≤7.5%	316	−0.7	−1.0	−0.2
>7.5–8.0%	226	−1.0	−1.3	−0.6
>8.0–8.5%	200	−1.2[a]	−1.8	−1.0
>8.5–9.0%	134	−1.4[a]	−2.1	−1.0
>9.0%	206	−2.0[a]	−2.6	−1.8
SUSTAIN 5: semaglutide vs placebo as add-on to basal insulin (30 weeks)				
All subjects	**396**	**−1.4**	**−1.8**	**−0.1**
≤7.5%	81	−0.7	−1.1	0.2
>7.5–8.0%	70	−1.4	−1.5	−0.1
>8.0–8.5%	91	−1.5	−2.0	−0.3
>8.5–9.0%	64	−1.5	−2.0	−0.1
>9.0%	90	−2.2	−2.5	−0.1

[a]Non-significant vs comparator – all other values significant vs comparator ($p<0.05$). Values are estimated means from a mixed model for repeated measurements analysis, with an interaction between treatment and subgroup variables as fixed factors and baseline value as covariate. Exenatide ER, exenatide extended release; IGlar, insulin glargine.

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Semaglutide-induced reductions in insulin resistance are mediated primarily via weight loss in subjects with type 2 diabetes (SUSTAIN 1-3)

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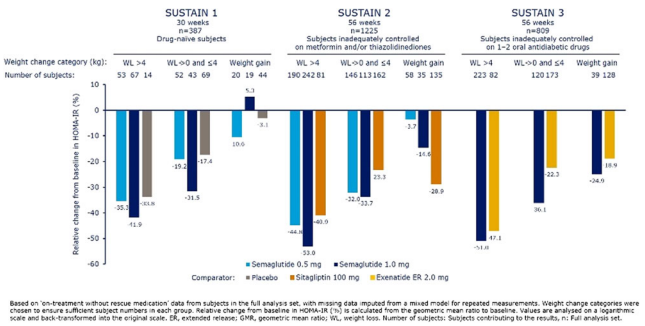
Background and aims: Semaglutide, a glucagon-like peptide-1 (GLP-1) analogue in development for the once-weekly treatment of type 2 diabetes (T2D), has demonstrated significant and clinically meaningful weight loss (WL) vs placebo and active comparators. As WL improves insulin resistance (IR), the present *post-hoc* analysis explored the association between these two parameters across three of the pivotal semaglutide trials, SUSTAIN 1-3, where it was scientifically relevant to measure IR.
Materials and methods: In the phase 3a SUSTAIN 1-3 trials, subjects were randomised to semaglutide s.c. 0.5 mg (SUSTAIN 1 and 2 only) or 1.0 mg once weekly. Comparators were placebo, sitagliptin 100 mg once daily and exenatide extended release (ER) 2.0 mg once weekly in SUSTAIN 1, 2 and 3, respectively. Change from baseline to end of treatment (30-56 weeks) in body weight and IR (assessed using homeostasis model assessments, HOMA-IR) were calculated. Change in HOMA-IR by weight change was evaluated. A mediation analysis was performed to quantify the extent of the improvement in HOMA-IR mediated by weight change (natural indirect effect) and that not mediated by weight change (natural direct effect).
Results: Mean body weight across the SUSTAIN 1-3 trials (baseline 89.5-95.8 kg) decreased significantly by 3.7-4.3 kg and 4.5-6.1 kg with semaglutide 0.5 mg and 1.0 mg, respectively, vs 1.0 kg, 1.9 kg and 1.9 kg with placebo, sitagliptin and exenatide ER ($p<0.0001$ vs all comparators). IR decreased by 27-36% and 32-46% with semaglutide 0.5 mg and 1.0 mg, respectively, vs 17%, 28% and 28% with placebo, sitagliptin and exenatide ER. Analysis of the reduction in IR by weight change showed that greater reductions in body weight were generally associated with greater reductions in IR (Figure; $p\leq0.0001$). In SUSTAIN 1, the reductions in IR were 15% and 21% greater with semaglutide 0.5 mg and 1.0 mg vs comparator, respectively, with 10% and 7% mediated by WL. Corresponding values for SUSTAIN 2 were 12% and 25%, with 9% and 23% mediated by WL. For semaglutide 1.0 mg in SUSTAIN 3, the values were 23%, with 16% mediated by WL. Across the trials, the effect mediated by WL was significant, except for semaglutide 1.0 mg in SUSTAIN 1. The effect not mediated by WL was 2-15% and not significant.
Conclusion: Semaglutide treatment consistently reduced body weight and IR in subjects with T2D in the SUSTAIN 1-3 trials. Greater WL was generally associated with greater reductions in IR. The reductions in IR observed with semaglutide vs comparators were primarily mediated by a significant effect on body weight.



Based on 'on-treatment without rescue medication' data from subjects in the full analysis set, with missing data imputed from a mixed model for repeated measurements. Weight change categories were chosen to ensure sufficient subject numbers in each group. Relative change from baseline in HOMA-IR (%) is calculated from the geometric mean ratio to baseline. Values are analysed on a logarithmic scale and back-transformed into the original scale. ER, extended release; GMR, geometric mean ratio; WL, weight loss. Number of subjects: Subjects contributing to the results; n: Full analysis set.

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More semaglutide-treated subjects achieved HbA_{1c} below 7% without weight gain, hypoglycaemia, and gastrointestinal adverse events vs comparators in the SUSTAIN 1-5 trials

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Background and aims: Semaglutide is a glucagon-like peptide 1 (GLP-1) analogue in development for the treatment of type 2 diabetes (T2D). The SUSTAIN 1-5 trials evaluated s. c. semaglutide in subjects with T2D vs placebo (as monotherapy and as an add-on to insulin), sitagliptin, exenatide extended release, and insulin glargine over 30-56 weeks. Across the trials, significantly more semaglutide-treated subjects achieved HbA_{1c} targets vs comparators, as well as clinically relevant reductions in body weight (BW). However, it is important to weigh the benefits of achieving HbA_{1c} targets and weight loss against the risk of hypoglycaemia and gastrointestinal (GI) adverse events (AEs). To evaluate the potential of semaglutide in helping subjects achieve HbA_{1c} targets, additional analyses were performed with data from the SUSTAIN 1-5 trials using composite endpoints which included HbA_{1c} targets, weight gain, hypoglycaemia, and GI AEs.
Materials and methods: Analyses were performed on randomised and treated subjects using a logistic regression for two composite endpoints at the end of treatment. The first endpoint (pre-specified) was the proportion of subjects achieving HbA_{1c} <7% (53 mmol/mol), no weight gain and no severe or blood-glucose (BG)-confirmed symptomatic hypoglycaemia. The second endpoint (*post-hoc*) was the proportion of subjects achieving the pre-specified endpoint without moderate/severe GI AEs.
Results: Significantly more subjects treated with semaglutide 0.5 mg and 1.0 mg once weekly achieved the composite endpoint of HbA_{1c} <7% without weight gain or severe/BG-confirmed symptomatic hypoglycaemia vs comparators (all $p<0.0001$, Table). For the *post-hoc* endpoint, significantly more semaglutide-treated subjects achieved HbA_{1c} <7% without weight gain, severe/BG-confirmed symptomatic hypoglycaemia, and without moderate/severe GI AEs vs comparators (all $p<0.0001$, Table).
Conclusion: In a broad range of subjects with T2D participating in the SUSTAIN 1-5 trials, significantly more subjects achieved HbA_{1c} <7% with semaglutide vs comparators without weight gain or hypoglycaemia. Moreover, semaglutide remained favourable vs comparators when the composite endpoint included the component of no moderate/severe GI AEs.

Table. Proportion of subjects achieving composite endpoints across the SUSTAIN 1–5 trials

Endpoint SUSTAIN trial vs comparator (background medication, FAS)	Semaglutide 0.5 mg			Semaglutide 1.0 mg			Comparator
	%	OR	[95% CI]	%	OR	[95% CI]	%
HbA_{1c} <7% (53 mmol/mol), no weight gain and no severe/BG-confirmed symptomatic hypoglycaemia							
SUSTAIN 1 vs placebo (treatment naive, n=387)	66	12.69*	[6.57; 24.52]	65	12.45*	[6.46; 23.99]	19
SUSTAIN 2 vs sitagliptin (1–2 OADs, n=1225)	63	4.84*	[3.51; 6.68]	74	9.52*	[6.75; 13.43]	27
SUSTAIN 3 vs exenatide ER (1–2 OADs, n=809)				57	4.03*	[2.90; 5.59]	29
SUSTAIN 4 vs IGlar (1–2 OADs, n=1082)	47	5.39*	[3.72; 7.81]	64	12.88*	[8.73; 19.02]	16
SUSTAIN 5 vs placebo (basal insulin±MET, n=396)	54	17.90*	[8.26; 38.78]	67	29.93*	[13.65; 65.61]	7
HbA_{1c} <7% (53 mmol/mol), no weight gain, no severe/BG-confirmed symptomatic hypoglycaemia, and no moderate/severe GI AEs (post-hoc)							
SUSTAIN 1 vs placebo (treatment naive, n=387)	55	8.05*	[4.26; 15.21]	55	6.98*	[3.74; 13.01]	19
SUSTAIN 2 vs sitagliptin (1–2 OADs, n=1225)	54	3.46*	[2.53; 4.73]	64	5.71*	[4.14; 7.86]	25
SUSTAIN 3 vs exenatide ER (1–2 OADs, n=809)				46	2.90*	[2.10; 4.01]	25
SUSTAIN 4 vs IGlar (1–2 OADs, n=1082)	40	4.12*	[2.85; 5.96]	53	7.70*	[5.30; 11.20]	14
SUSTAIN 5 vs placebo (basal insulin±MET, n=396)	48	14.55*	[6.71; 31.55]	60	21.42*	[9.85; 46.61]	7

*p<0.0001 vs comparator. ADA, American Diabetes Association; AE, adverse events; BG, blood glucose; Exenatide ER, exenatide extended release; FAS, full analysis set (randomised and treated subjects); GI, gastrointestinal; IGlar, insulin glargine; MET, metformin; OAD, oral anti-diabetic drug.

Severe or BG-confirmed symptomatic hypoglycaemia: an episode that was severe according to the ADA classification or BG confirmed by a plasma glucose value <3.1 mmol/L (56 mg/dL) with symptoms consistent with hypoglycaemia. 'On-treatment without rescue medication' data are presented. Logistic regression with treatment, trial-specific stratification, and country as fixed factors and baseline HbA_{1c} and body weight as covariate. Missing data are imputed from the mixed model for repeated measurements for change from baseline; post-baseline data are analysed with treatment, trial-specific stratification and country as fixed factors and baseline as covariate, nested within visit.

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Superior glycaemic control with semaglutide across SUSTAIN 1-5 clinical trials

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Background and aims: Semaglutide, a glucagon-like peptide 1 (GLP-1) analogue in development for once-weekly treatment of type 2 diabetes (T2D), demonstrated superior reductions in HbA_{1c} vs placebo and active comparators in the SUSTAIN 1-5 clinical trials. Pre-specified analyses included change from baseline in HbA_{1c} and fasting plasma glucose (FPG), and the proportions of subjects achieving HbA_{1c} targets (<7.0% or ≤6.5%) for semaglutide 0.5 mg and 1.0 mg.
Materials and methods: In these phase 3a trials, 3918 subjects with T2D (HbA_{1c} 7.0-10.0/10.5%) were randomised to once-weekly s.c semaglutide 0.5 mg or 1.0 mg vs placebo for 30 weeks (n=388; SUSTAIN 1); vs once-daily sitagliptin 100 mg for 56 weeks (n=1231; SUSTAIN 2); vs once-weekly exenatide extended release 2.0 mg for 56 weeks (n=813; SUSTAIN 3; semaglutide 1.0 mg only); vs once-daily insulin glargine (treat-to-target) for 30 weeks (n=1089; SUSTAIN 4); or vs placebo as an add on to basal insulin for 30 weeks (n=397; SUSTAIN 5). Background medication consisted of 1-2 oral antidiabetic drugs (metformin, sulphonylureas, thiazolidinediones) in SUSTAIN 2-4 and basal insulin ± metformin in SUSTAIN 5.
Results: Semaglutide consistently reduced mean HbA_{1c} from baseline to end of treatment vs all comparators. Mean HbA_{1c} decreased by 1.2-1.5% with semaglutide 0.5 mg and 1.5-1.8% with semaglutide 1.0 mg, vs 0.02-0.9% with comparators (all p<0.0001). Furthermore, a significantly higher proportion of subjects achieved HbA_{1c} targets of <7% and ≤6.5% with semaglutide 0.5 mg and 1.0 mg than with comparators (all p<0.0001; **Table 1**). Mean FPG reductions were greater with semaglutide vs comparators: 1.6-2.5 mmol/L with semaglutide 0.5 mg and 2.3-2.8 mmol/L with semaglutide 1.0 mg vs 0.5-2.1 mmol/L with comparators (all p≤0.0002 except semaglutide 0.5 mg vs insulin glargine).
Conclusion: Once-weekly s.c. semaglutide provided significant improvements in glycaemic control across the diabetes treatment continuum, from treatment-naïve subjects to those with advanced diabetes receiving insulin. No new safety or tolerability issues were observed with semaglutide in the SUSTAIN 1-5 trials.

Table 1. Proportion of subjects achieving HbA_{1c} targets of <7.0% and ≤6.5% across the SUSTAIN trials

		Semaglutide 0.5 mg		Semaglutide 1.0 mg		Comparator
		%	OR [95% CI]	%	OR [95% CI]	%
Proportion of subjects achieving HbA_{1c} <7.0%	SUSTAIN 1 vs placebo (treatment naïve)	74	16.92* [8.44; 33.89]	72	15.70* [8.00; 30.83]	25
	SUSTAIN 2 vs sitagliptin (on 1–2 OADs)	69	4.16* [3.02; 5.74]	78	7.92* [5.59; 11.22]	36
	SUSTAIN 3 vs exenatide ER (on 1–2 OADs)	N/A		67	3.88* [2.80; 5.38]	40
	SUSTAIN 4 vs IGlar (on 1–2 OADs)	57	2.39* [1.73; 3.28]	73	5.78* [4.08; 8.19]	38
	SUSTAIN 5 vs placebo (add on to insulin)	61	14.68* [7.43; 29.02]	79	34.28* [16.59; 70.83]	11
Proportion of subjects achieving HbA_{1c} ≤6.5%	SUSTAIN 1 vs placebo (treatment naïve)	59	15.99* [7.82; 32.68]	60	18.34* [8.96; 37.54]	13
	SUSTAIN 2 vs sitagliptin (on 1–2 OADs)	53	4.39* [3.15; 6.12]	66	8.99* [6.36; 12.72]	20
	SUSTAIN 3 vs exenatide ER (on 1–2 OADs)	N/A		47	3.73* [2.66; 5.23]	22
	SUSTAIN 4 vs IGlar (on 1–2 OADs)	37	3.02* [2.11; 4.33]	54	6.86* [4.76; 9.89]	18
	SUSTAIN 5 vs placebo (add on to insulin)	41	15.61* [6.47; 37.64]	61	35.84* [14.72; 87.27]	5

*p<0.0001 vs comparator, based on a logistic regression model with treatment and country as fixed factors (SUSTAIN 1–4) or treatment, country and stratification variable (HbA_{1c} level at screening [≤8.0% or >8.0%] crossed with use of metformin [Yes or No]; 2 by 2 levels) as fixed factors (SUSTAIN 5) and the baseline HbA_{1c} value as covariate.

SUSTAIN 4 IGlar starting dose 10 IU once daily uptitrated to SMPG target of 72–99 mg/dL. All subjects in SUSTAIN 5 were receiving stable treatment with basal insulin (minimum of 0.25 IU/kg/day and/or 20 IU/day of: insulin glargine, insulin detemir, insulin degludec and/or neutral protamine Hagedorn insulin). CI, confidence interval; exenatide ER, exenatide extended-release; IGlar, insulin glargine; OAD, oral antidiabetic drug; OR, odds ratio; SMPG, self-measured plasma glucose.

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Treatment with semaglutide provides superior body weight reduction vs comparators in subjects with type 2 diabetes across the SUSTAIN 1-5 trials

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Background and aims: Semaglutide is a glucagon-like peptide 1 (GLP-1) analogue in development for the treatment of type 2 diabetes (T2D). The SUSTAIN 1-5 trials evaluated s.c. semaglutide vs comparators. This review aims to characterise the effects of semaglutide treatment on body weight (BW) in a broad range of subjects with T2D across SUSTAIN 1-5.
Materials and methods: SUSTAIN 1-5 were five phase 3a, multicentre, randomised trials involving 3,918 subjects with T2D. Subjects were treatment-naïve (n=388, placebo; SUSTAIN 1), on metformin and/or thiazolidinediones (n=1231, sitagliptin; SUSTAIN 2), taking up to two oral antidiabetic drugs (n=813, exenatide extended release [ER]; SUSTAIN 3), insulin-naïve on stable treatment with metformin ± sulphonylureas (n=1089, insulin glargine; SUSTAIN 4), or on basal insulin ± metformin (n=397, placebo; SUSTAIN 5). The primary endpoint for all trials was change from baseline (BL) in HbA_{1c}. Pre-specified analyses included change from BL in BW, proportion of subjects achieving ≥5% and ≥10% BW loss from BL, and change in waist circumference.
Results: Mean BW was significantly reduced from BL by 3.5-6.4 kg with semaglutide vs a 1.9 kg reduction to 1.2 kg increase with comparators (p<0.05 for all; Table). Significantly more semaglutide-treated subjects achieved ≥5% and ≥10% BW loss from BL vs comparators (Proportion with ≥10% BW loss from BL, OR [95% CI]: SUSTAIN 1, 0.5 mg 3.60 [1.09; 11.95], 1.0 mg 6.23 [1.98; 19.61] vs placebo; SUSTAIN 2, 0.5 mg 4.09 [2.26; 7.40], 1.0 mg 8.85 [5.01; 15.61] vs sitagliptin; SUSTAIN 3, 1.0 mg 5.39 [3.20; 9.07] vs exenatide ER; SUSTAIN 4, 0.5 mg 6.35 [2.42; 16.69], 1.0 mg 14.51 [5.70; 36.92] vs basal insulin; SUSTAIN 5, 0.5 mg 3.18 [1.05; 9.63], 1.0 mg 12.80 [4.51; 36.33] vs placebo; p<0.05 for all; Table). Furthermore, semaglutide treatment led to significantly greater reductions in waist circumference from BL (cm) vs comparators (estimated treatment difference (ETD) [95% CI]: SUSTAIN 1, 0.5 mg -1.84 [-3.40; -0.28], 1.0 mg -2.18 [-3.74; -0.61] vs placebo; SUSTAIN 2, 0.5 mg -2.10 [-2.91; -1.29], 1.0 mg -3.67 [-4.48; -2.87] vs sitagliptin; SUSTAIN 3, 1.0 mg -2.76 [-3.63; -1.89] vs exenatide ER; SUSTAIN 4, 0.5 mg -3.42 [-4.24; -2.59], 1.0 mg -4.76 [-5.59; -3.93] vs basal insulin; SUSTAIN 5, 0.5 mg -1.46 [-2.83; -0.09], 1.0 mg -4.05 [-5.42; -2.67] vs placebo; p<0.05 for all).
Conclusion: Semaglutide treatment consistently provided significant reductions in BW, BMI and waist circumference vs comparators in a broad range of subjects with T2D.

Table. Changes from baseline in BW-related parameters across SUSTAIN trials

Endpoint SUSTAIN trial vs comparator (background medication)	Semaglutide 0.5 mg		Semaglutide 1.0 mg		Comparator
Change from baseline in BW by trial	kg	ETD [95% CI]	kg	ETD [95% CI]	kg
SUSTAIN 1 vs placebo (treatment naive)	-3.7	-2.75* [-3.92; -1.58]	-4.5	-3.56* [-4.74; -2.38]	-1.0
SUSTAIN 2 vs sitagliptin (1-2 OADs)	-4.3	-2.35* [-3.06; -1.63]	-6.1	-4.20* [-4.91; -3.49]	-1.9
SUSTAIN 3 vs exenatide ER (1-2 OADs)			-5.6	-3.78* [-4.58; -2.98]	-1.9
SUSTAIN 4 vs basal insulin (1-2 OADs)	-3.5	-4.62* [-5.27; -3.96]	-5.2	-6.33* [-6.99; -5.67]	+1.2
SUSTAIN 5 vs placebo (basal insulin±MET)	-3.7	-2.31* [-3.33; -1.29]	-6.4	-5.06* [-6.08; -4.04]	-1.4
Proportion of subjects with ≥5% BW loss	%	OR [95% CI]	%	OR [95% CI]	%
SUSTAIN 1 vs placebo (treatment naive)	37	7.88* [3.65; 17.04]	45	12.01* [5.53; 26.07]	7
SUSTAIN 2 vs sitagliptin (1-2 OADs)	46	3.76* [2.72; 5.19]	62	7.47* [5.38; 10.37]	18
SUSTAIN 3 vs exenatide ER (1-2 OADs)			52	5.12* [3.68; 7.11]	17
SUSTAIN 4 vs basal insulin (1-2 OADs)	37	13.37* [7.71; 23.20]	51	23.94* [13.80; 41.50]	5
SUSTAIN 5 vs placebo (basal insulin±MET)	42	5.91* [3.08; 11.31]	66	16.59* [8.52; 32.30]	11

*p<0.05 vs comparator. Analyses were performed on the full-analysis sets (randomised and received ≥1 dose of treatment). Missing data were imputed from a mixed model for repeated measurements. Observed proportion of subjects with ≥5% BW loss are shown. ORs were obtained using a logistic regression model. SUSTAIN 4: insulin glargine starting dose 10 IU once daily uptitrated to SMPG target of 72–99 mg/dL. SUSTAIN 5: All subjects were receiving stable treatment with basal insulin (minimum of 0.25 IU/kg/day and/or 20 IU/day of insulin glargine, insulin detemir, insulin degludec and/or neutral protamine Hagedorn insulin) ± metformin. BW, body weight; exenatide ER, exenatide extended release; OAD, oral antidiabetic drugs.

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Semaglutide consistently reduces both fasting and postprandial glucose levels across SUSTAIN 1-5 clinical trials

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Background and aims: Improvements in both fasting plasma glucose (FPG) and postprandial glucose (PPG) levels are major contributors to achieving HbA_{1c} targets in subjects with type 2 diabetes (T2D). Short-acting glucagon-like peptide-1 receptor agonists (GLP-1 RAs) generally demonstrate greater improvements in PPG following injection, while long-acting GLP-1 RAs have shown greater effects on FPG with more modest effects on PPG. Semaglutide is a GLP-1 analogue in development for the treatment of T2D. Semaglutide demonstrated superior HbA_{1c} reductions (1.2-1.5% with semaglutide 0.5 mg and 1.5-1.8% with semaglutide 1.0 mg) vs comparators across the SUSTAIN 1-5 clinical trials when used as monotherapy, add-on to metformin, with 1-2 oral anti-diabetic medications or with basal insulin. The aim of this analysis was to assess the effect of once-weekly s.c. semaglutide on FPG and PPG across the SUSTAIN 1-5 trials.
Materials and methods: In these phase 3a trials, a total of 3918 subjects (HbA_{1c} 7.0-10.0/10.5%) with T2D were randomised to semaglutide 0.5 mg, 1.0 mg or placebo (n=388, SUSTAIN 1); sitagliptin (n=1231, SUSTAIN 2); exenatide extended release [ER] (n=813, SUSTAIN 3, vs semaglutide 1.0 mg only); insulin glargine [IGlar] (n=1089, SUSTAIN 4); or placebo as add-on to basal insulin ± metformin (n=397, SUSTAIN 5); for 30 or 56 weeks. The effect of semaglutide 0.5 and 1.0 mg vs comparators on FPG and PPG (mean and postprandial increments), derived from laboratory values and from the 7/8-point self-measured plasma glucose (SMPG) profile, was assessed (vs baseline) at the end of treatment based on the full analysis set using on-treatment without rescue medication data.
Results: Semaglutide reduced mean FPG from baseline (**Table**). Reductions in FPG were significantly greater with semaglutide 1.0 mg vs comparators (all p≤0.0002). Reductions were also significantly greater with semaglutide 0.5 mg vs placebo and sitagliptin (all p≤0.0002), but not vs IGlar. Semaglutide reduced mean PPG increments (**Table**). The reductions in PPG increments were significantly greater with semaglutide 1.0 mg vs comparators (all p<0.02). Reductions were also greater with semaglutide 0.5 mg vs IGlar and placebo when added to insulin (both p<0.004), but not vs placebo (monotherapy) and sitagliptin (p=0.0807 and p=0.0926, respectively). Mean SMPG levels were significantly reduced with semaglutide 0.5 and 1.0 mg vs all comparators (p<0.0001), with the exception of semaglutide 0.5 mg vs IGlar.
Conclusion: Semaglutide administered once weekly consistently reduced FPG and PPG in subjects with T2D across the SUSTAIN 1-5 trials, suggesting that both components contribute to significantly better glycaemic control vs comparators.

Table. Reduction in mean FPG and PPG increments (mmol/L) from baseline to end of treatment: semaglutide 0.5 and 1.0 mg, vs comparators

	SUSTAIN 1 Semaglutide vs placebo n=388 30 weeks	SUSTAIN 2 Semaglutide vs sitagliptin n=1231 56 weeks	SUSTAIN 3 Semaglutide vs exenatide ER n=813 56 weeks	SUSTAIN 4 Semaglutide vs IGlar n=1089 30 weeks	SUSTAIN 5 Semaglutide add-on to basal insulin vs placebo n=397 30 weeks
FPG $[ETD]_{0.5\ mg}$	−1.96*	−0.97*	n/a	0.08	−1.14‡
FPG $[ETD]_{1.0\ mg}$	−1.79*	−1.49*	−0.84*	−0.61†	−1.88*
PPG $[ETD]_{0.5\ mg}$	−0.41	−0.18	n/a	−0.39†	−0.66†
PPG $[ETD]_{1.0\ mg}$	−0.74‡	−0.38†	−0.24†	−0.65*	−1.01*

*p<0.0001; †p<0.02; ‡p<0.004. FPG data was based on fasting laboratory values, while PPG (mean and PPG increments) was derived from the 7/8-point self-measured plasma glucose (SMPG) profile. ETD, estimated treatment difference; FPG, fasting plasma glucose; PPG, postprandial glucose.

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Efficacy and safety of once-weekly semaglutide in elderly subjects with type 2 diabetes: post hoc analysis of SUSTAIN 1-5 trials

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Background and aims: Semaglutide, a glucagon-like peptide 1 (GLP-1) analogue in development for treatment of type 2 diabetes (T2D), demonstrated superior reductions in HbA_{1c} and body weight (BW) across the SUSTAIN 1-5 clinical trials. This *post hoc* analysis evaluated the efficacy and safety of semaglutide vs comparators in elderly (≥65 years old) and non-elderly (<65 years old) subjects with T2D.
Materials and methods: In these phase 3a trials, subjects with T2D were randomised to once-weekly s.c. semaglutide 0.5 mg or 1.0 mg vs placebo (as monotherapy or as add-on to basal insulin), sitagliptin, exenatide extended release [ER], or insulin glargine.
Results: The analyses included a total of 854 subjects ≥65 years old (mean age: 69-70 years) and 3045 subjects <65 years old (mean age: 50-55 years). Semaglutide consistently reduced mean HbA_{1c} and BW from baseline to end of treatment in both age groups, and reductions were greater than with comparators (Table). The efficacy of semaglutide vs comparators in HbA_{1c} and BW reduction was comparable between age groups. In subjects receiving semaglutide 0.5 mg, 1.0 mg and comparators, the respective proportions of subjects reporting adverse events (AEs), pooled across trials, were comparable in the two age groups: 71.9%, 70.5% and 72.8% (≥65 years age group) vs 70.7%, 71.0% and 67.2% (<65 years age group). More subjects ≥65 years old reported serious AEs (7.1%, 7.6% and 8.9%) than <65 years old (6.3%, 7.2% and 5.0%), respectively. The severity of AEs was comparable between age groups; most AEs were mild-to-moderate. The respective proportions of subjects receiving semaglutide 0.5 mg, 1.0 mg and comparators and prematurely discontinuing treatment due to AEs were higher in the ≥65 years age group (11.1%, 10.6% and 3.8%) than in the <65 years age group (5.2%, 7.7% and 3.3%). Gastrointestinal (GI) AEs were more frequent with semaglutide than with comparators across both age groups. GI AEs were reported in 41.3%, 45.3% and 26.4% of subjects in the ≥65 years age

group receiving semaglutide 0.5 mg, 1.0 mg and comparators, respectively, and in 39.6%, 39.5% and 21.5% of subjects in the <65 years age group. Reductions in renal function, measured by the change from baseline in eGFR at week 30, were 3.2, 2.2 and 2.5 mL/min/1.73 m^2 in subjects ≥65 years of age receiving semaglutide 0.5 mg, 1.0 mg and comparators, respectively, and were 4.8, 5.4 and 3.4 mL/min/1.73 m^2 in subjects <65 years of age.

Conclusion: Semaglutide demonstrated consistent improvements in HbA_{1c} and BW vs comparators in elderly subjects ≥65 years old compared with those <65 years old. Despite a higher frequency of premature treatment discontinuation in subjects ≥65 years old, semaglutide had a similar safety profile to that of other GLP-1 receptor agonists with no clinically relevant risks observed in elderly subjects.

Table. Reduction in HbA_{1c} and body weight in subjects <65 and ≥65 years of age receiving semaglutide or comparators

		Number of subjects <65/≥65 years	Semaglutide 0.5 mg (Baseline <65/≥65 years) Change from baseline <65/≥65 years	Semaglutide 0.5 mg ETD <65 years [95% CI]	Semaglutide 0.5 mg ETD ≥65 years [95% CI]	Semaglutide 1.0 mg (Baseline <65/≥65 years) Change from baseline <65/≥65 years	Semaglutide 1.0 mg ETD <65 years [95% CI]	Semaglutide 1.0 mg ETD ≥65 years [95% CI]	Comparator (Baseline <65/≥65 years) Change from baseline <65/≥65 years
HbA_{1c} (%)	SUSTAIN 1: Semaglutide vs placebo	317/70	(8.2/7.7) −1.43/−1.50	−1.40 [−1.72; −1.08]	−1.35 [−2.01; −0.69]	(8.1/8.0) −1.59/−1.23	−1.55 [−1.87; −1.24]	−1.08 [−1.77; −0.38]	(7.9/8.0) −0.03/−0.15
	SUSTAIN 2: Semaglutide vs sitagliptin 100 mg	993/232	(8.0/8.0) −1.33/−1.31	−0.83 [−0.99; −0.66]	−0.59 [−0.94; −0.24]	(8.1/7.9) −1.63/−1.54	−1.12 [−1.29; −0.95]	−0.83 [−1.17; −0.48]	(8.2/8.1) −0.50/−0.71
	SUSTAIN 3: Semaglutide vs exenatide ER 2.0 mg	614/195				(8.4/8.2) −1.53/−1.65	−0.62 [−0.83; −0.42]	−0.68 [−1.04; −0.33]	(8.4/8.1) −0.90/−0.97
	SUSTAIN 4: Semaglutide vs IGlar	840/242	(8.2/7.8) −1.20/−1.25	−0.37 [−0.53; −0.21]	−0.43 [−0.73; −0.13]	(8.3/8.1) −1.75/−1.32	−0.92[a] [−1.08; −0.76]	−0.50[a] [−0.80; −0.19]	(8.2/7.9) −0.83/−0.82
	SUSTAIN 5: Semaglutide vs placebo	281/115	(8.5/8.1) −1.50/−1.27	−1.50 [−1.80; −1.19]	−1.00 [−1.45; −0.55]	(8.4/8.1) −1.87/−1.83	−1.87 [−2.17; −1.57]	−1.55 [−2.04; −1.06]	(8.5/8.3) −0.01/−0.27
Body weight (kg)	SUSTAIN 1: Semaglutide vs placebo	317/70	(90.8/85.8) −3.77/−3.64	−2.81 [−4.13; −1.50]	−2.25 [−5.00; 0.49]	(99.4/83.1) −4.56/−4.09	−3.61 [−4.92; −2.30]	−2.71 [−5.61; 0.19]	(92.0/76.2) −0.96/−1.38
	SUSTAIN 2: Semaglutide vs sitagliptin 100 mg	993/232	(90.8/86.2) −4.28/−4.60	−2.22 [−3.01; −1.43]	−3.15 [−4.81; −1.48]	(90.6/83.2) −6.16/−6.10	−4.10 [−4.90; −3.31]	−4.66 [−6.29; −3.02]	(91.2/81.4) −2.06/−1.45
	SUSTAIN 3: Semaglutide vs exenatide ER 2.0 mg	614/195				(98.1/89.6) −5.44/−6.66	−3.74 [−4.68; −2.81]	−4.92 [−6.56; −3.29]	(97.8/88.6) −1.70/−1.73
	SUSTAIN 4: Semaglutide vs IGlar	840/242	(94.6/91.0) −3.32/−4.03	−4.41 [−5.16; −3.67]	−5.49 [−6.87; −4.10]	(93.9/94.4) −5.03/−5.55	−6.12 [−6.86; −5.37]	−7.01 [−8.43; −5.59]	(93.8/88.5) 1.09/1.46
	SUSTAIN 5: Semaglutide vs placebo	281/115	(94.5/88.7) −3.68/−3.76	−2.48 [−3.75; −1.22]	−2.10 [−3.97; −0.23]	(94.3/86.2) −6.38/−6.61	−5.18 [−6.42; −3.94]	−4.95 [−6.99; −2.92]	(92.7/84.8) −1.20/−1.66

[a]Test for treatment by subgroup interaction (<65/≥65 years) was significant (p<0.02); all other interactions were non-significant. Values are estimated using a mixed model for repeated measurements analysis using on-treatment without rescue medication data from all randomised and exposed subjects, with treatment and baseline age subgroup as fixed factors, interaction between treatment and baseline age subgroup, and baseline HbA_{1c} (%) or body weight (kg) respectively as covariate, all nested within visit. CI, confidence interval; ETD, estimated treatment difference vs comparator; exenatide ER, exenatide extended release; IGlar, insulin glargine.

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Responder analysis of subjects achieving HbA_{1c} ≥1% and weight loss ≥5% across SUSTAIN 1-5 clinical trials

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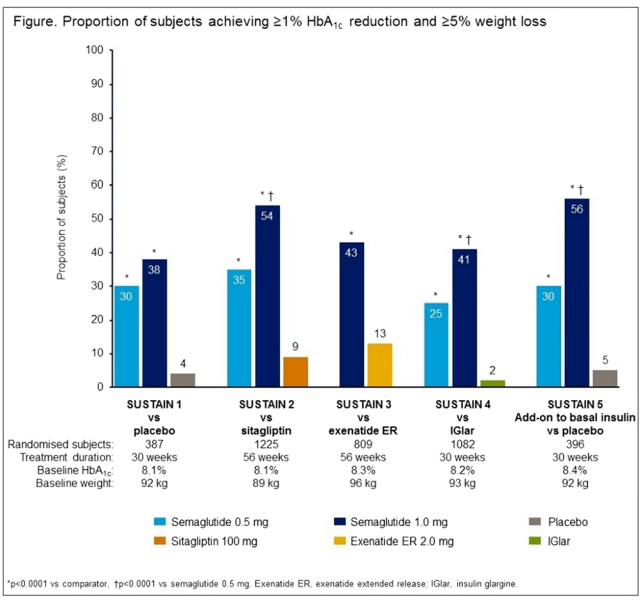
Background and aims: Semaglutide is a glucagon-like peptide-1 (GLP-1) analogue in development for once-weekly s.c. treatment of type 2 diabetes (T2D). Semaglutide demonstrated superior HbA_{1c} and body weight reductions vs comparators across the phase 3a SUSTAIN 1-5 clinical trials. We now report a *post-hoc* analysis of these trials evaluating the proportion of subjects who achieved a composite endpoint of both ≥1% (8.6 mmol/mol) reduction in HbA_{1c} and ≥5% weight loss.

Materials and methods: In the SUSTAIN 1-5 clinical trials, once-weekly s.c. semaglutide 0.5 mg and 1.0 mg was evaluated in 3918 subjects (HbA_{1c} 7.0-10.0% for SUSTAIN 1, 4 and 5, and 7.0-10.5% for SUSTAIN 2 and 3) with T2D. Semaglutide was evaluated vs placebo for 30 weeks (n=388; SUSTAIN 1); vs once-daily sitagliptin 100 mg for 56 weeks (n=1231; SUSTAIN 2); vs once-weekly exenatide extended release 2.0 mg for 56 weeks (n=813; SUSTAIN 3; semaglutide 1.0 mg only); vs once-daily insulin glargine (treat-to-target) for 30 weeks (n=1089; SUSTAIN 4); or vs placebo as an add-on to basal insulin for 30 weeks (n=397; SUSTAIN 5). The binary composite endpoint of subjects achieving ≥1% HbA_{1c} reduction and ≥5% weight loss was analysed by a logistic regression model, using on-treatment without rescue medication data with missing values at end of treatment, imputed from a mixed model for repeated measurements and subsequently dichotomised.

Results: The proportion of subjects achieving both ≥1% HbA_{1c} reduction and ≥5% weight loss was significantly greater with semaglutide 0.5 mg (25-35%) and 1.0 mg (38-56%) than with comparators (2-13%; p<0.0001 for all comparisons; **Figure**). The proportion of subjects achieving the composite endpoint was greater with semaglutide 1.0 mg vs 0.5 mg (p<0.0001 for SUSTAIN 2, 4 and 5; p=0.17 for SUSTAIN 1), indicating a dose-dependent effect. In SUSTAIN 1-4, severe or blood glucose-confirmed symptomatic hypoglycaemia events were fewer or similar with semaglutide vs comparators, while in SUSTAIN 5, on a background of basal insulin, more events were observed with semaglutide than with placebo. In all five trials, semaglutide was well tolerated, with a safety profile similar to that reported for other GLP-1 receptor agonists.

Conclusion: With semaglutide treatment, the proportion of subjects achieving the clinically meaningful composite endpoint of ≥1% HbA_{1c} reduction and ≥5% weight loss was statistically significantly (p<0.0001) greater than with comparators.



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The impact of gastrointestinal adverse events on weight loss with semaglutide in subjects with type 2 diabetes

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Background and aims: Semaglutide, a glucagon-like peptide 1 analogue in development for treatment of type 2 diabetes (T2D), demonstrated superior reductions in HbA_{1c} and body weight vs placebo and active comparators across the SUSTAIN 1-5 trials. The most common adverse events reported with semaglutide were gastrointestinal-related. In a phase 1 trial in obese subjects, semaglutide 1.0 mg was shown to reduce body weight, appetite and energy intake vs placebo. This post hoc analysis was undertaken to assess the relationship between weight loss and nausea and/or vomiting in SUSTAIN 1-5.

Materials and methods: In these phase 3a trials, subjects with inadequately controlled T2D who were drug-naïve (SUSTAIN 1), on metformin and/or thiazolidinediones (SUSTAIN 2), on 1-2 oral antidiabetic drugs (SUSTAIN 3), on metformin ± sulphonylureas (SUSTAIN 4) or on basal insulin ± metformin (SUSTAIN 5) were randomised to semaglutide s.c. 0.5 mg (excluding SUSTAIN 3) or 1.0 mg (all trials) once weekly. The populations were divided into subgroups based on reporting of any nausea and/or vomiting. A mediation analysis was also performed to separate the overall effect on weight into direct or indirect (mediated by nausea or vomiting) effects.

Results: Across the five trials, 15.2-24.0% and 21.5-27.2% of subjects experienced nausea or vomiting with semaglutide 0.5 mg and 1.0 mg, respectively, vs 6.0-14.1% with comparators. Weight loss ≥5% was achieved by 36.7-46.0% and 44.6-65.6% of subjects with semaglutide 0.5 mg and 1.0 mg, respectively, vs 7.0-18.4% with comparators. With semaglutide 0.5 mg, ≥5% weight loss was achieved by 43.7-55.0% of subjects experiencing nausea or vomiting vs 32.7-44.2% of those without these adverse events. The corresponding values for semaglutide 1.0 mg were 51.5-86.7% and 42.3-60.1%, respectively. Regardless of nausea or vomiting, weight loss was consistently greater with semaglutide vs comparators (all $p<0.01$) (Table). Overall, weight reductions with semaglutide were greater vs comparators by 2.3-6.3 kg. Only 0.07-0.5 kg of this difference was due to nausea or vomiting (indirect effects) and these effects were non-significant in most trials (except for semaglutide 1.0 mg in SUSTAIN 4 and 5); 2.2-5.9 kg was not mediated by nausea or vomiting (effects were all statistically significant).

Conclusion: Across the SUSTAIN 1-5 trials, weight loss with semaglutide 0.5 mg and 1.0 mg was consistently greater vs comparators, and the contribution to the weight loss mediated by nausea or vomiting was small, indicating that these events were not the primary cause of weight loss.

Table: Summary of changes in body weight from baseline by nausea or vomiting across the SUSTAIN 1–5 trials

	Change in body weight from baseline (kg)					
	Semaglutide 0.5 mg		Semaglutide 1.0 mg		Comparator	
Nausea or vomiting	With	Without	With	Without	With	Without
SUSTAIN 1 vs placebo n=387	−5.1 (n=27)	−3.3 (n=101)	−5.6 (n=33)	−4.3 (n=97)	0.0 (n=10)	−1.0 (n=119)
SUSTAIN 2 vs sitagliptin 100 mg QD n=1225	−5.3 (n=83)	−4.1 (n=326)	−6.8 (n=88)	−5.9 (n=321)	−2.6 (n=34)	−1.8 (n=373)
SUSTAIN 3 vs exenatide ER 2.0 mg QW n=809	-	-	−6.9 (n=97)	−5.4 (n=307)	−2.7 (n=57)	−1.6 (n=348)
SUSTAIN 4 vs titrated IGlar QD n=1082	−4.4 (n=87)	−3.2 (n=275)	−6.3 (n=98)	−4.8 (n=262)	2.2 (n=22)	1.1 (n=338)
SUSTAIN 5 vs placebo n=396	−4.2 (n=20)	−3.6 (n=112)	−8.0 (n=30)	−6.0 (n=101)	0.1 (n=8)	−1.4 (n=125)

ER, extended release; IGlar, insulin glargine; QD, once daily; QW, once weekly. Data represent the 'on-treatment without rescue medication' observation period from the full analysis set. Missing data for body weight imputed from a mixed model for repeated measures with treatment, region and stratum as fixed factors and baseline value as covariate, all nested within visit. Numbers of subjects in each trial are those exposed to at least one dose of trial drug.

Clinical Trial Registration Number: NCT02054897, NCT01930188, NCT01885208, NCT02128932, NCT2305381
Supported by: Novo Nordisk A/S
Disclosure: **S. Atkin:** None.

PS 064 Incretins in special situations

822

Pharmacokinetics and tolerability of semaglutide in subjects with hepatic impairment

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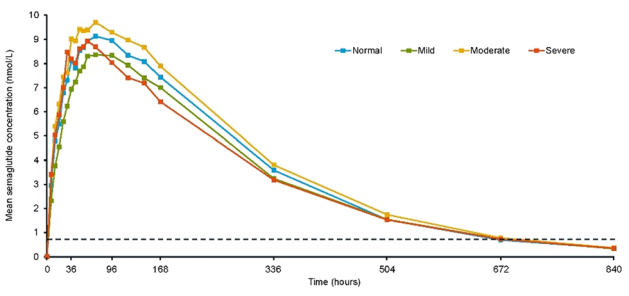
Background and aims: Semaglutide is a glucagon-like peptide-1 receptor agonist in development for the treatment of type 2 diabetes (T2D). Impaired hepatic function may affect the pharmacokinetics (PK) of drugs. This trial investigated whether the PK of semaglutide was altered in subjects who had various degrees of hepatic impairment (HI), versus subjects with normal hepatic function.

Materials and methods: This was a multicentre, open-label, parallel-group trial in which subjects with varying degrees of HI or normal hepatic function received a single, subcutaneous dose of 0.5 mg semaglutide. Using the Child-Pugh criteria, subjects with stable HI were assigned to one of three HI function groups (mild, moderate or severe). Subjects with normal hepatic function were enrolled for comparison. Semaglutide plasma concentrations were assessed regularly for up to 35 days (840 hours) post-dose. The primary PK endpoint was area under the semaglutide plasma concentration-time curve from time zero to infinity ($AUC_{0-\infty}$). 'No effect' was declared if the 90% confidence interval [CI] for the ratio between the HI and normal function groups was within the interval 0.70-1.43. Secondary endpoints included maximum plasma semaglutide concentration (C_{max}), time to reach C_{max} (t_{max}), terminal elimination half-life ($t_{1/2}$), renal clearance (CL/R), and protein binding (*in vitro* assessments on pre-dose plasma samples). The safety and tolerability of semaglutide was also assessed.

Results: Forty-four subjects were allocated to four HI function groups: normal (n=19), mild (n=8), moderate (n=10), and severe (n=7). Semaglutide exposure was similar across all groups (**Figure**), as the $AUC_{0-\infty}$ met the criterion of 'no effect' for all three HI groups versus the group with normal hepatic function ($AUC_{0-\infty}$ treatment ratios for: mild/normal 0.95 [90% CI, 0.77-1.16]; moderate/normal 1.02 [0.93-1.12]; severe/normal 0.97 [0.84-1.12]). In addition, semaglutide C_{max} did not appear to be influenced by hepatic function, as treatment ratios were: mild/normal 0.99 [0.80-1.23]; moderate/normal 1.02 [0.88-1.18]; and severe/normal 1.15 [0.89-1.48] (sensitivity analysis of the latter, which excluded one extreme semaglutide concentration for one subject: 1.05 [0.88-1.25]). Median t_{max} and $t_{1/2}$ were similar (54-78 hours and 150-163 hours, respectively) in all groups. Plasma protein binding was >99% in all subjects. A total of 12 adverse events were reported in 10 subjects; none were serious and all were mild or moderate, and transient. No new safety or tolerability issues were observed.

Conclusion: Semaglutide exposure was not affected by hepatic impairment. Semaglutide was well tolerated and there were no new safety issues. The results suggest that no dose adjustment of semaglutide is necessary in subjects with hepatic impairment.

Figure: Semaglutide profiles in subjects with normal hepatic function or hepatic impairment after administration of a single dose of semaglutide – geometric mean plot



Dotted line represents the lower limit of quantification; values below this limit are imputed.

Clinical Trial Registration Number: NCT02210871
Supported by: Novo Nordisk A/S
Disclosure: **G. Arold:** None.

823

The effect of liraglutide on bone turnover during exercise training in patients with type 2 diabetes

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Background and aims: Type 2 diabetes (T2D) is associated with normal bone mineral density (BMD), but an increased fracture risk. We investigated the effect of liraglutide treatment combined with exercise training on the bone formation markers osteocalcin and procollagen type-1 amino-terminal propeptide (P1NP), the bone resorption marker C-terminal telopeptide of type 1 collagen (CTX), whole-body BMD and whole-body bone mineral content (BMC) in patients with T2D.
Materials and methods: Nineteen middle-aged, dysregulated, obese, untrained patients with T2D treated with diet and/or metformin were randomized to receive once-daily injection with liraglutide 1.8 mg (n=12) or placebo (n=7) during 16 weeks. Both groups had three supervised 60-minute training sessions per week: two spinning sessions and one session with resistance training. All patients underwent a liquid meal test and a dual energy X-ray absorptiometry scan before and after the intervention.
Results: Significant increases in fasting plasma osteocalcin from 9.4±3.2 (mean±SD) to 11.0±3.6 μg/l (P=0.005) and CTX from 0.107±0.1 to 0.161±0.1 μg/l (P=0.001) were observed in the liraglutide group whereas no changes were observed in the placebo group (10.7±1.9 to 11.6±2.9 μg/l (P=0.297) and 0.155±0.1 to 0.222±0.1 μg/l (P=0.250), respectively). In the liraglutide group, area under the curve for osteocalcin during a meal test increased from 2.386±824 to 2.714±944 μg/l × min (P=0.005) and for CTX from 17.7±7.3 to 27.6±9.1 μg/l × min (P=0.003); no changes were observed in the placebo group (2.346±762 to 2.669±1016 μg/l (P=0.156) and 21.1±8.4 to 24.7±19.8 μg/l (P=0.313), respectively). Plasma levels of P1NP were unaffected in both groups. Whole-body BMD and total BMC were unchanged in both groups.
Conclusion: In conclusion, liraglutide treatment combined with exercise for 16 weeks seems to increase bone turnover without affecting whole-body BMD and BMC, respectively, in untrained patients with T2D.
Clinical Trial Registration Number: NCT01455441
Disclosure: **P. Mensberg:** Grants; Unrestricted grant from Novo Noridisk.

824

Steady state blood glucose control for once weekly dulaglutide during peak and trough concentration days

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Background and aims: Dulaglutide (DU), a GLP-1 receptor agonist, with a half-life of ~5 days, is administered once weekly in patients with type 2 diabetes. In this post hoc analysis of AWARD-3, the sustainability of DU glycaemic effect over a 7-day interval at steady state (attained between 2-4 weeks of dosing) was assessed. AWARD-3 was considered for this analysis as it was the only monotherapy study of DU.
Materials and methods: Within 1 week prior to the Week 26 visit, patients were asked to collect 8-point self-monitored blood glucose (SMBG) profiles on any 2 non-consecutive days. All evaluable SMBG profiles with valid dosing dates were segregated according to Days 1-7 of a dosing interval. SMBG data on DU peak (Day 2/3) and trough (Day 6/7) plasma concentration days were tested for equivalence. A mixed effect model with random intercept and the time of SMBG collection (peak/trough) as a single covariate, was fitted for statistical analysis.
Results: Mean daily SMBG concentrations were found to be equivalent between DU peak and trough days for both doses (Table). Model-predicted DU concentrations (90% prediction interval) also remained above the minimum effective concentration throughout the 7-day period.
Conclusion: Throughout the weekly dosing interval at steady state, DU has a similar effect on blood glucose control during peak and trough plasma concentration days, as assessed by the change in mean daily SMBG concentrations.

	Mean Daily SMBG Concentration (mmol/L) After Once Weekly Dulaglutide Administration at Week 26[1]				
	Peak (Day 2/3) Mean ± SD	Trough (Day 6/7) Mean ± SD	Mean Difference Between Peak and Trough (90% CI)	Equivalence Margin of ±10% of Lower Mean Peak/Trough[2]	Equivalence Established Between Peak and Trough (Yes/No)
DU 1.5 mg	7.9 ± 2.0 N=48	8.3 ± 1.9 N=41	-0.4 (-0.7, -0.1)	±0.8	Yes
DU 0.75 mg	7.8 ± 2.1 N=53	7.8 ± 1.8 N=32	0.1 (-0.5, 0.1)	±0.8	Yes

Abbreviations: SMBG = self-monitored blood glucose, DU = dulaglutide, SD = standard deviation, N = number of subjects, CI = confidence interval.
[1]The regimen for SMBG 8-point profile was pre-meal and 2 hours post-meal (morning, midday, evening), bedtime and 3 AM or 5 hours after bedtime.
[2]Equivalence was established only if 90% CI was contained within ±10% of lower mean daily peak/trough SMBG concentrations.

Clinical Trial Registration Number: NCT01126580
Disclosure: **H. Patel:** Employment/Consultancy; Eli Lilly and Company. Stock/Shareholding; Eli Lilly and Company.

825

Acute effects of lixisenatide on gastric emptying, glycaemia and 'postprandial' blood pressure responses to an oral glucose load in healthy subjects and type 2 diabetes

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Background and aims: Postprandial hypotension (PPH), a fall in systolic blood pressure (SBP) of >20mmHg after a meal, occurs frequently in older people and patients with type 2 diabetes (T2DM) and is associated with considerable morbidity and increased mortality. Current management of PPH is suboptimal. We have shown that the magnitude of the postprandial fall in BP is greater when gastric emptying (GE) is relatively more rapid and that intravenous (iv) administration of glucagon-like peptide-1 (GLP-1), in addition to slowing GE, attenuates the postprandial fall in BP and rise in splanchnic blood flow in T2DM. We have now evaluated the effects of the short-acting GLP-1RA, lixisenatide (LIXI), on GE and the BP, superior mesenteric artery (SMA) blood flow, and glycaemic responses to a 75g oral glucose load in healthy and T2DM subjects.
Materials and methods: Fifteen healthy subjects (9M, 6F; age: 67.2 ± 2.3 yr; BMI: 25.4 ± 0.8 kg/m^2) and 15 patients with T2DM managed by diet or metformin alone (9M, 6F; age: 61.9 ± 2.3 yr; BMI: 30.3 ± 0.7 kg/m^2; duration of known diabetes: 5.3 ± 1.2 yr; HbA_1c: 6.9 ± 0.2 % (51.8 ± 2.3 mmol/mol)) had concurrent measurements of GE (scintigraphy), BP (automated machine), SMA blood flow (Doppler ultrasound) and plasma glucose for 180 min after a 75g glucose drink labelled with 20MBq

^{99m}Tc-Calcium Phytate on 2 separate days. All subjects received LIXI (10mcg sc) or placebo (PLAC) in a randomised, double-blind, crossover fashion 30 min before the drink. Data are mean values ± SEM.

Results: LIXI slowed GE (% retention at 120 min) markedly in both healthy subjects (LIXI: 72 ± 5.7% vs PLAC: 38 ± 4.1%; P<0.01) and T2DM (LIXI: 68 ± 5.5% vs PLAC: 33 ± 2.6%; P<0.01) and attenuated the fall in systolic BP (P<0.05; Figure) compared to PLAC in both healthy subjects and T2DM, with no difference between the groups. The maximum rise in SMA flow was attenuated by LIXI in both the healthy subjects (LIXI: 521 ± 68 ml/min vs PLAC: 1031 ± 173 ml/min; P<0.01) and T2DM (LIXI: 187 ± 38 ml/min vs PLAC: 360 ± 72 ml/min; P<0.01), but was greater in the healthy subjects than T2DM on both the PLAC and LIXI days (P<0.001 for both). The iAUC 0-120min for plasma glucose was greater in T2DM (P<0.005) than healthy subjects, and reduced by LIXI in both groups (Healthy subjects LIXI 60 ± 24 mmol/l*min vs PLAC 348 ± 36 mmol/l*min; P<0.001 and T2DM LIXI 204 ± 60 mmol/l*min vs PLAC 600 ± 72 mmol/l*min; P<0.001).

Conclusion: In health and T2DM, the marked slowing of GE of glucose and consequent reduction in glycaemia induced by lixisenatide (10mcg sc) are associated with attenuation of the rise of SMA flow and fall in SBP. Accordingly, lixisenatide may be useful in the management of PPH.

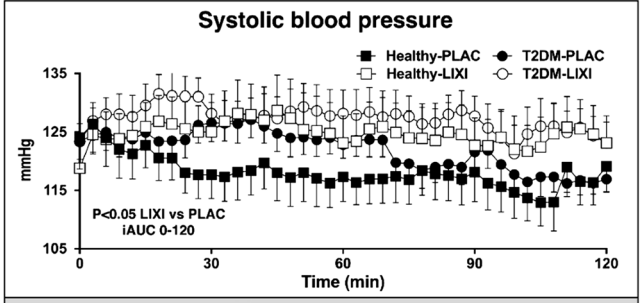

Figure: Effect of lixisenatide (10mcg sc) on systolic blood pressure in healthy subjects (n=15) and T2DM (n=15)

Clinical Trial Registration Number: NCT02308254
Supported by: Sanofi-Aventis Australia Pty Ltd, NHMRC (APP627011)
Disclosure: **K.L. Jones:** Grants; Sanofi-Aventis Pty Ltd; NHMRC CDA Research Fellowship (APP627011).

826

Autonomic neuropathy is associated with the lack of glucagon-like peptide-1 receptor agonists treatment efficacy in patients with type 2 diabetes

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Background and aims: While glucagon-like peptide-1 receptor agonists (GLP-1 RA) are widely used in the treatment of type 2 diabetes (T2DM), factors contributing to the lack of their efficacy have not been fully elucidated. We investigated the potential influence of cardiac autonomic neuropathy (CAN) in patients not responding to GLP1-RA.

Materials and methods: Two hundred and 14 patients with T2DM; 142 (66%) on liraglutide and 72 (34 %) on exenatide, both added to co-existing antidiabetic therapy, were divided into 2 groups (GLP-1 RA responders and non-responders) and followed for 40 months. Non-responding was defined by switching to insulin therapy at any time after intervention during 40 months of treatment. Exenatide was started as 5 μg and increased up to 10 μg twice daily. Liraglutide was started as 0.6 mg and increased up to 1.8 mg once daily. Testing of CAN was carried out on VAGUS 2100 (Sigma Medizin Technik, Thum, Germany) using a standard battery of cardiovascular tests based on heart rate variation (HRV): HRV at rest, HRV during deep breathing, Valsalva maneuver, active orthostatic test and blood pressure response to standing. The statistical data were processed by standard, vector and spectral analysis and following parameters were obtained: the coefficient of variation at rest (HRV-CV), the spectrum of low frequency power (LF) and high frequency power (HF), the coefficient of variation during deep breathing (dbHRV-CV), E / I ratio, 30 : 15 ratio and the Valsalva ratio. The resulting parameters were compared with normal values for the appropriate age, and a pathological finding of two or more parameters was considered as CAN.

Results: Non-responders (n=47), compared to responders (n=167), were older (62 vs 59 years, p=0.002), had longer diabetes duration o (14.9 vs 12.7 years, p<0.001), higher systolic (147.5 vs 143.3 mmHg, p=0.001), diastolic blood pressure (88.1 vs 86.4 mmHg, p= 0.002) and heart rate (81.1 vs 71.7 beats/min., p=0.001). The non-responders group compared to responders showed increased CAN presence (9.4% vs 4.7% and 53.1% vs 19.2%, p<0.001): significantly lower HRV-CV at rest, dbHRV-CV and Valsalva ratio, a significant attenuation for both LF and HF bands indicating lower parasympathetic or higher sympathetic nerve activity. The Cox regression model including the possible confounding variables revealed that higher HRV-CV, dbHRV-CV, E/I ratio, LF and LF/HF were associated with GLP-1 RA therapy success. For each increase in HRV-CV and dbHRV-CV of 1%, the 40 months chance for achieving glucoregulation with GLP-1RA increased by 15.6% and 12.3% respectively. The analysis of the time to insulin dependency according to cardiac autonomic function revealed that patients without CAN required insulin in 32.05 (27.25-36.87) months while patients with bordeline and pathological autonomic test results ended up with insulin therapy in 22.32 (13.85-30.78) and 21.21 (16.96-25.47) months (p<0.001 and 0.899, respectively).

Conclusion: Lack of GLP1-RA efficacy appears to be associated with CAN leading to the shortening of the time of insulin dependency in T2DM. As autonomic dysfunction could represent a significant factor leading to treatment failure, early identification of the CAN presence might be of special clinical interest. The predictive value of the relationship between GLP-1 RA efficacy and autonomic disbalance merits to be further investigated.

Clinical Trial Registration Number: 7459
Supported by: HrZZ
Disclosure: **L. Duvnjak:** None.

827

Efficacy of dulaglutide 1.5 mg combined with insulin in older, poorly controlled insulin-treated patients with long-standing type 2 diabetes: a post-hoc analysis

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Background and aims: This post-hoc analysis assessed the efficacy and safety of once weekly dulaglutide (DU) 1.5 mg combined with insulin in patients (pt) with type 2 diabetes (T2D), categorised by subgroups of age (<65 and ≥65 years; yr), duration of diabetes (DoD; <10 and ≥10 yr) and baseline (BL) HbA_{1c} [≤9% (≤74.9 mmol/mol) and >9% (>74.9 mmol/mol)] at 6 months (mo).

Materials and methods: A pooled analysis was conducted for efficacy of two Phase 3 randomised trials (AWARD-4 and -9) that assessed the use of DU in a similar T2D population with a pooled mean BL age: 59 yr, DoD: 13 yr and HbA_{1c}: 8.4% (68.3 mmol/mol). Weight and hypoglycaemia were analysed by individual trial. In AWARD-4, DU + lispro TID was assessed against glargine + lispro TID. In AWARD-9, DU added to glargine was assessed against placebo added to glargine. Insulins were titrated to target in both trials.

Results: At 6 mo, DU 1.5 mg significantly reduced HbA_{1c} in all subgroups (p<0.001) with the highest reduction observed in pt with BL

HbA_{1c} >9% (>74.9 mmol/mol; Table). All except 3 subgroups [DoD <10 yr, AWARD-4; BL HbA_{1c} >9% (>74.9 mmol/mol), both trials] demonstrated weight loss. The incidence of documented symptomatic hypoglycaemia was similar in all subgroups in AWARD-4 study; and in AWARD-9 study, the incidence was numerically greater in subgroups of ≥10 yr DoD, and >9% baseline HbA_{1c}. The most common adverse events observed in each trial were gastrointestinal in nature.

Conclusion: DU 1.5 mg combined with basal or prandial insulin is efficacious for pt with T2D independent of age, DoD and BL HbA_{1c}.

Baseline characteristics, Δ HbA_{1c} and percent to goal (HbA_{1c} <7% [<53 mmol/mol]) at 6 months of dulaglutide 1.5 mg patients categorised by baseline HbA_{1c}, age and duration of diabetes

	HbA_{1c}		Age		Duration of Diabetes	
	≤9% (≤74.9 mmol/mol) N=325	>9% (>74.9 mmol/mol) N=118	<65 Years N=313	≥65 Years N=132	<10 Years N=158	≥10 Years N=287
Baseline age, yr	60.4 (9.3)	56.3 (9.6)	54.8 (7.2)	70.1 (4.1)	55.1 (10.0)	61.6 (8.4)
Baseline duration of diabetes, yr	13.3 (7.8)	11.5 (5.4)	11.6 (6.3)	15.9 (8.5)	5.9 (2.3)	16.7 (6.2)
Baseline HbA_{1c}						
%	8.0 (0.6)	9.7 (0.6)	8.5 (1.1)	8.3 (0.9)	8.5 (1.0)	8.4 (1.0)
mmol/mol	63.9 (6.6)	82.5 (6.6)	69.4 (12.0)	67.2 (9.8)	69.4 (10.9)	68.3 (10.9)
Δ HbA_{1c}						
%	-1.3 (0.1)**	-2.5 (0.1)**	-1.5 (0.1)**	-1.6 (0.1)**	-1.6 (0.1)**	-1.6 (0.1)**
mmol/mol	-14.2 (1.1)	-27.3 (1.1)	-16.4 (1.1)	-17.5 (1.1)	-17.5 (1.1)	-17.5 (1.1)
Percent to goal HbA_{1c} <7%	235 (72.3)	54 (45.8)	203 (64.9)	87 (65.9)	113 (71.5)	177 (61.7)

Baseline HbA_{1c}, age and duration of diabetes are presented as mean (SD), Δ HbA_{1c} is presented as LS mean (SE) and percent to goal is presented as n (%); ITT population

**p<0.001 from baseline

AWARD=Assessment of Weekly AdministRation of LY2189265 (dulaglutide) in Diabetes; Δ=change

Clinical Trial Registration Number: NCT01191268, NCT02152371
Supported by: Eli Lilly and Company
Disclosure: **L. Fernández Landó:** Employment/Consultancy; Eli Lilly and Company. Stock/Shareholding; Stock/shareholder.

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The effect of liraglutide on oxidative nucleic acid modifications in women with prior gestational diabetes

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Background and aims: Animal studies have shown that liraglutide, a glucagon-like peptide-1 analogue, lowers oxidative modifications. RNA oxidation, measured by urinary excretion of 8-oxo-7,8-dihydroguanosine (8-oxoGuo), is associated with mortality in patients with type 2 diabetes. Here, we investigated the effect of liraglutide on oxidative nucleic acid modifications in women with prior gestational diabetes mellitus (pGDM).
Materials and methods: Women with pGDM (n=104) were randomised to one-year, double-blinded intervention with liraglutide (1.8 mg, once-daily) (n=49) or placebo (n=55) followed by a one-year, open-label intervention. An age and BMI matched control group of women without pGDM was also studied (n=15) at baseline. The urinary excretion of 8-oxoGuo and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) was determined at baseline, after 53 weeks (52 weeks intervention followed by one week wash out), and after 104 weeks by ultra-performance liquid chromatography tandem mass-spectrometry as a measurement of whole body RNA and DNA oxidation, respectively.
Results: At baseline, similar urinary excretion of 8-oxoGuo/creatinine (mean±SD: 1.58±0.40 vs. 1.61±0.53 nmol/nmol, p=0.86) and 8-oxodG/creatinine (1.06±0.33 vs 1.20±0.54 nmol/nmol, p=0.39) was seen in women with and without pGDM. Treatment with liraglutide for 52 weeks did not affect urinary excretion of 8-oxoGuo/creatinine (delta values (mean±SD): -0.01±0.36 vs. -0.00±0.52 nmol/nmol, p=0.91) or 8-oxodG/creatinine (-0.01±0.25 vs. 0.08±0.45 nmol/nmol, p=0.31) vs. placebo treatment after a one-week washout period. Subsequent, open-label treatment with liraglutide for additionally 52 weeks vs. no further treatment did not affect urinary excretion of 8-oxoGuo/creatinine (delta values from baseline (mean±SD): 0.05±0.29 vs. 0.13±0.43 nmol/nmol, p=0.45) or 8-oxodG/creatinine (0.15±0.25 vs. 0.30±0.33 nmol/nmol, p=0.10).
Conclusion: In conclusion, women with and without pGDM exhibit similar RNA and DNA oxidations, and liraglutide treatment for two years does not seem to affect oxidative nucleic acid modifications in women with pGDM.
Clinical Trial Registration Number: EUDRACT(2012-001371-37)
Supported by: Unrestricted grant from Novo Nordisk
Disclosure: **E.L. Larsen:** Grants; Unrestricted grant from Novo Nordisk.

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Efficacy of liraglutide QD vs sitagliptin QD vs insulin glargine on liver fat when combined with metformin in type 2 diabetes subjects with NAFLD (Light-on Study)

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) has become epidemic in type 2 diabetes mellitus (T2DM), yet limited data are available on efficacy and safety of anti-hyperglycemic agents for liver injury. This study investigated the effects of liraglutide versus sitagliptin versus insulin glargine on liver fat combined with metformin in NAFLD patients with T2DM.
Materials and methods: Seventy-five patients with NAFLD and T2DM inadequately controlled on metformin monotherapy (at least 1500 mg/d) were randomized to receive liraglutide or sitagliptin or insulin glargine therapy for 26 weeks. The liver fat index (MRI estimated proton density fat fraction, MRI-PDFF) was measured by MRI IDEAL IQ (Iterative Decomposition of water and fat with Echo Asymmetry and Least-squares estimation). The Chi-square test, paired t test and one way ANOVA (least significant difference) were used for statistical analysis.
Results: A total of 68 patients finished the study. Baseline characteristics were similar among the three groups. There was significant improvement in glycated hemoglobin (HbA1c) in three groups (liraglutide, 7.3±0.7 % to 6.1±0.6%, P=0.001; sitagliptin, 7.5±0.9 % to 6.6±0.9%, P=0.001; glargine, 7.7±1.0% to 6.8±1.1 %, P=0.018), whereas the change of HbA1c (ΔHbA1c) from baseline was not significantly different among three groups (P>0.05). Both liraglutide and sitagliptin treatment were associated with a significant decrease in MRI-PDFF (liraglutide,17.8 ±6.8% to 13.0±7.0%, P=0.004; sitagliptin,15.9±6.0% to 12.1±4.8%, P=0.002) and weight (liraglutide, 87.9±12.5 kg to 83.9±10.9 kg, P=0.004; sitagliptin, 88.2±13.9 kg to 86.5±13.5 kg, P=0.004), but MRI-PDFF showed no significant change in glargine treatment (14.2

±5.2% to 13.3±7.1%, *P*> 0.05), nor did weight (85.2±13.8 kg to 84.1 ±14.6 kg, *P*>0.05). The changes of weight (Δweight) and MRI-PDFF(ΔMRI-PDFF) from baseline in the liraglutide group were significantly higher than those in the glargine group(*P*=0.030 and *P*=0.009 respectively), whereas Δweight and ΔMRI-PDFF from baseline has no significant difference between the liraglutide group and sitagliptin group (*P*=0.074 and *P*=0.337 respectively).

Conclusion: The liraglutide or sitagliptin therapy combined with metformin reduced the liver fat in T2DM patients with NAFLD.

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Dyslipidaemia impairs effects of GLP-1 on nitric oxide action and oxidative stress in human platelets

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Background and aims: Glucagon-like peptide 1 (GLP-1) exerts important effects on the glucose homeostasis and influences the cardiovascular system. Platelets are very sensitive to the inhibitory effects of nitric oxide (NO), which is impaired and contributes to platelet hyperreactivity in Type 2 Diabetes (T2D), thus contributing to increased thrombotic events. We have recently demonstrated that, in platelets from healthy subjects, the bioactive GLP-1(7-36) and the GLP-1 analogue Liraglutide increase platelet sensitivity to NO/cGMP/PKG/VASP pathway and reduce oxidative stress. We have also found that these GLP-1 effects are reduced in T2DM. Aim of this study was to evaluate if dyslipidaemia without hyperglycemia affects GLP-1-related peptides activity on platelet response to NO and reactive oxygen species (ROS) synthesis in platelets.

Materials and methods: In platelet samples obtained from 26 non diabetic patients affected by dyslipidaemia (M/F: 13/13; age 49.4±1.9 years; BMI: 24.9±0.8 kg/m2; fasting glucose: 91.7±1.6 mg/dl, total cholesterol: 273.5±8.5 mg/dl, HDL-cholesterol: 64.8±4.1 mg/dl, triglycerides 166.7 ±21.0 mg/dl, LDL-cholesterol:182.3±7.7 mg/dl) and from 18 healthy controls (M/F:10/8; age:49.2±3.6 years; BMI: 24.3±0.8 kg/m^2), we evaluated the GLP-1(7-36) or Liraglutide (100 nmol/l) ability to influence: i)the anti-aggregating effects of the NO donor Na-nitroprusside (SNP, 5 μmol/l) with the platelet agonists ADP (10 μmol/l), collagen (4 mg/l) and arachidonic acid (AA, 1 mmol/l) (Born's method); ii)the SNP-induced VASP (pVASP) phosphorylation at ser239 (Western Blot); and iii)the ROS production induced by AA (100 μmol/l)(DCF-DA fluorescence assay).

Results: In dyslipidaemic subjects compared with healthy subjects, the effects of GLP-1(7-36) and Liraglutide on platelet response were significantly lower. In particular, in the presence of GLP-1(7-36): the percent increase of the anti-aggregating effects of SNP were 7.0±1.9 vs 23.2±4.5 (p=0.0001) with ADP, 8.3±6.8 vs 30.8±5.3 (p=0.02) with collagen and 6.6±3.2 vs 31.8±5.5 (p=0.0001) with AA; the percent increase of the SNP-induced pVASP levels was 3.5±4.3 vs 33.1±3.9 (p=0.0001); the percent reduction of ROS synthesis was 10.1±5.2 vs 30.2±7.9 (p=0.03). In the presence of Liraglutide: the percent increase of the anti-aggregating effects of SNP were 9.6±2.9 vs 35.3±4.9 (p=0.0001) with ADP, 15.0±5.2 vs 36.8±5.6 (p=0.008) with collagen, 17.0±5.2 vs 36.8±5.1 (p=0.01) with AA; the percent increase of the SNP-induced pVASP was 5.1±4.2 vs 40.1 ±5.5 (p=0.0001); the percent reduction of ROS synthesis was 15.6±5.7 vs 40.2±7.8 (p=0.01).

Conclusion: In platelets from non diabetic dyslipidaemic subjects, the ability of GLP-1(7-36) and Liraglutide to enhance the inhibitory pathway NO/PKG/VASP and reduce ROS production was greatly impaired. Our findings are suggestive for a role of dyslipidaemia per se to cause a resistance to GLP-1 effects on platelet function and for dyslipidaemia being a determinant of impaired GLP-1 action on platelets which we also observed in T2D.

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Autonomic regulation may modify the weight reducing effect of liraglutide in overweight patients with type 1 diabetes

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Background and aims: The exact mechanisms by which glucagon-like peptide-1 (GLP-1) exerts anorectic effects are unclear, but peripheral activation of vagal neurons as well as brain GLP-1 receptors seems to be involved. Vagal neurons may also be involved in the effect of GLP-1 on the pancreatic islet function. The role of the autonomic nervous system (ANS) in the efficacy of long-acting GLP-1 receptor agonists, such as liraglutide, in patients with type 1 diabetes is unknown. We assessed the effect of autonomic neuropathy on liraglutide-induced weight loss, insulin requirement and rates of hypoglycaemia. We investigated the effect of liraglutide treatment on ANS function.

Materials and methods: Lira-1 was a randomised, double-blind, placebo-controlled trial assessing the efficacy of 1.8 mg liraglutide once-daily for 24 weeks in obese and poorly controlled type 1 diabetes. ANS function was assessed by cardiovascular reflex tests (CARTs): heart rate response to deep breathing (E/I ratio), to standing (30/15 ratio) and to the Valsalva manoeuvre. Participants with two or three pathological CARTs were diagnosed as having cardiovascular autonomic neuropathy (CAN). In addition, ANS function was assessed by resting heart rate variability indices by parasympathetic measures (RMSSD and high frequency power (HF)) mixed sympathetic and parasympathetic measures (SDNN, low frequency power (LF) and total power (TP).

Results: 100 patients aged 48 (SD 12) years, with a mean HbA_{1c} of 72 mmol/mol (SD 7) and a mean BMI of 30 (SD 3) kg/m^2 were assigned to liraglutide or placebo. 30 percent of all participants had CAN. Patients with and without CAN had a similar liraglutide induced weight loss of 6.1 kg (SD 4.0) and 5.8 kg (SD 2.9) (p=0.9), respectively. One SD higher level in baseline values of the 30/15 ratio, SDNN and TP was associated with increased weight reduction by liraglutide of 0.97 kg (95% CI: 0.25;1.69, p<0.01), 0.77 kg (95 % CI 0.13;1.40, p=0.02) and 0.69 kg (95% CI -0.001;1.39, p=0.06) respectively. CAN measures were not associated with change in insulin requirements. In the liraglutide group, the rate of hypoglycaemic events was higher in patients with CAN vs. patients with no CAN. The between group rate ratio (RR) was 1.32 (95% CI 1.13; 1.54 p<0.001). A one SD higher level of HF and RMSSD was associated with lower rates of hypoglycaemia (RR<0.8, p<0.0001 for both). In the placebo group, CAN and increased values of the E/I ratio, SDNN, LF, TP and RMSSD were associated with less events (data not shown). Liraglutide treatment did not induce change in CARTs or HRV indices.

Conclusion: During treatment with liraglutide in obese type 1 diabetes patients, autonomic dysfunction but not the diagnosis of CAN was associated with less weight loss. The E/I ratio (a measure of vagal function) was not associated with weight reduction suggesting that the weight loss may be independent of the vagus nerve activity. Autonomic neuropathy

measures were not associated with insulin requirements and only parasympathetic measures were associated with hypoglycaemia in the liraglutide group.

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PS 065 Incretin mechanisms

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Liraglutide treatment reduces the NA and SNP relaxant effect on the rat isolated gastric fundus: comparison with a model of non-obese type 2 diabetes

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Background and aims: GLP-1R agonists (GLP-1RA) have been developed for the treatment of type 2 diabetes (T2D) and obesity because of their beneficial metabolic and anorexic effects. Regarding the gastrointestinal tract, they delay gastric emptying and inhibit gut motility through activation of GLP-1R on enteric neurons and via nitric oxide release. The main goal of this study was to understand and pharmacologically characterize the effects of GLP-RA on gastric fundus motility, in the absence and presence of T2D.
Materials and methods: Gastric fundus strips isolated from 16 weeks Wistar rats and non-obese type 2 diabetic Goto-Kakizaki (GK) rats were used to study the isometric contractile responses to GLP-1, exenatide and liraglutide in the presence and the absence of Exendin-3 (selective GLP-1R antagonist), and N^G-nitro-L-Arginine, (L-NNA, non-selective inhibitor of nitric oxide synthase). In addition 14 week male Wistar and GK rats were injected twice a day with liraglutide (200μg/Kg s.c.) or saline for 14 days, after which they were sacrificed and isometric relaxation induced by sodium nitroprusside (SNP) and noradrenaline (NA) was studied using gastric fundus strips from all 4 groups. Samples were also collected to determine GLP-1R, nNOS and p-nNOS density (Western Blotting).
Results: Functional studies showed that GLP1-RA induced concentration-dependent non-cholinergic contraction of rat gastric fundus, partially inhibited by Exendin-3 and not altered by L-NNA. Moreover GLP-1 showed more intrinsic activity in diabetic rats (E_{max} 9.09±1.44 mN, n=12) than in control rats (E_{max} 5.6±0.69 mN, n=16) ($p<0.05$, Student *t* test) and according to ANOVA followed by Tukey's multiple comparison test, exenatide showed more intrinsic activity and liraglutide was the most potent of the three GLP-1RA studied (pEC_{50} of 9.62±0.23, n=5 *versus* exenatide pEC_{50} of 8.78±0.16 and GLP-1 pEC_{50} of 8.33±0.16, n= 12). *In vivo* studies showed a significant decrease in caloric intake and body weight both in diabetic and non-diabetic liraglutide treated-rats. Additionally, a significant decrease in SNP- and NA-induced relaxation of gastric fundus strips isolated from both treated animal groups was observed: NA showed less intrinsic activity and potency in Wistar treated-rats and less potency in GK treated-rats, indicating that NA-induced relaxation was reduced by both treatment and pathology. Finally, a significant decrease of GLP-1R levels was observed in gastric fundus from treated-rats (compared to non-treated ones), but no significant changes were observed in nNOS and p-nNOS levels between the 4 groups.
Conclusion: The increased gastric fundus tonus induced by GLP-1RA may constitute a peripheral mechanism by which GLP-1RA can reduce food intake.
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Effect of liraglutide on time in various glycaemic ranges in persons with type 2 diabetes treated with multiple daily insulin injections

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Background and aims: We have earlier shown that liraglutide compared to placebo improves glycaemic control (HbA1c) and reduces body weight in persons with type 2 diabetes (T2D) treated with multiple daily insulin injections (MDI). In the current evaluation of the data from the study we further analysed effects of liraglutide compared to placebo on various glycaemic ranges including time in hypoglycaemia, euglycaemia and hyperglycaemia using masked continuous glucose monitoring (CGM).
Materials and methods: Data from the MDI-liraglutide trial randomizing 124 persons with T2D treated with MDI having an HbA1c of ≥58 mmol/mol (7.5%) and ≤102 mmol/mol (11.5%), and body mass index of 27.5-45 kg/m^2 were used. At 24 weeks 99 (80%) of patients had masked CGM data. We categorized glycaemic ranges into hypoglycemic, euglycaemic and hyperglycaemic ranges (Table).
Results: The mean percent time in hypoglycaemia was similar for persons receiving liraglutide and placebo at 24 weeks treatment, Table. The mean time in euglycaemia was greater in liraglutide group compared to placebo, 431 versus 243 min/24h (p=0.0012) and 960 versus 696 min/24h (p<0.001) for the two glycaemic cut-offs (4-7 mmol/l and 4-10 mmol/l) respectively, see Table. In contrast, the mean time in hyperglycaemia was lower in the liraglutide group compared to placebo, 456 versus 723 min/24h (p<0.001) and 134 versus 264 min/24h (p=0.0094) for the two evaluated glycaemic cut-offs (>10 mmol/l and >14 mmol/l), see Table.
Conclusion: Using masked CGM we found that adding liraglutide to persons with T2D treated with MDI increases time in euglycaemia, reduces time in hyperglycaemia without the risk of increased time in hypoglycemia.

Table 1. Time (min/24h) with hypoglycaemia, euglycaemia and hyperglycaemia measured by CGM (ITT population)

	Baseline			24 Weeks			
Variable	Liraglutide (n=63)	Placebo (n=59)	p-value	Liraglutide (n=63)	Placebo (n=59)	p-value	Adjusted p-value*
Time (min/24h) with hypoglycaemia <3.6 mmol/l	2.89 (9.05) 0.00 (0.00; 57.75) n=55	5.48 (27.83) 0.00 (0.00; 200.73) n=53	0.75	3.46 (7.45) 0.00 (0.00; 30.22) n=52	4.32 (11.24) 0.00 (0.00; 62.08) n=47	0.67	0.67
Time (min/24h) with hypoglycaemia <3.9 mmol/l	14.5 (35.0) 0.0 (0.0; 224.5) n=55	14.4 (37.5) 0.0 (0.0; 244.4) n=53	0.99	19.5 (34.2) 3.8 (0.0; 163.4) n=52	19.3 (32.3) 5.1 (0.0; 165.8) n=47	0.97	0.95
Time (min/24h) with euglycaemia 4-7 mmol/l	219.7 (199.5) 183.5 (0.0; 863.2) n=55	220.7 (205.8) 171.0 (0.0; 830.3) n=53	0.98	430.2 (304.8) 334.3 (15.7; 1205.2) n=52	244.0 (203.6) 224.6 (0.0; 745.1) n=47	0.0007	0.0012
Time (min/24h) with euglycaemia 4-10 mmol/l	631 (347) 636 (3; 1299) n=55	679 (320) 729 (53; 1222) n=53	0.47	960 (346) 1007 (95; 1408) n=52	696 (363) 691 (0; 1264) n=47	0.0007	0.0001
Time (min/24h) with hyperglycaemia >10 mmol/l	792 (362) 800 (7; 1437) n=55	745 (338) 695 (200; 1387) n=53	0.49	456.8 (361.7) 429.3 (0.0; 1346.0) n=52	723 (409) 723 (122; 1440) n=47	0.0011	0.0002
Time (min/24h) with hyperglycaemia >14 mmol/l	309.3 (306.0) 201.1 (0.0; 1149.2) n=55	258.3 (263.8) 149.5 (0.0; 926.6) n=53	0.36	133.6 (251.4) 29.8 (0.0; 1179.8) n=52	263.9 (312.4) 143.1 (0.0; 1246.6) n=47	0.023	0.0094

For continuous variables Mean (SD) / Median (Min; Max) / n= is presented. For comparison between groups the Fisher's Non Parametric Permutation Test was used for continuous variables. Logistic regression with group as dependent variable is used to adjust for baseline value.

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Dulaglutide treatment reduces glycaemic excursions in people with type 2 diabetes: a post-hoc analysis of 6 phase 3 randomised clinical trials

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Background and aims: The once-weekly glucagon-like peptide-1 receptor agonist dulaglutide (DU) demonstrated a significant HbA1c reduction and the potential for weight loss in adults with uncontrolled type 2 diabetes (T2D) in the AWARD phase 3 clinical programme. DU has also demonstrated significant reductions in fasting, pre- and postprandial glucose levels.
Materials and methods: To further understand the glycaemic effects of DU 1.5 mg and 0.75 mg, we performed a post-hoc analysis examining glycaemic excursions (morning, midday, evening and daily mean) obtained by self-monitored blood glucose testing at 6 or 12 month time points. Analysis was performed by individual study (AWARD-1, -2, -3, -6, -8 and -9), given the different background therapies. Glycaemic excursions were derived by subtracting blood glucose values before each meal from blood glucose values 2 hours after the meal.
Results: Treatment with DU 1.5 mg resulted in significant reductions from baseline in daily mean glucose excursions (change: -0.39 to -0.83 mmol/L, p<0.05) in all six trials. The effect of DU 1.5 mg on glycaemic excursions was most evident in the morning followed by the evening (Table). The trend of the glycaemic excursion reductions from baseline with DU 1.5 mg and DU 0.75 mg was similar.
Conclusion: The effect of DU treatment on glycaemic excursions may contribute to overall improvements in glycaemic control in patients with T2D treated with DU.

Study (Time Point)	Mean Change in Glycaemic Excursions From Baseline to Endpoint (mmol/L) DU 1.5 mg			DU 0.75 mg		
	Morning	Mid-Day	Evening	Morning	Mid-Day	Evening
AWARD-1 (6 mos) In combination w/ MET + PIO	−0.83±0.17*	−0.11±0.17	−0.78±0.17*	−0.89±0.17*	0.06±0.17	−0.11±0.17
AWARD-2 (12 mos) In combination w/ MET + GLIM	−0.67±0.17*	−0.11±0.22	−0.17±0.22	−0.72±0.17*	−0.11±0.22	−0.89±0.22*
AWARD-3 (6 mos) Monotherapy	−0.89±0.17*	−0.39±0.17*	−0.67±0.17*	−1.11±0.17*	−0.28±0.17	−0.56±0.17*
AWARD-6 (6 mos) In combination w/ MET	−1.05±0.11*	−0.39±0.17*	−0.67±0.17*	—	—	—
AWARD-8 (6 mos) In combination w/ GLIM	−0.83±0.17*	−0.39±0.17*	−0.33±0.17	—	—	—
AWARD-9 (6 mos) In combination w/ insulin glargine ± MET	−1.33±0.22*	−0.61±0.22*	−0.50±0.22*	—	—	—

Values are mean ± standard error; intent to treat population, without post-rescue visits, last observation carried forward
*p<0.5 for change from baseline
Abbreviations: 1°=primary; DU=dulaglutide; GLIM=glimepiride; MET=metform; mos=months; PIO=pioglitazone; w/=with

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Disclosure: **V. Pechtner:** None.

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Effects of exenatide once weekly and dapagliflozin in combination and alone on a composite of HbA_{1c}, weight, and SBP across baseline BMI subgroups in the DURATION-8 trial

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Background and aims: In the randomised, double-blind, active-controlled DURATION-8 study, the combination of exenatide once weekly (QW) + dapagliflozin once daily (QD) led to improvements in glycaemic measures, weight, and systolic blood pressure (SBP) in adult patients with type 2 diabetes inadequately controlled by metformin monotherapy. For exenatide QW, it is known that weight loss increases with higher BMI and may be associated with greater improvements in HbA1c and SBP. We hypothesised that improvements in a composite of HbA1c, weight, and SBP may be associated with baseline BMI and thus investigated treatment effects on these endpoints alone and as a composite of all 3 across baseline BMI subgroups.
Materials and methods: Patients received exenatide QW 2 mg + dapagliflozin 10 mg QD (N=228), exenatide QW 2 mg + placebo (N=227), or dapagliflozin 10 mg QD + placebo (N=230) added-on to metformin. All endpoints were studied at 28 weeks. In this post hoc analysis, patients in the intent-to-treat population were analysed by

baseline BMI subgroups: <25, ≥25 to <30, ≥30 to <35, ≥35 to <40, and ≥40 kg/m^2. The number and percentage of patients achieving reductions in HbA1c ≥1%, weight ≥2 kg, SBP ≥2 mmHg, and the composite of these 3 were calculated for each treatment and BMI subgroup.

Results: At baseline, mean HbA1c was 9.3% in all groups; in the exenatide QW + dapagliflozin, exenatide QW, and dapagliflozin groups, mean weight was 91.8, 89.8, and 91.1 kg, respectively, mean BMI was 33.2, 32.0, and 33.0 kg/m^2, respectively, and mean SBP was 130.5, 129.6, and 129.7 mmHg, respectively. Within each baseline BMI subgroup, proportions of patients achieving individual endpoints were generally greater for the combination than exenatide or dapagliflozin alone (Table). A greater proportion of patients treated with exenatide QW + dapagliflozin achieved the composite endpoint compared with exenatide QW or dapagliflozin alone across BMI subgroups. Within each treatment group, in general, greater proportions of patients achieved the composite endpoint as baseline BMI increased. The greatest composite benefit was observed in patients with BMI ≥35 kg/m^2 treated with exenatide QW + dapagliflozin.

Conclusion: Exenatide QW + dapagliflozin was associated with a greater proportion of patients achieving a composite endpoint of HbA1c reduction, weight loss, and SBP reduction than exenatide QW or dapagliflozin alone across all baseline BMI subgroups. More obese patients achieved the composite endpoint of HbA1c, weight, and SBP compared with overweight patients.

Table. Percentage of patients achieving specific HbA1c, weight, and/or SBP reductions in BMI subgroups

Treatment, endpoint	Baseline BMI, kg/m²*			
Patients achieving endpoint, (%)	25 to <30	30 to <35	35 to <40	≥40
ExQW + DAPA	**N=71**	**N=65**	**N=43**	**N=32**
HbA1c reduction†	67.6	58.5	83.7	75.0
Weight loss‡	52.1	44.6	65.1	68.8
SBP reduction§	46.5	50.8	58.1	62.5
Composite of all 3	25.4	27.7	41.9	40.6
ExQW + PBO	**N=78**	**N=71**	**N=43**	**N=18**
HbA1c reduction†	55.1	57.7	60.5	66.7
Weight loss‡	19.2	32.4	41.9	61.1
SBP reduction§	38.5	39.4	44.2	50.0
Composite of all 3	7.7	9.9	14.0	33.3
DAPA + PBO	**N=57**	**N=88**	**N=38**	**N=32**
HbA1c reduction†	52.6	56.8	63.2	56.2
Weight loss‡	31.6	51.1	50.0	59.4
SBP reduction§	36.8	47.7	55.3	40.6
Composite of all 3	10.5	20.5	23.7	15.6

*There were too few patients in the BMI <25 kg/m^2 subgroup for interpretation of the results. †HbA1c reduction ≥1%. ‡Weight loss ≥2 kg. §SBP reduction ≥2 mmHg.

BMI, body mass index; DAPA, dapagliflozin; ExQW, exenatide once weekly; PBO, placebo; red., reduction; SBP, systolic blood pressure.

Clinical Trial Registration Number: NCT02229396
Supported by: The DURATION-8 study and this analysis were sponsored by AstraZeneca.
Disclosure: **E. Repetto:** Employment/Consultancy; AstraZeneca employee.

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Characteristics associated with the choice of first injectable therapy among U.S. patients with type 2 diabetes

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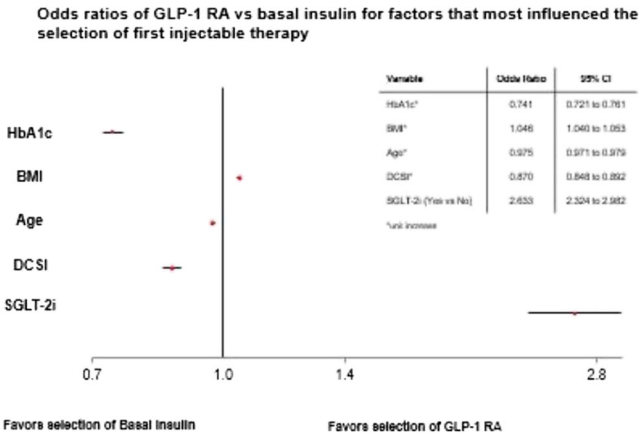
Background and aims: The objective of this retrospective observational study was to identify and describe clinical and demographic characteristics associated with the choice of first injectable therapy (glucagon-like peptide-1 receptor agonist [GLP-1 RA] or basal insulin [BI]) by physicians among patients with type 2 diabetes (T2D).

Materials and methods: This analysis included adults with T2D who initiated GLP-1 RA or BI (index date) between November 2014 and February 2016 using data from the Practice Fusion electronic health record database. Patients with ≥1 diagnosis of T2D and ≥1 office visit between 6 and 18 months prior to the index date who were naïve to GLP-1 RAs and BI and had ≥1 HbA1c result in the 6-month baseline period were included. Gradient boosting method (GBM) and bootstrapped logistic regression were used to identify variables that influenced selection of treatment.

Results: The study included 3,546 and 7,507 GLP-1 RA and BI initiators, respectively. Descriptive analyses showed that at baseline, GLP-1 RA initiators were significantly younger (mean 58 vs 63 years) and had lower HbA1c (mean 8.2 vs 9.1%), lower Diabetes Complications Severity Index (DCSI) scores (mean 1.0 vs 1.7), and higher BMI (mean 36 vs 33 kg/m^2) compared to BI initiators. Variables selected by GBM with the highest relative importance (≥5%) in the selection of GLP-1 RA or BI were HbA1c, BMI, age, sodium-glucose cotransporter-2 inhibitor (SGLT-2i) use and DCSI score. The same variables were identified by logistic regression in all the bootstrapped samples with the addition of race. The figure below shows the odds ratio (OR) of selecting GLP-1 RA vs BI as first injectable therapy.

Conclusion: There were relevant clinical and demographic differences between the two cohorts, suggesting GLP-1 RAs and BI are prescribed to different types of patients with T2D. Key predictors that favored the choice of a GLP-1 RA as first injectable were higher BMI and previous use of an SGLT-2i; predictors that favored the choice of initiating BI were worse glycemic control, older age, and higher disease burden.



Supported by: Eli Lilly and Company fully supported this study
Disclosure: **M. Yu:** Employment/Consultancy; Eli Lilly and Company.

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Blockade of glucagon-like peptide-1 receptors by exendin(9-39) attenuates the increase in heart rate during small intestinal glucose infusion in type 2 diabetes

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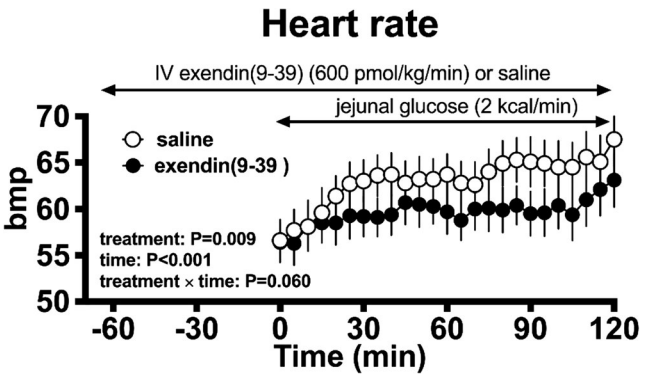
Background and aims: Glucagon-like peptide-1 (GLP-1) and its mimetics have been shown to increase heart rate (HR) in both health and type 2 diabetes. However, it remains unclear whether this effect is attributable to GLP-1 receptor signaling and, if so, whether endogenous GLP-1 modulates cardiovascular function through this pathway, particularly during the postprandial phase. Given that postprandial GLP-1 secretion is determined in part by the rate of gastric emptying, which varies substantially between individuals and is also modulated by GLP-1, we evaluated theHR and blood pressure (BP) responses to a standardised jejunal glucose infusion, in the absence and presence of the GLP-1 receptor antagonist, exendin(9-39), in patients with type 2 diabetes.
Materials and methods: 10 patients with type 2 diabetes (age 69.5 ± 2.9 years; 8 male; BMI 26.6 ± 0.9 kg/m^2; HbA1c 6.6 ± 2.2% (48.9 ± 2.3 mmol/mol); duration of known diabetes 13.6 ± 3.4 years), managed by diet or metformin alone, were each studied on two days, separated by at least 7 days, in a double-blind, randomised fashion. On each study day, a nasal-jejunal catheter was positioned with an infusion port located 50 cm below the pylorus. An intravenous (IV) infusion of exendin(9-39) (600 pmol/kg/min) or 0.9% saline was commenced 60 min before, and maintained during, a 120-min intrajejunal glucose infusion (2 kcal/min). HR and BP were measured every 5 min, using an automated device. On one day, autonomic function was assessed using standardised cardiovascular reflex tests.
Results: None had cardiovascular autonomic dysfunction. Prior to jejunal glucose infusion, neither HR (IV saline: 57 ± 3 bpm vs. IV exendin(9-39): 56 ± 2 bpm) nor BP (130 ± 7 and 71 ± 2 mmHg vs. 132 ± 6 and 73 ± 3 mmHg for systolic and diastolic BP, respectively) differed between the two study days. During jejunal glucose infusion, HR increased on both days, and the magnitude of increase was less with IV exendin(9-39) compared with IV saline (treatment effect: P = 0.009). Systolic and diastolic BP both decreased slightly during jejunal glucose infusion, without any difference between the two study days.
Conclusion: In relatively well controlled, normotensive patients with type 2 diabetes, without autonomic dysfunction, blockade of GLP-1 receptors by exendin(9-39) attenuates the HR response to small intestinal glucose infusion. These observations are indicative of a physiological role of GLP-1 receptor signalling in the regulation of postprandial cardiovascular function in type 2 diabetes.



Clinical Trial Registration Number: ACTRN12615001239550
Supported by: Australian NHMRC project grant APP1066815
Disclosure: **C.K. Rayner:** Grants; NHMRC project grant APP1066815.

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A calculator to predict durability of HbA_{1c} response with DPP4 inhibitors, sulfonylureas and thiazolidinediones: a MASTERMIND precision medicine study

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Background and aims: Precision diabetes aims to target patients with the most effective treatment based on their characteristics. Common second-line type 2 diabetes therapies are DPP4 inhibitors (DPP4i, 27% of current UK second/third line oral prescriptions), sulfonylureas (SU, 51% of prescriptions) and thiazolidinediones (TZD, 8% of prescriptions), but there is little guidance as to which therapy is best for which individuals. We aimed to develop a calculator to predict individual durability of good glycaemic control for these therapies based on routinely measured clinical characteristics.
Materials and methods: To measure glycaemic durability we developed time to glycaemic failure survival models for each therapy in UK primary care data (Clinical Practice Research Datalink (CPRD), n=102,830). Failure was defined as time to a patient reaching a confirmed HbA1c threshold of 8.5%. For SU and TZD we validated survival models in individual participant data from two head-to-head randomised trials (ADOPT n=2,725, RECORD n=2,222). For DPP4i we replicated associations between clinical characteristics and HbA1c in a prospective study (PRIBA, n=254), in which change in HbA1c at 6 months was the outcome.
Results: Lower baseline BMI was associated with greater glycaemic durability with DPP4i and SU (both p<0.001), but shorter glycaemic durability with TZD (p<0.001). With SU, males maintained good glycaemic control for longer compared to females (p<0.001), whilst females had greater glycaemic durability with TZD over males (p<0.001). There was no association between sex and glycaemic control with DPP4i (p=0.44). For all therapies, lower baseline HbA1c (p<0.001) and older age (p<0.001 were associated with greater glycaemic durability but to different degrees. Model discrimination using these characteristics was good for each therapy model (C-statistic DPP4i 0.72, SU 0.70, TZD 0.76). SU and TZD survival models validated well in external trial data (ADOPT: C-statistic 0.68 SU, 0.73 TZD) and consistent predictors of DPP4i HbA1c response were identified in the prospective PRIBA study. Findings translate into significant differences in glycaemic durability between therapies: If a female age 65 years with a starting HbA1c of 8.5% and a BMI of 35kg/m^2 remains on stable therapy the likelihood of good control (HbA1c < 8.5%) at 3 years is greatest at 84.5% (95%CI 84-85%) with TZD compared to DPP4i (66%, 95%CI 65-67%) and SU (68.5%, 95%CI 68-69%).
Conclusion: A simple calculator can predict glycaemic durability with common second-line therapies. Extending our calculator to encompass other second-line therapies and drug-specific side effects could help clinicians adopt a more personalised approach to type 2 diabetes therapy.
Supported by: MRC (MR/N00633X/1)
Disclosure: **J.M. Dennis:** None.

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Influence of breakfast on clock gene and AMPK mRNA expression and postprandial glycaemia in healthy and type 2 diabetes

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University of Jerusalem, [5]Department of Molecular Genetics, Weizmann Institute of Science, Weizmann Institute, Israel.

Background and aims: The circadian clock regulates glucose metabolism by mediating the activity of metabolic enzymes, hormones, and transport systems. Meal timing not aligned with the circadian clock i.e. breakfast skipping and/or eating at hours assigned to sleep, have been associated with high HbA1C and postprandial hyperglycemia after lunch and dinner. Our aim was to explore the effect of breakfast consumption or omission on glucose homeostasis and clock gene, Ampk, and Sirt1 mRNA expression in healthy and type 2 diabetes individuals.
Materials and methods: In a crossover design, 18 healthy and 18 T2D volunteers with 14.5±1.5yr diabetes, BMI 30.7±1.1 kg/m2 and HbA1C: 7.6 ± 0.1 % were randomly assigned to a test day with breakfast and lunch (YesB) and a test day with only lunch (NoB). Postprandial clock genes (Clock, Bmal1, Per1, Per2, Cry1, Rev-erbα, Rorα) Ampk and Sirt1 mRNA expression, in white blood cells (WBC), and plasma glucose, insulin, intact glucagon-like peptide-1 (iGLP-1) and dipeptidyl peptidase IV (DPP-IV) plasma activity were assessed after breakfast and lunch.
Results: In healthy people, YesB day led to 26% upregulation of Bmal1, the positive loop of clock gene expression ($p<0.005$), to 34-43% increase of Ampk and Sirt1 ($p<0.005$), and to 30% downregulation of negative feedback loop Per2 after lunch ($p<0.05$). In the diabetic group, YesB day led to 25-42% upregulation of Bmal1, Ampk, Per1, Cry1 and Rev-erbα ($p<0.005$). Breakfast skipping (NoB day) in healthy and type 2 diabetic individuals, led to downregulation of Bmal1 and Ampk ($p<0.005$) and it was associated with ~15% elevation of AUC of glucose response after lunch and impaired insulin and iGLP-1 responses to lunch ($p<0.0001$).
Conclusion: Breakfast skipping adversely affects the expression of clock, clock-related genes and Ampk mRNA expression after lunch and was correlated with reduced insulin and iGLP-1 and increased postprandial glycemic response after lunch in both healthy and diabetic individuals. These results suggest that intake of breakfast is important for maintaining the clock gene regulation of the overall glucose metabolism both in healthy and in type 2 diabetes.
Clinical Trial Registration Number: NCT01939782
Supported by: D-Cure and Ministry of Health, Israel
Disclosure: **D. Jakubowicz:** None.

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Effect of meal frequency on glucose and insulin responses in obese people with impaired glucose tolerance and with type 2 diabetes: a randomised trial

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Background and aims: Recent studies have shown that lifestyle intervention in prediabetes reduces the progression to type 2 diabetes (DM2). However, the impact of meal frequency on glucose metabolism remains unknown. The aim of the study was to compare the effects of two isocaloric meal patterns (three- vs six meals per day) on glucose and insulin levels during an oran glucose tolerance test (OGTT) in subjects with: 1) impaired glucose tolerance (Group-A, 2-hour-blood-glucose: 140-199mg/dL), 2) type 2 diabetes (Group-B) and 3) with 2-hour-blood-glucose: 140-199mg/dl and/or blood-glucose >200mg/dL at 30 or 60 or 90min (Group-C).
Materials and methods: In a randomized, crossover, 24-weeks study, 2x2 factorial design, Group-A (n=15, aged 44(4)y, body mass index (BMI) 33(1)kg/m2), Group-B (n=20, aged 52(3)y, BMI 33(1)kg/m2) and Group-C (n=12, aged 52(4)y, BMI 32(2)kg/m2), followed a weight maintenance diet (%carbohydrates:protein:fat:45:25:35), consumed either as a three-or a six-meal pattern, for 12 weeks. Antropometric measurements, diet compliance and subjective hunger, satiety and desire to eat were assessed biweekly. All subjects underwent a 75g glucose-OGTT for measurements of blood glucose and insulin (every 30min for 120min) at the beginning and end of each intervention. HaemoglobinA1c (HbA1c), blood lipids and hepatic enzymes were measured at the beginning and end of each intervention.
Results: Body weight remained stable throughout the study. HbA1c, 2-hour postprandial blood glucose and insulin resistance (HOMA-IR) were decreased with 6-meals vs 3-meals in Group-B ($p=0.006$, $p=0.01$ and $p=0.049$, respectively). Time to peak for blood glucose was decreased with 6-meals vs 3-meals in Group-C ($p=0.046$). Subjective hunger and desire to eat were significantly lower after 6-meals vs 3-meals in all groups ($p=0.03$ and $p=0.029$, respectively). No differences were found before and after the 3-and-6-meal patterns for fasting glucose, fasting insulin, iAUC for glucose, iAUC for insulin, blood lipids and hepatic enzymes (p for all>0.05).
Conclusion: The six-meal pattern improved glycemic control in people with DM2 compared to isocaloric three-meals. The six-meal pattern decreased subjective hunger and desire to eat in all groups compared to isocaloric three-meals. As a result, increased meal frequency may be an important component of medical nutrition therapy in people with impaired glucose metabolism.
Clinical Trial Registration Number: NCT02248272
Disclosure: **E. Papakonstantinou:** None.

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Caffeine consumption and mortality in diabetes: an analysis of NHANES 1999-2010

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Background and aims: An inverse relation between coffee consumption and mortality has been reported in the general population. Coffee consumption has also been associated with a risk reduction for the development of type 2 diabetes. However, the association of caffeine consumption with mortality of patients with diabetes remains unclear.
Materials and methods: We examined the association of caffeine consumption with mortality among 1,568 women and 1,484 men with diabetes in a prospective nationwide cohort, using the continuous National Health and Nutrition Examination Survey (NHANES) 1999-2010. Caffeine consumption was assessed at baseline using 24h dietary recalls. Cox proportional hazard models were fitted to estimate hazard ratios (HR) for all-cause, cardiovascular and cancer-related mortality among women and men according to caffeine consumption and its source (coffee, tea or soft drinks), adjusting for potential confounders (age, race, education level, annual family income, smoking status, body mass index, daily carbohydrate consumption, alcohol consumption, years since diabetes diagnosis, hypertension, diabetic kidney disease, retinopathy, macrovascular complications and insulin treatment).
Results: A dose-dependent inverse association between caffeine consumption and total mortality was observed in women with diabetes

(p=0.002). Adjusted HR for death among women who consumed caffeine, as compared with women who did not, were: 0.49 (95% confidence interval [CI], 0.33-0.74) for less than 100mg of caffeine per day, 0.43 (95% CI, 0.26-0.70) for 100 to <200mg of caffeine, and 0.34 (95% CI, 0.20-0.57) for 200mg or more of caffeine per day (p=0.007). This association was not observed in men with diabetes (p=0.887). There was no significant association between total caffeine consumption and deaths from cardiovascular diseases or cancer, both in men and women. Regarding the source of caffeine consumption, women with diabetes who consumed more caffeine from coffee had reduced risk of all-cause (p=0.007) and cardiovascular death (p=0.041). Women who consumed more caffeine from tea had reduced mortality from cancer (p=0.009). No associations between source of caffeine consumption and all-cause, cardiovascular or cancer mortality were observed in men.

Conclusion: Our study showed a dose-dependent protective effect of caffeine consumption on all-cause mortality among women. The effect on mortality appears to depend on the source of caffeine, with a protective effect of coffee consumption on all-cause mortality and cardiovascular mortality, and a protective effect of caffeine from tea on cancer mortality among women with diabetes.

Disclosure: **J.S. Neves:** None.

PS 066 Novel approaches to glucose-lowering

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Efficacy and safety of diacerein in patients with inadequatedly controlled type 2 diabetes: a randomised controlled trial

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Background and aims: Diacerein is an immune-modulator anti-inflammatory drug with potential beneficial actions in type 2 diabetes. The objective was to assess the efficacy and safety of a 1-year diacerein treatment in improving glycemic control of patients with inadequately-controlled type 2 diabetes.

Materials and methods: It was a randomized, double-blinded, placebo-controlled trial performed on a tertiary-care university hospital. 512 patients with type 2 diabetes were screened, 142 with HbA_{1c} between 7.5% and 9.5% entered an 8-week run-in phase, and 84 were randomized to a 48-week treatment with placebo (41 patients) or diacerein 100 mg/day (43 patients).The primary outcome was the difference in mean changes of HbA_{1c} between treatment groups. Secondary outcomes were other efficacy and safety measurements (fasting glycemia, serum lipids, renal and hepatic function, hematological- inflammatory indices, weight and blood pressures). A general linear regression with repeated-measurement variables, adjusted for age, sex, diabetes duration and each baseline value, estimated the differences between groups. Both intention-to-treat and per-protocol analysis, excluding 10 patients who interrupted treatment (8 diacerein and 2 placebo), were performed.

Results: Mean baseline HbA_{1c} was 8.2% in both groups. Diacerein reduced HbA_{1c} in contrast to placebo by 0.35% (95% CI: -0.68 to -0.02; p=0.038) in intention-to-treat and by 0.41% (95% CI: -0.75 to -0.06%; p=0.023) in per-protocol analysis. The peak of diacerein effect occurred at the 24^{th}-week of treatment (-0.61%; p=0.014 and -0.71%; p=0.005, respectively), but it attenuated towards non-significant differences at the 48^{th}-week. No significant effect of diacerein was observed in other efficacy and safety measures. Diarrhea occurred in 65% of the patients using diacerein and caused treatment interruption in 16%. Mild hypoglycemic events were equally observed in both groups; however, more patients in the diacerein group reduced baseline anti-diabetic medication and more patients in the placebo group increased it during treatment.

Conclusion: Diacerein reduced overall mean HbA_{1c} levels, with peak of effect at the 24^{th}-week of treatment, but with attenuation towards the 48^{th}-week. The drug was well tolerated, without any serious adverse events, except for diarrhea; and it may be indicated as adjunct treatment in patients with type 2 diabetes, particularly in those with concomitant symptomatic osteoarthritis.

Clinical Trial Registration Number: NCT02242149
Supported by: CNPq Brazil; FAPERJ, Brazil

Disclosure: **C.R.L. Cardoso:** Grants; grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) and from the Fundação Carlos Chagas Filho de Amparo a Pesquisa do Estado do Rio de Janeiro (FAPERJ, Brazil).

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Imeglimin monotherapy in Japanese patients with type 2 diabetes: results from a randomised, 24-week, double-blind, placebo-controlled, phase IIb trial

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Background and aims: Imeglimin is the first in a novel class of glucose-lowering drugs. Imeglimin is acting on the two key defects in type 2 diabetes mellitus (T2DM) improving both insulin secretion in response to glucose and insulin sensitivity, through a unique mechanism of action targeting mitochondria.
This study was designed to determine the efficacy and safety/tolerability of imeglimin monotherapy at 3 doses compared to placebo in Japanese patients with T2DM after 24 weeks of treatment.
Materials and methods: In this randomized, double-blind, placebo-controlled dose ranging trial, Japanese adults with T2DM were randomized 1:1:1:1, using a web-based system, to twice daily oral imeglimin 500 mg, 1000 mg, 1500 mg or matched placebo. Randomization was stratified according to baseline glycosylated hemoglobin (HbA1c) and to previous andiabetic treatment status (naïve versus previously treated patients). All patients went through a wash-out/run-in period of 6 to 10 weeks before randomization. The primary efficacy endpoint was the placebo-adjusted dose-dependent reduction in HbA1c from baseline after 24 weeks of treatment, evaluated in all randomized treated patients with at least one post-baseline assessment. Secondary endpoints included changes in fasting plasma glucose, insulin, C-peptide concentrations, percentage of responders (HbA1c < 7 %) and patients requiring rescue therapy as well as safety and tolerability. Pre-dose PK was assessed at each visit and within 6 hours post-dose after 4 weeks of treatment.
Results: The clinical part of the study is completed. 299 patients were randomized. At baseline, preliminary results showed a ratio of 67% men, a mean age of 59.8 ± 9.7 years and mean body mass index of 25.5 ± 4.4 kg/m². Mean baseline HbA1c was 7.9 ± 0.7 %. Ninety % (268) of the patients completed the study up to 24 weeks. No suspected unexpected serious adverse reaction (SUSAR) were reported during the course of the study.
Conclusion: Full results will be available Q2 2017. This study will be presented in its integrality at the time of the congress.
Clinical Trial Registration Number: JapicCTI-153086
Disclosure: **J. Dubourg:** Employment/Consultancy; Poxel S.A.

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The dual amylin and calcitonin agonist KBP-042 is superior to both amylin and calcitonin monotherapy and able to override amylin resistance

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Background and aims: Amylin agonists induce weight loss and improve post-prandial glucose regulation, and the amylin analogue Pramlintide is approved as treatment of diabetes as a supplement to insulin; however, amylin lacks potency and has a very short-lived activity *in vivo*. Dual amylin and calcitonin receptor agonists (DACRAs), such as KBP-042, are novel candidates for treatment of T2D and obesity due to their beneficial effects on body weight, blood glucose and insulin sensitivity, effects that are superior to those of amylin. However, it is unclear whether the dual agonism has beneficial effects on metabolism, independent of the amylin receptor mediated effects. Here we compared the effects of the dual agonist KBP-042 to effects of the selective receptor agonists amylin and calcitonin on regulation of body weight, blood glucose and insulin sensitivity. In addition, we evaluated KBP-042 mediated effects following initial amylin therapy.
Materials and methods: High-fat fed Sprague Dawley rats were treated 4 weeks by subcutaneous continuous infusion with either dual agonist KBP-042 (5 μg/kg/d), rat amylin (300 or 1000 μg/kg/d), rat calcitonin (300 or 1000 μg/kg/d) or vehicle. In addition a group received KBP-042 (5 μg/kg) by a daily subcutaneous injection. After 4 weeks of treatment, all treatment groups were switched to receive KBP-042 (5 μg/kg/d) for 10 days. Food intake and body weight were monitored throughout the study, while glucose tolerance, insulin secretion and gastric emptying were assessed before the treatment switch.
Results: KBP-042 induced a large weight reduction in the initial phase of the study, then the body weight stabilized and a sustained reduction in body weight was obtained (~15% vehicle corrected, $p<0.01$) independent of delivery route. Initially, both doses of amylin had a marked effect on body weight while calcitonin only had a slight effect (ns), however, the high dose of amylin (1000 μg/kg/d) lost efficacy compared to the low dose. All treatments significantly reduced food intake in the initial phase of the study, KBP-042 and amylin being superior to calcitonin. Calcitonin and the high dose of amylin lost the effect on food intake within few days of treatment, corresponding to the observed regain in body weight. KBP-042 and the lower dose of amylin (300 μg/kg/d) showed a prolonged significant reduction in food intake compared to vehicle. Moreover, KBP-042 and low dose amylin improved oral glucose tolerance, although only KBP-042 did so significantly ($p<0.05$). In addition, KBP-042 reduced the rate of gastric emptying to 59.6 % of vehicle. Switching treatment to KBP-042 induced a transient drop in food intake and a sustained reduction in body weight, in all groups not pretreated with KBP-042. KBP-042 even rescued the reduced amylin response and induced a full response on body weight despite amylin resistance.
Conclusion: In conclusion, the dual agonist KBP-042 had beneficial effects on body weight, appetite, gastric emptying and insulin sensitivity superior to activating either the amylin or the calcitonin receptor alone. Infusion of a high dose of amylin lost efficacy, indicating amylin resistance, an effect not seen with the more efficacious dose of KBP-042. Interestingly, KBP-042 was able to induce a full response on body weight independent on the former treatment and even despite amylin resistance.
Supported by: The Danish Research Foundation (Den Danske Forskningsfond).
Disclosure: **A.T. Larsen:** None.

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The MetAP2 inhibitor ZGN-1061 improves glycaemia in high fat diet-induced obese mice

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Background and aims: Methionine aminopeptidase 2 inhibitors (MetAP2i) are a promising new therapeutic approach for the treatment of diabetes. Beloranib is a prototype MetAP2i which, when tested in obese type 2 diabetes subjects, resulted in 2.0% reduction in HbA1c and 13% body weight loss from baseline following 26 weeks of treatment. A novel MetAP2i, ZGN-1061, is in Phase 1 clinical testing. ZGN-1061 is a potent and selective inhibitor of MetAP2 enzyme activity and shows similar effects on obesity as beloranib in animal models, but has an improved safety profile in model systems of thrombotic risk. The aim of this study was to evaluate the anti-diabetic potential of ZGN-1061 in diet-induced obese (DIO) and relate these changes to weight loss and change of body composition.
Materials and methods: Male C57BL/6J mice were placed on a high fat diet (45% kcal as fat) for 34 weeks then were treated with ZGN-1061 at doses of 0.03, 0.1 and 0.3 mg/kg (SC, QD) or beloranib at 0.1 mg/kg (SC, QD) for 4 weeks (n=10/group). Body weight, food intake, DEXA body composition and oral glucose tolerance test (OGTT) were assessed.
Results: ZGN-1061 dose dependently reduced body weight by 4.4%, 17.0%, and 29.5% after 29 days of treatment. In comparison, beloranib achieved 31.5% weight loss. Mice in the 0.3 mg/kg 1061 and 0.1 mg/kg beloranib dose groups were still losing weight over the final week of the study suggesting dosing longer than one month could show greater weight loss. Energy intake (kJ/gram lean mass) was transiently reduced in the two higher ZGN-1061 dose groups as well as the beloranib group by 23%, 28% and 31% at day 16, respectively. However, by the end of the study energy intake was no different from vehicle in all drug treated groups. At day 27 body composition as measured by DEXA show ZGN-1061 reduced the % fat and increased the % lean mass at both the mid and high dose groups with no change in the low dose group. On day 30 an OGTT was performed. Baseline fasted glucose was reduced relative to vehicle by 12.3%, 16.4% and 27.1% in the ZGN-1061 dose response. Beloranib had a 23.7% reduction of fasted glucose. Oral glucose tolerance

was improved with treatment as shown by 12.0%, 27.2% and 33.2% reductions of the glucose AUC 0-120 and 25.4%, 44.4% and 60.1% reductions of insulin AUC 0-120 across the ZGN-1061 doses. Calculation of HOMA-IR reveal a marked drug effect to reduce the insulin resistance index at all dose levels of ZGN-1061, where the lowest dose improved HOMA-IR by 40.3% and the highest dose by 81.2%.

Conclusion: The MetAP2i ZGN-1061 dose-dependently improves glycemic control and insulin sensitivity in DIO mice. Weight loss at the higher doses likely contributes to the improvement of glucose control, however, glucose was reduced even at the lowest dose which was weight neutral and had no effect on fat mass after one month of dosing.

Group	Dose (mg/kg)	BW (g)	% Fat mass	% Lean mass	Glucose (mM)	Glucose AUC 0-120	Insulin (ng/ml)	Insulin AUC 0-120
DIO Vehicle	0	50.2	43.2	56.8	12.1	37.9	2.63	11.3
DIO ZGN-1061	0.03	48.1 a	42.5	57.5	10.6 a	33.4 a	1.79 c	8.4 b
DIO ZGN-1061	0.1	42.0 c	37.6 c	62.4 c	10.1 b	27.6 c	1.37 c	6.3 c
DIO ZGN-1061	0.3	36.0 c	27.5 c	72.5 c	8.8 c	25.4 c	0.68 c	4.5 c
DIO beloranib	0.1	35.1 c	25.9 c	74.1 c	9.2 c	25.8 c	1.27 c	5.4 c
Lean C57BL/J6	0	32.1 c	22.8 c	77.2 c	10.8	33.3 a	0.72 c	2.6 c

DEXA body composition from day 27; Body weights (BW) from day 29; OGTT glucose and insulin from day 30: a, p<0.05; b, p<0.01, c, p<0.001

Disclosure: **B.F. Burkey:** Employment/Consultancy; Zafgen.

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A novel GPR40 agonist HD-6277 comparison to Fasiglifam and LY2922470 in type 2 diabetic rats

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Background and aims: G protein-coupled receptor 40/free fatty acid receptor 1 (GPR40/FFA1), is highly expressed in pancreatic β-cells and mediates free fatty acid-induced insulin secretion. Recently, new drugs, i.e., agonists of GPR40, have been used for preventing and treating type 2 diabetes mellitus (T2DM). HD-6277 is one such novel oral agonist of GPR40 under development as a once-a-day drug for the treatment of T2DM. Here, we studied the pharmacological effect of HD-6277 and compared the glucose-lowering effects with fasiglifam (TAK-875), a selective, potent, and orally bioavailable GPR40 agonist terminated in phase III clinical trials. Additionally, we compared the pharmacological potency between HD-6277, fasiglifam, and LY2922470.

Materials and methods: hGPR40-CHO, INS-1, and NCI-H716 cell lines were used to assess the effects of HD-6277 in vitro. The in vivo effect of HD-6277 was examined in Sprague-Dawley (SD) rats, Zucker Diabetic Fatty (ZDF) rats and Zucker Fatty (ZF) rats, using an oral glucose tolerance test (OGTT) and an insulin tolerance test (ITT). We also measured the GLP-1 levels on cynomolgus monkeys.

Results: HD-6277 showed more effective in vitro profile than fasiglifam in calcium influx assay (EC_{50} = 13 nM) and IP accumulation assay (EC_{50} = 7.5 nM). In comparison of pharmacological effects between HD-6277, fasiglifam and LY2922470, HD-6277 increased glucose-stimulated insulin secretion (GSIS) in INS-1 cells and SD rats. In fasted SD rats, while glibenclamide (10 mg/kg, p.o.) induced hypoglycemia, HD-6277 (10-100 mg/kg, p.o.) neither caused hypoglycemia nor enhanced insulin secretion. HD-6277 increased GLP-1 secretion in in vitro and in vivo, whereas fasiglifam did not. Then we evaluated the efficacy of HD-6277 in type 2 diabetic rats. In subchronic study with male ZDF rats, HD-6277 improved glycemic control (OGTT) and increased GLP-1 and insulin secretion. In male ZF rats, an oral administrations for 10~11 weeks, HD-6277 improved insulin sensitivity (ITT) and HbA_{1c} level. The impaired insulin action was recovered by HD-6277 treatment in liver and adipose from the rats. In the cynomolgus monkey study, HD-6277 also showed a significant increase in GLP-1 secretion (P<0.05).

Conclusion: The pharmacological potency of HD-6277 was higher than fasiglifam and similar to LY2922470. HD-6277 improved glycemic control in type 2 diabetic rats, which was related to enhancement of insulin signaling and GLP-1 secretion. Therefore, we propose HD-6277 as an effective candidate for the treatment of T2DM.

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Disclosure: **C. Kim:** None.

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Efficacy and safety of DS-8500a, a GPR119 agonist, in Japanese patients with type 2 diabetes: a randomised, double-blind, placebo-controlled, 12-week study

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Background and aims: G-protein coupled receptor 119 (GPR119) is a promising target for the treatment of type 2 diabetes mellitus (T2DM). Pancreatic β-cells and intestinal L cells express GPR119, enabling a single drug to promote both insulin and GLP-1 secretion. In this study, the efficacy and safety of 12-week treatment with the GPR119 agonist DS-8500a was compared with placebo and sitagliptin 50 mg in Japanese patients with T2DM.

Materials and methods: We conducted a randomized, double-blind, parallel-group Phase 2b comparison study across 25 sites in Japan. In total, 368 Japanese patients aged ≥20 years with T2DM and HbA1c levels ≥7.0% and <10.0% were randomized to receive placebo, DS-8500a (25, 50, or 75 mg once daily), or sitagliptin 50 mg once daily for 12 weeks. The primary efficacy endpoint was change in HbA1c from baseline to week 12. Secondary endpoints included change in fasting plasma glucose (FPG), $AUC_{0\text{-}3h}$ for glucose during a meal tolerance test (MTT), 2-hour postprandial glucose (2hr-PPG), and change in lipid parameters (total, LDL- and HDL-cholesterol; triglycerides) from baseline to week 12. Safety endpoints included adverse events, occurrence of hypoglycemia, and clinical and laboratory variables.

Results: Patient demographics did not differ significantly across the groups. DS-8500a demonstrated dose-dependent lowering of HbA1c compared with placebo at week 12 (25 mg, −0.21% [p=0.0233]; 50 mg, −0.34% [p=0.0004]; 75 mg, −0.45% [p<0.0001]). DS-8500a 50 mg and 75 mg also demonstrated significant lowering effects on FPG compared with placebo (50 mg, −12.7 mg/dL [p=0.0004]; 75 mg −14.4 mg/dL [p<0.0001]), as well as glucose $AUC_{0\text{-}3h}$ (50 mg, −50.6 mg/dL×h [p=0.0020]; 75 mg, −59.7 mg/dL×h [p=0.0003]) and 2hr-PPG (50 mg, −18.5 mg/dL [p=0.0102]; 75 mg, −22.0 mg/dL [p=0.0022]). Although the glucose-lowering effects of other GPR119 agonists are typically abrogated after 2 to 4 weeks of treatment, the decrease in FPG by DS-8500a administration was maintained over time up to 12 weeks. However, DS-8500a (at any dose) did not lower any of the above parameters to a greater extent than sitagliptin. Compared with placebo and sitagliptin, DS-8500a 50 and 75 mg significantly reduced total cholesterol (−7.0% [p<0.0001], −6.2% [p=0.0002]), LDL-cholesterol (−7.3% [p=0.0044], −8.1% [p=0.0014]), and triglycerides (−32.7% [p<0.0001], −30.9% [p<0.0001]), and significantly increased HDL-cholesterol (5.9%, [p=0.0032], 6.6%,[p=0.0009], respectively). All doses of DS-8500a were well tolerated. Two cases of clinically relevant drug-related hypoglycemia occurred in the DS-8500a 50 mg group.

Conclusion: DS-8500a was well tolerated, and demonstrated significant glucose-lowering effects and favorable changes in lipid profile up to 12 weeks compared with placebo in Japanese patients with T2DM.

Clinical Trial Registration Number: NCT02628392
Supported by: Daiichi Sankyo Co., Ltd.

Disclosure: **Y. Yamada:** Other; Daiichi Sankyo Co., Ltd., Kyowa Hakko Kirin Co., Ltd., Ono Pharmaceutical Co., Ltd., Mitsubishi Tanabe Pharma Corporation, Taisho Toyama Pharmaceutical Co., Ltd., Takeda Pharmaceutical Co. Ltd., Teijin Pharma Ltd., Nippon Boehringer Ingelheim Co., Ltd., Novartis Pharma K.K., Novo Nordisk Pharma Ltd., MSD K.K., Dainippon Sumitomo Pharma Co., Ltd., Kowa Pharmaceutical Company Ltd., Sanofi K.K.

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GLP-1 receptor variants markedly differentiate glycaemic response to GLP-1 receptor agonists: a DIRECT study

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Background and aims: Glycaemic response to GLP-1 Receptor Agonist (GLP-1RA) treatment varies markedly among patients with Type 2 Diabetes (T2D) yet the mechanism for this variation is uncertain and only short-term glycaemic response can predict who will have a marked mid-long-term response and who will respond less. Common missense variants in the GLP-1R have previously been reported to alter GLP-1 mediated insulin secretion in non-diabetic individuals. We aimed to investigate how GLP-1R variants alter glycaemic response to the GLP-1RA.
Materials and methods: We performed a meta-analysis across the DIRECT, PRIBA and GoDARTS cohorts. A total of 1156 T2D subjects were followed-up for 6 months after initiation of GLP-1RA treatment. Patients were treated predominantly with Exenatide or Liraglutide. The association of GLP-1R variants, rs6923761 (Gly168Ser) and rs10305420 (Pro7Leu), with reduction in glycated haemoglobin (HbA1c) after treatment were assessed using multiple linear regression in an additive model.
Results: Gly168Ser and Pro7Leu variants in the GLP-1R were independently associated with reduced efficacy of GLP-1RA to lower HbA1c (Gly168Ser β (HbA1c change per allele) = -0.18%, p = 0.001, Pro7Leu β = -0.14%, p = 0.01). We then derived a genetic risk score, summing up these two variants. The 22% of the population who carry no risk allele in either of the variants had a mean HbA1c reduction of 1.39% [1.21-1.58] (15.2 mmol/mol [13.2-17.3]) in response to GLP-1RA. In contrast the 17% percent of the population who carry 3 or more risk alleles had a much lower glycaemic response to GLP1RA treatment 0.86% [0.66-1.13] (9.4 mmol/mol [7.0 - 11.9]), a difference of 0.53% [5.8mmol/mol] ($p < 0.001$). There was no significant impact of these SNPs on weight change in response to GLP-1RA suggesting the variants impact on the glycaemic lowering mechanisms of GLP-1RA rather than weight lowering mechanisms.
Conclusion: Missense variants in the GLP-1R have a large clinical impact on glycaemic response to GLP-1RA. The frequencies of these variants are not rare, making this the largest, most common pharmacogenetic effect described to date in T2D.
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Disclosure: **A.Y. Dawed:** Grants; DIabetes REseraCh on patient straTification (DIRECT) has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement n° 115317.

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The changing landscape of diabetes drug therapy in a nationwide population of people with type 2 diabetes

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Background and aims: Recent years have seen the availability of several new drug classes for treating type 2 diabetes (T2DM) as well as recommendations for earlier intensification of therapy. Here in the total population of Scotland with T2DM, we examined the change over the past 10 years in prevalent treatment intensity as proxied by numbers of diabetes drugs. We also report the current class-specific usage of diabetes drugs.
Materials and methods: Using the Scottish Care Information Diabetes national registry, which covers the entire Scottish population with T2DM, we describe the prevalence of issued prescriptions for diabetic drug usage in two periods: mid-2005 to mid-2006, and mid-2015 to mid-2016. Differences in proportions were tested by direct comparison of the age-standardised estimates and their 95% CIs.
Results: Table 1 shows the prevalence of numbers of drugs being used in the two time periods. The age-standardised prevalence of intensive therapy (3+ non-insulin drugs or insulin) increased from 13.77% (95% CI 13.59 - 13.94) to 18.11% (17.95 - 18.28). Among those who initiated intensive therapy during the given year, the average duration of diabetes by time of initiation was slightly longer in 2015/16 than 2005/6. In both periods examined, the majority of patients were receiving monotherapy or dual therapy, largely comprising metformin and sulphonylureas. In 2005/6, metformin, sulphonylureas, and insulin comprised 45.08%, 29.27%, and 13.40% of all prescriptions, respectively. In 2015/16, metformin, sulphonylureas, and insulin comprised 46.43%, 22.07%, and 11.56% of all prescriptions, respectively. Among the remaining prescriptions, the next most prevalent classes were dipeptidyl peptidase-4 (DPP-IV) inhibitors (9.75%), thiazolidinediones (4.04%), sodium-glucose co-transporter-2 (SGLT2) inhibitors (3.23%), glucagon-like peptide 1 (GLP-1) receptor agonists (2.80%), and other (0.12%). Of those who initiated a non-metformin, non-sulphonylurea, non-insulin drug within 2015/16 (n=19655), 6.44%, 39.77% and 38.88% of users had been treated previously in this period with 0, 1 or 2 drugs before their initiation, respectively.
Conclusion: Over the past 10 years, as new drug classes have become available, there has been an increase in the prevalence of intensive therapy of diabetes. However, the duration of diabetes at which intensification occurs has not reduced. New drug classes are largely being used as second and third line treatments.

	2005/6	2015/16
N	173565	263843
0 drugs	35.52%	34.61%
1 non-insulin drug	31.06%	30.14%
2 non-insulin drugs	19.66%	17.14%
3+ non-insulin drugs	3.32%	8.20%
Insulin only	5.31%	3.66%
Insulin + other drug(s)	5.14%	6.25%
Mean, 95% CI of duration of diabetes at initiation of insulin (years)	12.050 (12.048, 12.053)	13.819 (13.817, 13.822)
Mean, 95% CI of duration of diabetes at initiation of intensive therapy (years)	10.647 (10.646, 10.648)	11.500 (11.499, 11.501)

Supported by: MRC
Disclosure: **L. Brierley:** None.

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Single and multiple dose evaluation of a novel MetAP2 inhibitor: results of a randomised, double-blind, placebo-controlled clinical trial

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Background and aims: Methionine aminopeptidase 2 (MetAP2) inhibitors reduce fat biosynthesis and increase fat oxidation and lipolysis, resulting in weight loss and reduced hunger. A MetAP2 inhibitor, beloranib, resulted in 13% weight loss and 2.0% reduction in A1C from baseline in midstage clinical trials but development was halted due to an imbalance of venous thromboembolic events in beloranib-treated subjects vs. placebo. Nonclinical studies of a second generation MetAP2 inhibitor, ZGN-1061, demonstrate rapid absorption and clearance of ZGN-1061, which is hypothesized to reduce exposure at endothelial cell targets and minimize prothrombotic effect.
Materials and methods: This first-in-human clinical trial of ZGN-1061 subcutaneous injection assessed safety, pharmacokinetics (PK), and preliminary efficacy. The clinical trial included a single ascending dose (SAD) phase in healthy subjects (BMI 23-<30 kg/m^2) and a multiple ascending dose (MAD) phase in subjects with BMI 27-40 kg/m^2. Target engagement was assessed using ZGN-1061 bound to MetAP2 and evidence of MetAP2 inhibition. Results of the completed SAD doses (0.2, 0.6, 1.2, 2.4, 3.6, and 4.8 mg) and the MAD doses (0.2, 0.6, and 1.8 mg twice weekly for 4 weeks) are reported.
Results: The SAD phase included 39 subjects (ZGN-1061 N=28, placebo N=11), primarily male with mean age 31 and BMI 26 kg/m^2. ZGN-1061 maximum plasma concentrations (C_{max}) increased linearly with dose and occurred within 30 minutes of dosing, while half-life was less than 1 hour, indicating rapid absorption and clearance. ZGN-1061 was well tolerated across the doses tested. The most common adverse events (AEs) were headache and procedural site irritation; no dose-related patterns were observed. There were no serious AEs and no evidence of venous thromboembolism. MetAP2 binding and evidence of MetAP2 inhibition increased with dose; maximal values were observed at doses ≥1.2 mg. The MAD phase included 29 subjects (ZGN-1061 N=22, placebo N=7), primarily male with mean age 40 and BMI 33 kg/m^2. PK results were consistent with SAD findings although C_{max}, overall exposure, MetAP2 binding, and MetAP2 inhibition were increased after repeated dosing. Safety observations were comparable to the SAD phase. Efficacy measures indicated trends for greater weight loss and favorable biomarker changes with ZGN-1061 relative to placebo.
Conclusion: Consistent with preclinical studies, ZGN-1061 was rapidly absorbed and cleared. Preliminary safety results indicate that ZGN-1061 was well tolerated with no safety signals or venous thromboembolic events over the range of doses tested. These results support the evaluation of ZGN-1061 in larger clinical trials of longer duration.
Clinical Trial Registration Number: 2016-001605-17
Disclosure: **J. Malloy:** Employment/Consultancy; Zafgen, Inc. Stock/Shareholding; Zafgen, Inc.

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Co-administration of tesofensine/metoprolol: improvement in heart rate with significant body weight reduction in overweight or obese subjects with type 2 diabetes

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Background and aims: Tesofensine (TESO) is a presynaptic reuptake inhibitor of the neurotransmitters noradrenaline, dopamine, and serotonin, suppressing appetite and reward centres in the brain and increasing energy expenditure. In earlier trials, TESO showed statistically and clinically meaningful weight reduction in obese subjects, but also increases in heart rate (HR) and blood pressure (BP) at therapeutically relevant doses. In this double-blind, randomized, placebo-controlled trial we examined the effect of co-administration of 0.5 mg TESO with 100 mg of the β1-selective beta-blocker metoprolol (MET) on HR, BP, body weight, tolerability and safety in overweight or obese subjects with type 2 diabetes (T2D) on metformin therapy with or without one additional anti-diabetic oral agent.
Materials and methods: Sixty subjects with T2D (39M/21F; mean ± SD age: 63 ± 6 yrs; weight: 96 ± 16 kg; HbA1c 7.7 ± 0.4%; HR: 66 ± 8 bpm; SBP: 134 ± 7 mmHg; DBP: 84 ± 5 mmHg), were randomly assigned to receive either TESO+MET or matching placebo (PLAC) once daily for 90 days. Fifty-eight subjects completed the study. HR over 24 hrs (primary endpoint) was measured by continuous telemetry and BP in regular intervals during an inpatient stay. Body weight and safety assessments were performed every 2 or 4 weeks, laboratory assessments at the baseline and at end of treatment (EOT). Least square mean differences were compared between treatments using repeated measures ANCOVA with baseline as covariate and treatment as fixed effect.
Results: TESO+MET led to a statistically significant reduction in mean 24-h HR by 3.8 bpm vs. PLAC, and a reduction in SBP and DBP which did not reach statistical significance. Also, a significant reduction in body weight, which exceeded the one observed with PLAC by 3.5 kg (table). TESO+MET was well tolerated. The most frequently reported adverse events were sweating, nausea, dry mouth and headache. There were no clinically significant findings in ECG, haematology or biochemistry. There was only one serious adverse event observed on the PLAC.
Conclusion: Addition of metoprolol to tesofensine fully mitigated the increases in HR/BP observed with tesofensine alone and still led to significant weight loss in subjects with T2D without any structured background weigh loss program. TESO+MET was well tolerated with no new or unexpected safety findings and may therefore offer a favourable benefit/risk profile in obese subjects with T2D.

	TESO		PLAC		Difference in change from baseline (95% CI)	p-value
	Baseline	EOT	Baseline	EOT		
24-h HR [bpm]	72 ± 7	68 ± 7	70 ± 8	70 ± 9	-3.8 (-6.4; -1.3)	0.0038
SBP [mmHg]	126 ± 8	123 ± 12	129 ± 9	127 ± 10	-3.1 (-7.5; 1.2)	0.152
DBP [mmHg]	76 ± 9	74 ± 9	77 ± 10	76 ± 10	-2.1 (-4.9; 0.7)	0.138
Body weight [kg]	99 ± 19	96 ± 20	94 ± 12	93 ± 13	-3.5 (-4.7; -2.3)	<.0001

Clinical Trial Registration Number: NCT02737891
Supported by: Saniona A/S
Disclosure: **R.V. Dvorak:** Employment/Consultancy; employee.

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Sustained improvement of type 2 diabetes in 250 hypogonadal men with and without testosterone therapy (TTh) for 9 years: real-life data from a registry study

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Background and aims: The prevalence of hypogonadism in men with T2DM can be as high as 50%. In an ongoing registry on effectiveness and safety of testosterone undecanoate injections (TU) started in 2004 in a urological setting, more than 1/3 of patients have T2DM.
Materials and methods: Of 696 men with testosterone deficiency (total testosterone ≤12.1 nmol/L), 250 (36%) have T2DM. T2DM had been diagnosed and is treated by their family physician. In the urology office, 133 men received TU 1000 mg/12 weeks (T-group), 117 had opted

against TTh and served as controls (CTRL). Most measurements were performed 1-4 times a year for up to 11 years. 8-year data are reported. Mean changes over time between groups were compared by mixed effects model for repeated measures with random effect for intercept and fixed effects for time, group and their interaction and adjusted for age, weight, waist circumference, fasting glucose, blood pressure and lipids to account for baseline differences between groups.

Results: Mean age (years): 61.8±5.4 (T-group), 64.9±4.3 (CTRL). Mean HbA_{1c} progressively decreased from 8.8±0.9 to 6.1±0.4% after 8 years in the T-group ($p<0.0001$). The decrease was statistically significant vs. previous year for all 8 years. In CTRL, mean HbA_{1c} increased from 7.5 ±0.5 to 8.2±0.5% ($p<0.0001$). The estimated adjusted difference between groups was -3.1% ($p<0.0001$). Fasting glucose decreased from 7.6±1.1 to 5.3±0.1 mmol/L ($p<0.0001$) in the T-group and slightly increased from 5.8±0.3 to 5.9±0.3 mmol/L in CTRL (NS). The estimated adjusted difference between groups was -0.8 mmol/L ($p<0.0001$). HOMA-IR was only available for the T-group. It decreased from 10.2±2.0 to 3.6±0.8 after 8 years ($p<0.0001$). The decrease was statistically significant vs. previous year for the first 3 years. Insulin in serum was only available for the T-group. It decreased from 29.6±4.2 to 15±3.4 mU/L ($p<0.0001$) with statistical significance vs. previous year for the first 4 years. At baseline 54 patients in the T-group were on insulin and received a mean dose of 32.4 ±12.1 units. The mean dose requirement declined to 19.7±11.0 ($p<0.0001$) with statistical significance vs. previous year for the first 4 years. In the T-group, 106 (80%) achieved HbA_{1c} <6.5%. In CTRL, only 1 patient achieved HbA1c <6.5%. All but 9 men experienced an increase in HbA_{1c}. Waist circumference progressively decreased from 111.1±7.4 to 100.7±5.5 cm in the T-group and increased from 110.1±7.2 to 111.7 ±7.3 cm in CTRL. The adjusted estimated difference between groups at 8 years was -13 cm ($p<0.0001$ for all). Weight change from baseline was -19.4±4.8% in the T-group and +2.0±2.8% in CTRL. Estimated adjusted difference between groups: -20.2% ($p<0.0001$ for all). Since all injections were administered in the doctor's office and documented, there was a 100% adherence to TTh.

Conclusion: Long-term TTh with TU in hypogonadal men with T2DM improved glycaemic control compared to untreated controls. In patients in the testosterone group receiving insulin, the dose could be substantially reduced. The observed effects may have been mediated by reductions in weight and waist circumference. Correcting hypogonadism in men with T2DM sustainably supports standard diabetes treatment.

Supported by: Bayer AG

Disclosure: **K.S. Haider:** None.

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No progression from prediabetes to type 2 diabetes in 45 hypogonadal men receiving testosterone therapy (TTh) for up to 9 years: real-life data from a registry study

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Background and aims: In individuals aged 45 years, the lifetime risk to progress from prediabetes to diabetes was recently reported as 74.0%. The recommended prevention strategy is weight loss. Since long-term TTh in hypogonadal men results in sustained weight loss, we studied hypogonadal men with Prediabetes defined by HbA_{1c} of 5.7-6.4% in the context of an ongoing registry on effectiveness and safety of testosterone undecanoate injections (TU) started in 2004 in a urological setting.

Materials and methods: Of 400 men with symptomatic testosterone deficiency (total testosterone ≤12.1 nmol/L), 45 (11.3%) had prediabetes. They were all treatment-naïve regarding antidiabetic therapy. The majority had presented to the urology office with complaints of erectile function or urinary function. All men men received TU 1000 mg/12 weeks (T-group) following an initial 6-week interval. No structured recommendations for lifestyle modifications were provided. Most measurements were performed 2 to 4 times a year for up to 11 years. 9-year data are presented.

Results: Mean age was 55.2±8.0 years. Mean HbA_{1c} steadily decreased from 5.9±0.2 to 5.2±0.2% after 9 years ($p<0.0001$). Fasting glucose decreased from 5.5±0.2 to 5.3±0.1 mmol/L ($p<0.05$). The triglyceride:HDL ratio, a surrogate marker of insulin resistance, decreased from 7.3±1.8 to 3.5±0.7 ($p<0.0001$). The TyG index, another surrogate parameter of insulin resistance and predictor for the risk of incident diabetes, decreased from 9.5±0.2 to 9.1±0.1 ($p<0.0001$). Weight progressively decreased from 99.7±14.2 to 85.1±7.4 kg ($p<0.0001$). The decrease was statistically significant compared to the previous year for the first 7 years. Waist circumference decreased from 102.4±6.7 to 94.5±5.6 cm ($p<0.0001$). The decrease was statistically significant compared to the previous year for the first 4 years. BMI decreased from 32.0±48 to 27.7±2.9 kg/m^2 ($p<0.0001$). The decrease was statistically significant compared to the previous year for the first 7 years. Mean weight change from baseline was -17.9% ($p<0.0001$). No patient progressed from prediabetes to T2DM during the entire observation period. 44 patients' last measured HbA_{1c} was <5.7% which is below the prediabetes range. Only 1 patient's last HbA_{1c} was 6.0%. Since all testosterone injections were administered in the doctor's office and documented, there was a 100% adherence to TTh.

Conclusion: Long-term TTh with TU in hypogonadal men with prediabetes completely prevented progression from prediabetes to type 2 diabetes. This effect may have been mediated by weight loss which was demonstrated by sustained reductions in weight and waist circumference. In addition, it is known that testosterone increases lean body mass and reduces fat mass, changes in body composition which are beneficial over and beyond weight loss alone.

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Prevalence of type 2 diabetes and prediabetes in hypogonadal men and effects of testosterone treatment: real-life experience from a controlled registry study

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Background and aims: The high prevalence of hypogonadism in men with T2DM is well established. There is less information about the prevalence of hypogonadism in prediabetes.

Materials and methods: In a controlled registry study of 505 hypogonadal men in a urology office, 147 (29.1%) had T2DM. Their mean age at baseline was 62.7±8.3 years. 94 men received testosterone therapy (TTh, T-group) with testosterone undecanoate injections (TU) 1000 mg/12 weeks following an initial 6-week interval for up to 12 years. 53 men had opted against TTh and served as controls (CTRL). 253 (50.2%) men had prediabetes, defined by an HbA_{1c} from 5.7-6.4%. Mean age: 61.5±9.6 years. 175 men received TTh for up to 12 years, 78 served as controls (CTRL). 8-year data are reported.

Results: T2DM: Weight (kg) decreased from 107.8±13.2 to 97.7±12.3 in the T-group ($p<0.0001$) and increased from 95.8±10.7 to 99.5±15.5 in CTRL ($p<0.0001$ for both). Waist circumference (cm) decreased from 114.0±10.7 to 105.6±10.8 in the T-group and increased from 103.2±8.9 to 105.0±12.2 in CTRL ($p<0.0001$ for both). BMI (kg/m^2) decreased from 34.1±4.1 to 30.8±3.9 in the T-group and increased from 30.5±3.8 to 32.3 ±5.0 in CTRL ($p<0.0001$ for both). Fasting glucose (mmol/L) decreased from 7.9±2.3 to 5.9±1.7 in the T-group and from 6.9±2.6 to 6.2±3.9 in CTRL ($p<0.0001$ for both). HbA_{1c} decreased from 7.9±1.0 to 7.0±1.2% in the T-group ($p<0.0001$) and remained stable from 6.8±0.9 to 6.7±1.0% in CTRL. 74 of 94 patients (78.7%) with T2DM in the T-group had their last

measured HbA_{1c} <6.5%, but only 9 of 52 (17.3%) men in CTRL, regardless of concomitant treatment modalities. Prediabetes: Weight (kg) decreased from 96.0±11.7 to 89.3±9.8 in the T-group (p<0.0001) and increased from 92.1±9.9 to 98.2±6.3 in CTRL (p<0.0001 for both). Waist circumference (cm) decreased from 104.7±7.1 to 98.4±6.6 in the T-group and increased from 99.9±9.0 to 106.0±3.2 in CTRL (p<0.0001 for both). BMI (kg/m^2) decreased from 30.5±3.9 to 28.3±3.4 in the T-group and increased from 29.5±2.8 to 30.4±2.2 in CTRL (p<0.0001 for both). Fasting glucose (mmol/L) decreased from 5.3±0.9 to 4.9±0.7 in the T-group (p<0.0001) and increased from 4.9±1.4 to 5.0±0.9 in CTRL (p<0.05). HbA_{1c} decreased from 5.9±0.2 to 5.6±0.4% in the T-group and increased from 5.9±0.2 to 6.1 ±0.6% in CTRL(p<0.0001 for both).. No patient in the T-group progressed from prediabetes to overt T2DM. 152/175 had their last measured HbA_{1c} <5.7% and were no longer in the prediabetes category. In CTRL, 30/ 78 (38.5%) men in CTRL progressed from prediabetes to T2DM.

Conclusion: Long-term TTh with TU in unselected hypogonadal men with a high prevalence of T2DM and prediabetes resulted in weight loss and improvements in glycemic control whereas untreated controls experienced worsening of all parameters. Testosterone treatment of hypogonadal men fully prevented progression from prediabetes to T2DM.

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Early results of the first Brazilian patients with generalised congenital lipodystrophy on treatment with metreleptin

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Background and aims: Generalized congenital lipodystrophy (GCL) is an autosomal recessive disease characterized by difficulty in storing body fat and, consequently, very low serum levels of leptin. They present with diabetes, hypertriglyceridemia and ectopic fat deposit, as in the liver. In Brazil, we have one of the largest series of this rare disease.

Materials and methods: Eleven patients (6 men and 5 women; 5 adults and 6 children, 12.6 ± 9.3 years of age) with GCL (3 Type 1, 5 type 2 and 3 non-genotyped patients) were treated with subcutaneous metreleptin injections. Only the adults had diabetes (n = 5). To quantify the appetite, we used the simplified nutritional appetite questionnaire. Blood tests were performed before the start of treatment and, 1 and 3 months later. Parametric data are shown as mean ± SD or as median (minimum-maximum) if nonparametric. Comparisons were made between the results of baseline and the 3 months after treatment. A p-value < 0.05 was considered statistically significant.

Results: The mean dose of metreleptin was 0.46±0.28 mg/day. It was well tolerated and no patient stopped the use. Five patients had some skin reactions in the local of injection and one child patient had important anorexia needing to decrease the dose for one week. Fasting triglyceridemia [225(35-1473) vs. 111(48-309) mg/dL, p=0.01], aspartate aminotransferase [31(22-87) vs. 26(9-38) UI/L, p=0.009], alanine aminotransferase [51(25-152) vs. 26(11-63) UI/L, p=0.01] and gamma glutamyl transferase [25(12-66) vs. 16(11-45) UI/L, p=0.005)] reduced significantly. Fasting glycemia (151.5±108 vs. 107.5±38.9mg/dL, p=0.09), glycated hemoglobin (7.2±3.2 vs. 6.0±1.7%, p=0.09), total cholesterol (133±29.9 vs. 113.9±28.4 mg/dL, p=0.06) and HDL cholesterol (28.6±7.4 vs. 28.0±5.0 mg/dL, p=0.69) decreased, but it was not statistically significant. One diabetic patient stopped insulin use and started oral hypoglycemic pills; other patient decreased insulin dose and another patient decreased the oral hypoglycemic pill to half dose. Despite these reductions, fasting glycemia (239.4±107 vs. 142.6±30.9 mg/dl) and glycated hemoglobin (9.9±3.2 vs. 7.2±1.9%) reduced significantly in diabetic patients. The mean appetite score (maximal 20 points) was 18.3 and decreased to 14.9 after 1 month, but increased to 16.1 after 3 months. The BMI decreased significantly (18.2 ±3.5 to 17.0±3.3 kg/m2, p=0.01).

Conclusion: Metreleptin use can bring early improvements in glycemia, triglyceridemia, and liver enzymes. The effect decreasing the appetite is as precocious as one month of use, but in some patients the appetite increase at 3 months.

Disclosure: **J.G. Lima:** None.

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Impact of metreleptin on hepatomegaly in patients with generalised lipodystrophy

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Background and aims: Lipodystrophy syndromes are clinically heterogeneous, inherited or acquired, and often life-threatening. The underlying pathogenesis of generalized lipodystrophy (GL) is the irreversible widespread deficiency of adipose tissue leading to low leptin levels. Due to the lack of adipocytes, excess calories accumulate as triglycerides (TG) in ectopic locations such as the liver. Ectopic liver fat can progress to hepatomegaly, steatohepatitis, portal hypertension, cirrhosis, and liver failure. This study examined the effect of leptin replacement therapy (metreleptin, ML) on liver volume (LV) and key metabolic parameters in adult and pediatric patients with GL.

Materials and methods: This is a post hoc analysis of an open label, prospective study of ML in patients with GL conducted at the NIH. LV by MRI was assessed for all available patients enrolled from 2000-2008. Normal LV was calculated as 25 mL/kg of body weight. Mild, moderate, and severe hepatomegaly were defined as ≤1.25 multiple of normal (MN), 1.25-2.5 MN, and >2.5 MN, respectively. TG, A1c, AST, and ALT were measured at baseline and after ML treatment.

Results: Among the 34 evaluable patients with GL enrolled through 12/31/2008, 21 had both baseline and follow-up liver volumetric measurements. At baseline, all had enlarged livers with a mean ± SD LV of 3357.7 ± 1121.7 mL, ranging from 1.1-6 x normal. The majority of patients were female (67%), 57% had congenital GL, and the mean age was 24 ± 16 years. At baseline, 91% had diabetes, 76% had hypertriglyceridemia, and 2 had autoimmune hepatitis. For patients assessed within a year after initiating ML (n=21, mean treatment duration 9.8 ± 2.8 months), LV decreased by 24.5 ± 16.6%. Longer exposure (n=14, 46.7 ± 24.4 months) appeared to have a larger decrease in LV relative to baseline of 34.7 ± 18.9%. Treatment with ML for one year resulted in significant reductions in A1c [n=19, -2.1 ± 1.2%], AST [n=19, -46.8 ± 63.0 U/L], ALT [n=19, -69.7 ± 101.7 U/L], and TG [n=15, -43.7 ± 36.7%]. ML was generally well tolerated; most commonly reported adverse events were abdominal pain and hypoglycemia. Among the subgroup of 13 pediatric patients who were < 18 years (mean 14 ± 3 years), all had an enlarged liver at baseline (mean ± SD LV of 3459 ± 1178 mL), ranging from 1.2-6 x normal. For pediatric patients assessed within a year after initiating ML (n=13, mean treatment duration of 9.4 ± 3.2 months), LV decreased by 25.2 ± 15.4%. Longer exposure (n=9, 46.2 ± 26.9 months) appeared to have a larger decrease in LV relative to baseline of 34.3 ± 18.1%. Treatment with ML for 1 year resulted in significant reductions in A1c [n=11, -2.2 ± 1.4%], AST [n=11, -56.3 ± 75.3 U/L], ALT [n=11, -90.5 ± 128.6 U/L], and TG [n=9, -43.1 ± 30.8%]. Similar to the larger cohort, ML was generally well-tolerated in pediatric patients; gastrointestinal disorders including abdominal pain and pancreatitis were the most commonly reported adverse events. The 2 pediatric patients with pancreatitis also reported it in their past medical history, prior to ML treatment initiation.

Conclusion: Moderate to severe hepatomegaly, usually due to hepatic TG accumulation, is a common feature in patients with GL. In addition to its metabolic effects, this post hoc analysis provides additional evidence that ML may have a clinically significant and sustained effect in reducing LV in adult and pediatric patients with GL.

Clinical Trial Registration Number: NCT00025883

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Co-administration of tesofensine and metoprolol: improvements in body weight and liver fat content in patients with type 2 diabetes

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Background and aims: Tesofensine (TESO) is an inhibitor of the monoamine presynaptic reuptake of the neurotransmitters noradrenaline, dopamine and serotonin suppressing appetite and reward centres in the brain and increasing energy expenditure. TESO demonstrated strong weight reducing effects in Phase 2 clinical studies in obese subjects, but also an increase in blood pressure and heart rate at therapeutically relevant doses. In this double-blind, randomized, placebo-controlled trial we investigated the effect of co-administration of 0.5 mg TESO with 100 mg of the β1-selective beta-blocker metoprolol (MET) on body weight, body composition and metabolic endpoints in subjects with T2D on metformin therapy with or without one additional anti-diabetic oral agent.
Materials and methods: Sixty subjects with T2D [mean±SD age: 62.8±6 yrs; weight: 96.4±16.4 kg; BMI: 32.6±5.3 kg/m²; waist circumference: 111.4±11.8 cm; HbA1c 7.7±0.4%], were randomly allocated to once daily treatment with TESO+MET or matching placebos (PLAC) for 90 days. Fifty-eight subjects completed the study. Anthropometric assessments including body weight and waist circumference as well as vital signs and safety parameters were assessed every 7-14 days. Liver fat (by magnetic resonance spectroscopy) and metabolic endpoints were measured at baseline and end of treatment (EOT) in a sub-set of the patients.
Results: TESO+MET led to a progressive weight reduction which was significantly greater than the minor changes observed with PLAC (table). Accordingly, treatment with TESO+MET led to a significantly greater reduction in mean waist circumference compared with PLAC. While TESO+MET showed no significant advantages in glycaemic parameters (HbA1c, 1.5-Anhydroglucitol, fasting plasma glucose (FPG) and area under the curve of the 9-point glucose profile) or lipids, there was a strong trend to significantly larger improvements in liver fat which decreased with TESO+MET, but increased with PLAC. TESO+MET was well tolerated with nausea, hyperhidrosis, headache and dry mouth being the most frequent adverse events (AEs). No serious AE occurred with TESO+MET. Most importantly, there were no deleterious effects on heart rate and blood pressure with TESO+MET, but rather a significant reduction in mean 24 hr heart rate (LS-mean difference between treatments in change of baseline -3.8 bpm, p=0.0038).
Conclusion: This study demonstrates that a tesofensine/metoprolol co-administration significantly reduces body weight as well as waist circumference and trends to improve liver fat in patients with T2D without any negative effects on heart rate and blood pressure.

¤	TESO+MET¤		PLAC¤		Difference·between·treatments·in·change·from·baseline(95%·CI)¤	p-value¤
¤	Baseline¤	EOT¤	Baseline¤	EOT¤	¤	¤
Body·weight·[kg]¤	99·±·19¤	96·±·20¤	94·±·12¤	93·±·13¤	-3.5·(-4.7;·-2.3)¤	<.0001¤
Waist·circumference·[cm]¤	114·±·13¤	112·±·13¤	109·±·9¤	108·±·9¤	-2.3·(-3.9;·-0.7)¤	0.0070¤
HbA1c·[%]¤	7.5·±·0.5¤	7.3·±·0.6¤	7.7·±·0.4¤	7.4·±·0.7¤	0.05·(-0.2;·0.3)¤	0.7240¤
1.5-Anhydroglucitol·(mg/L)¤	9.8·±·6.0¤	11.8·±·6.1¤	6.7·±·3.6¤	8.2·±·5.0¤	0.8·(-0.9;·2.5)¤	0.3290¤
FPG·[mg/dL]¤	161·±·30¤	158·±·34¤	163·±·28¤	157·±·25¤	2·(-10;·14)¤	0.7331¤
Liver·fat·content·[%]¤	16·±·8¤	14·±·8¤	12·±·8¤	13·±·9¤	-2.7·(-5.5;·0.1)¤	0.0625¤
Cholesterol·[mmol/L]¤	5.2·±·1.0¤	4.9·±·0.8¤	4.7·±·0.8¤	4.5·±·0.7¤	0.08·(-0.2;·0.4)¤	0.5920¤
HDL·[mmol/L]¤	1.2·±·0.4¤	1.1·±·0.3¤	1.2·±·0.3¤	1.1·±·0.3¤	-0.02·(-0.1;·0.1)¤	0.6613¤
LDL·[mmol/L]¤	3.4·±·0.9¤	3.3·±·0.7¤	2.9·±·0.6¤	2.9·±·0.5¤	0.13·(-0.1;·0.4)¤	0.2537¤
Triglycerides·[mmol/L]¤	2.2·±·1.0¤	1.8·±·0.8¤	2.0·±·0.7¤	1.8·±·0.7¤	-0.07·(-0.3;·0.2)¤	0.5722¤

Clinical Trial Registration Number: NCT02737891
Disclosure: **G. Andersen:** None.

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Endoscopic proximal intestinal exclusion with EndoBarrier therapy is associated with improvement in gonadal function in obese men with diabetes

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Background and aims: Previous evidence has linked obesity with low testosterone levels and improvement in gonadal function with weight loss. Our aim was to ascertain whether weight loss with Endobarrier® was associated with and improvement in testosterone levels and hence gonadal function in obese men with Type 2 diabetes.
Materials and methods: This was a sub-study of a larger multi-centre, randomised controlled trial investigating the efficacy of Endobarrier® therapy, a 60cm endoluminal sleeve implanted in the proximal intestine. This was a proof-of-concept study. Testosterone levels were measured prior to initiating treatment and 12 months later prior to implant removal. Free testosterone was estimated using the Vermeulen equation.
Results: There were 22 male participants with mean age 49.1(±10.8) years and diabetes duration of 11.4(±5.2) years. 15/22 patients that received Endobarrier®, mean weight loss was 9.9 (SE2.1) Kg (p<0.001) with a maximum weight loss of 25.6kg. Only one patient did not lose weight; HbA1c improved by 22.1 (SE5.4) mmol/mol[2.0%; SE0.49] (p=0.001). The total testosterone levels improved by 1.9 (SE 0.78)nmol/L (p=0.03). The estimated free testosterone level after adjusting for the change in weight and HbA1c improved by 77% (p=0.001; adjR^2 = 0.65 for model).
Conclusion: The study indicates that Endobarrier® therapy is associated with a significant improvement in free testosterone levels in men, independent of change in glycaemic control and body weight. This may imply return of gonadal function in some men following therapy.

Table: Characteristics of the 22 male patients who completed treatment with Endobarrier . P-values reflect change from baseline with 5% significance

Parameter	Baseline	At Removal	Difference (95%CI)	P value (n=31)
Age (years)	48.1±10.8			
Diabetes Duration (years)	11.4 (4.6-24.0)			
Taking insulin (%)	50.0			
Weight (Kg)	126.9±16.8	120.3±19.9	-9.9 (-14.4,-5.1)	0.0003
HbA1c (mmol/mol)	87.4±16.0	65.3±18.0	-22.1 (-33.7,-10.6)	0.0011
HbA1c (%)	10.1±1.5	8.1±1.6	-2.0 (-3.1,-0.97)	0.0011
Total Testosterone nmol/L(unadjusted)	8.0±4.4	9.9±4.0	1.9 (0.19, 3.6)	0.03

Clinical Trial Registration Number: ISRCTN00151053
Disclosure: **A. Basu:** None.

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Jejunal diversion, a novel malabsorbtive procedure, improves metabolic control and augments the incretin effect in obese subjects with type 2 diabetes

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Background and aims: Jejunal diversion (JD) is a novel metabolic surgical procedure using side-to-side jejuno-jejunostomy to create a partial intestinal by-pass in a less invasive manner compared with other malabsorbtive operations, thus far used predominantly in experimental animals. The aim of our study was to assess the effects of JD on anthropometric, metabolic and hormonal parameters of patients with obesity and type 2 diabetes mellitus (T2DM).
Materials and methods: Fifteen obese T2DM subjects (8 males, aged 52.7 ± 6.1 years) were examined before and 3 and 12 months after JD. Postprandial effects of JD were evaluated using a 5-hour oral glucose tolerance test (oGTT).
Results: JD rapidly and sustainably reduced body weight (BMI 34.1 ± 3.5 vs. 31.9 ± 3.5 vs. 31.0 ± 3.7 kg/m^2 for 0 vs. 3 vs. 12 months, p<0.001) and improved glucose control (HbA_{1C} 78.9 ± 9.5 vs. 62.3 ± 11.4 vs. 54.1 ± 12.3 mmol/mol, p<0.001) and lipid profile (LDL 2.68 ± 0.72 vs. 1.94 ± 0.75 vs. 2.03 ± 0.80 mmol/l, p=0.014; triglycerides 3.67 ± 3.96 vs. 1.81 ± 0.99 vs. 1.86 ± 1.31 mmol/l, p=0.012). JD further augmented the incretin effect by increasing postprandial levels of GLP-1 along with the reduction of insulin and glucagon, while GIP, PP, amylin and ghrelin were not affected. Interestingly, bile acid levels peaked already 3 months after JD with a subsequent return to baseline at 12 months, in spite of no change being observed in their intestinal regulator fibroblast growth factor 19 (FGF-19). JD was accompanied by a reduction in serum ferritin (133.2 ± 97.6 vs. 66.9 ± 47.4 μg/l for 0 vs. 12 months, p=0.004), vitamin B12 (491.6 ± 216.6 vs. 264.3 ± 139.5 ng/l, p<0.001) and zinc (16.2 ± 1.6 vs. 14.0 ± 2.8 mmol/l, p=0.013) without influencing any other nutritional parameters.
Conclusion: In obese subjects with T2DM JD induces significant weight loss and improvement in metabolic control that persist for at least 12 months after operation. Changes in GLP-1 and bile acids might be at least partially responsible for these effects.
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Duodenal mucosal resurfacing demonstrates sustained improvement in glycaemic parameters in type 2 diabetes: 12 month data

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Background and aims: Duodenal exclusion via bariatric surgery has been shown to confer an insulin sensitizing metabolic benefit that is, in part, weight-independent. Duodenal Mucosal Resurfacing (DMR) is an endoscopic procedure that rejuvenates the duodenal mucosa through hydrothermal ablation and may confer similar metabolic benefits using a less invasive procedure. Here we report the 12 months glycaemia data following a single DMR procedure in patients with T2D.
Materials and methods: Data were extracted from a single arm, open label, multicentre study in which type 2 diabetes patients on oral glucose lowering medication with baseline HbA1c 7.5 - 10.0%, and age 25 - 75 years received a single DMR procedure. Sulfonylurea's were discontinued before DMR and other glucose regulating medication was kept stable for at least 6 months post DMR. Hereafter medication modifications were made at the treating physician's discretion following the local or international guidelines. Efficacy was analysed in a modified intent-to-treat cohort (patients who received ≥1 ablation) and a sub-group of patients (n=15) with preserved beta-cell function (baseline fasting plasma insulin, FPI >15 uU/mL). Change from baseline in HbA1c, fasting plasma glucose (FPG), and Homeostasis Model Assessment index (HOMA-IR) were analysed using paired t-tests. Data are reported as mean ± SD.
Results: Baseline parameters (n=27) were: age, 55.0 ± 9.0 y; diabetes duration, 6.0 ± 2.5 y; BMI, 32.5 ± 4.2 kg/m^2; HbA1c, 8.7 ± 1.0 %. On average, HbA1c remained lower than baseline over 12 months after a single DMR procedure; reductions at 6, 9 and 12 months were, -1.0 ± 1.0% (p < 0.001), -1.1 ± 1.5% (p = 0.001), -0.7 ± 1.2% (p=0.008). Similarly, FPG and HOMA-IR were lower relative to baseline, with reductions of -33.9 ± 48.7 mg/dL, and -3.8 ± 4.9 (p=0.002), at 12 months. In the cohort with baseline FPI >15 uU/mL, the mean HbA1c reductions were, -1.4 ± 1.1% (p < 0.0003), -1.6 ± 1.8% (p = 0.006), and -1.3 ± 1.3% (p=0.002), at 6, 9, and 12 months. A modest reduction in mean body weight was observed throughout 12 months (at 12 months; -1.5 ± 4.2 kg, p = 0.08) for the full cohort.
Conclusion: A single DMR procedure produced sustained reductions in HbA1c, FPG, and HOMA-IR out to 12 months, with robust glycaemic effects in patients with preserved beta-cell function. Larger, randomized controlled studies are planned to further establish the efficacy, safety and durability of the metabolic effects associated with DMR.
Clinical Trial Registration Number: NCT02413567
Supported by: Fractyl Laboratories, Inc.
Disclosure: **A.C.G. van Baar:** None.

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Mesenchymal stromal cells from the umbilical cord (UCX®): A role in protection against non-alcoholic fatty liver disease?

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Background and aims: Mesenchymal Stromal Cells (MSCs) have been shown to have promising therapeutic applications, mainly due to their immunomodulatory, anti-inflammatory, pro-angiogenic and regenerative properties. Umbilical cord tissue-derived expanded MSCs, UCX®, consists of a specific population of MSCs from the umbilical cord tissue (UC-MSCs), isolated according to a patented technology. Preclinical studies using UCX® have demonstrated the safety and quality of the cells, as well as their efficacy in animal models for several immune-related and cardiovascular diseases. However, the role of the UCX® in diet-induced obesity and non-alcoholic fatty liver disease (NAFLD) remains widely unknown.
Materials and methods: Male C57Bl/6 mice were used. The mice were divided in three experimental groups according to the type of diet and UCX® administration. The mice were given ad libitum access to a normal- (Chow) or hyper-caloric diet (HFat), from ages 6 to 18 weeks. The HFat group was further divided into two groups: HFat and HFat-UCX®, the latter being treated with UCX® every week for a total of 7 weeks (from ages 11 to 18, via an intraperitoneal injection of 10E6 cells). Mice were monitored weekly for body weight, blood glucose and food/calorie intake, as well as a close observation of their behavior, for distress signals. After 5 weeks of diet and at the end of the study both insulin sensitivity and glucose tolerance were determined. At the end of

the study several organs and tissues were collected for further analysis. Livers were stained by H&E (Hematoxylin & Eosin) and their content in cholesterol and triglycerides was determined.
Results: As expected HFat diet promoted body weight gain (20.53 ± 0.156g n=4 vs. 24.02 ± 0.759g n=9, p<0.05), fasting hyperglycemia (79.50 ± 3.617mg/dL n=4 vs. 109.9 ± 8.324mg/dL n=9, p<0.05) and glucose intolerance (24140 ± 2788 n=4 vs. 30110 ± 2118 AUC A.U. n=9), compared to the control littermates under a Chow diet. Mice were at this point considered as being in a pre-diabetic state. After the treatment HFat-UCX® animals were protected against body weight gain, compared to the untreated HFat mice (33.83 ± 3.676g vs. 31.85 ± 1.379g, n=4). HFat-UCX® animals had lower fasting glucose levels (134 ± 3.122mg/dL vs. 106.2 ± 2.956mg/dL, n=4/5, p<0.001) and improved glucose tolerance (44800 ± 5275 vs. 59023 ± 6919 AUC A.U., n=4, p<0.05). Food intake was unaffected by the UCX® treatment. HFat-UCX® animals show a decreased liver/body weight ratio and ectopic lipid accumulation, assessed by H&E staining. Compared to the untreated HFat mice, HFat-UCX® mice, show decreased accumulation of triglycerides (45.63 ± 1.896 vs. 37.90 ± 2.816 mg/g of liver weight n=4, p=0.0632) in the liver.
Conclusion: The preliminary data suggests that UCX® may have a protective role in diet-induced obesity and NAFLD. The mechanism through which the cells protect against lipid accumulation in the liver needs to be further investigated.
Supported by: iNOVA4Health - UID/Multi/04462/2013
Disclosure: **I. Sousa-Lima:** None.

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LY3298176, a novel long-acting GIP/GLP-1 coagonist, shows enhanced activity on weight loss and energy utilisation whilst maintaining its efficacy for glycaemic control

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Background and aims: Commonly used glucagon-like peptide-1 receptor (GLP-1R) agonists provide very substantial reductions in $HbA1_c$ as well as clinically meaningful weight loss; however, it remains unknown if combining GLP-1R activity with glucose-dependent insulinotropic peptide receptor (GIPR) activity results in a treatment with improved efficacy. In this study we evaluated GLP-1R and GIPR activation, glucose lowering and weight loss in vitro and in vivo using a novel acylated long-acting GIP/GLP-1 coagonist peptide, LY3298176.
Materials and methods: Peptide analogues were made using an automated peptide synthesizer and evaluated in GIPR and GLP-1R binding and functional assays. Insulin secretion was studied in isolated primary rat islets as well as in vivo in rodents. Weight loss studies were carried out in high fat fed C57Bl6 mice and glycaemic control was evaluated at the end of a 2 week dosing period. Energy utilization was determined by the aid of Oxymax chambers and whole body QNMR was used to determine body composition at the end of the weight loss study.
Results: LY3298176 binds to human (h) GIPR and GLP-1R with an affinity (K_i) of 34 and 230 nM, respectively. Half-maximal concentrations in the functional cAMP generation assays in cells that over-express hGIPR and hGLP-1R were 11 and 71 nM, respectively. LY3298176 does not bind to or activate the human glucagon receptor at concentrations ≤7.5 μM. LY3298176 dose-dependently enhanced insulin secretion from rat or mouse islets. Furthermore, LY3298176 enhanced insulin secretion in islets from GIPR knockout mice as well as in islets from GLP-1R knockout animals. In rats, LY3298176 both enhanced insulin secretion in an intravenous glucose tolerance test as well as inhibited gastric emptying of a semi-liquid meal. Half maximal doses (ED_{50}) were 0.87 nmol/kg (insulin secretion) and 11.8 nmol/kg (gastric emptying). LY3298176, given every 72 hours (hrs) for 2 weeks, dose-dependently induced weight loss in high-fat diet induced obese mice. The weight loss amounted to 38% at the highest dose administered (100 nmol/kg) and the ED_{50} was estimated to 5 nmol/kg. The weight loss was predominantly loss of fat mass (>80%) and was associated with reduced food intake and a 10-15% increase in energy utilisation. Improved plasma and liver lipids were also observed. Interestingly LY3298176, contrary to what is known for GLP-1, didn't increase corticosterone secretion in rats. The terminal elimination half-lives of subcutaneously administered LY3298176 were ≈10hrs and 146hrs in rats and dogs, respectively.
Conclusion: Our data suggest that LY3298176 is a potent and efficacious glucose-lowering agent that provides substantial weight loss. We see weight loss that appears to be associated with increased energy utilisation, something typically not seen using GLP-1 analogues alone. The 146hr elimination half-life in dogs suggests that LY3298176 is suitable for once-weekly dosing.
Disclosure: **K. Bokvist:** Employment/Consultancy; Eli Lilly and Company. Stock/Shareholding; Eli Lilly and Company.

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Crossover study comparing bioavailability of a capsule formulation of the glucagon receptor antagonist LGD-6972 to an oral solution formulation in healthy subjects

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Background and aims: LGD-6972 is a novel, orally bioavailable small molecule glucagon receptor antagonist (GRA) being developed as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus (T2DM). In a single- dose Phase 1 study using an oral solution formulation in Captisol (betadex [β-cyclodextrin] sulfobutylether sodium), LGD-6972 demonstrated favorable safety, tolerability and pharmacokinetics (PK) in normal healthy volunteers (NHV) and in subjects with T2DM, and dose dependent reductions in fasting plasma glucose (FPG). PK/PD modeling predicted an efficacious exposure and dose range for a subsequent multiple dose Phase 1 study. Multiple doses of LGD-6972 oral solution demonstrated favorable safety, tolerability and PK in T2DM subjects and a robust, dose-dependent reduction of FPG (maximal decrease of 3.15 mmol/L in baseline adjusted values on day 14). This Phase 1, single-center, randomized, open-label, single-dose, 2-period complete crossover study was conducted to compare the relative bioavailability of an oral capsule formulation of LGD-6972 to the solution formulation in NHV.
Materials and methods: A total of 12 subjects were enrolled in the study. In 2 treatment periods, subjects received each of the following treatments as a single oral dose, under fasting conditions (6 subjects per treatment in each period): Treatment A - 15 mg LGD-6972 administered as 3 × 5 mg capsules; Treatment B - 15 mg LGD-6972 administered as 4 mL × 3.75 mg/mL solution. Serial blood samples were collected for LGD-6972 plasma concentration determination, and safety was assessed during the 48 hours following each dose. Subjects were discharged from the study site on day 3 and returned on days 4, 7, and 14 for follow-up procedures.
Results: Bioequivalence between Treatment A and Treatment B was demonstrated for area under the plasma concentration versus time curve from time 0 (predose) to the last measurable concentration ($AUC_{0\text{-last}}$), AUC from time 0 (predose) to 312 hours postdose ($AUC_{0\text{-}312h}$), and AUC from time 0 (predose) to infinity (extrapolated) ($AUC_{0\text{-}\infty}$) (90% confidence intervals [CI] = [83.2, 109], [83.9, 108], and [83.9, 108], respectively). Bioequivalence was not demonstrated for maximum observed concentration (C_{max}) (90% CI = [75.6, 103]); however, a sensitivity analysis indicated that this may have been due to high variability in individual subject PK concentrations with the sampling schedule and small sample size for the design. Other PK parameters, including time to maximum observed concentration (T_{max}), half-life ($t_{1/2}$), elimination rate constant (λ_z), oral

clearance (CL/F), and apparent volume of distribution (V_d/F), were similar between treatments. Fasting glucose in subjects receiving Treatment A or Treatment B was reduced compared to baseline at 24 and 48 hours post-dose, returning to baseline by day 7. All treatment-emergent adverse events during the study were mild in severity, and no subject had a serious adverse event or discontinued from the study due to an adverse event.
Conclusion: Overall, single oral doses of 15-mg LGD-6972, administered as capsules or solution, were safe and well tolerated. The observed PK profile for the capsule formulation supports its use in the 12-week, Phase 2a study in subjects with T2DM currently underway.
Clinical Trial Registration Number: NCT02672839, NCT02851849
Supported by: Ligand Pharmaceuticals Incorporated
Disclosure: **J.D. Pipkin:** Employment/Consultancy; Ligand Pharmaceuticals Incorporated.

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The novel glucagon receptor agonist SAR438544, first in human safety, pharmacokinetic and pharmacodynamic data from a study in healthy volunteers

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Background and aims: SAR438544 is a novel synthetic agonist of the glucagon receptor in a stable formulation for subcutaneous (SC) injection to treat hypoglycemia. A first-in-human, randomized, double-blind, glucagon and placebo-controlled, single ascending dose study to evaluate safety, tolerability, PK and PD of SAR438544 was conducted in healthy volunteers.
Materials and methods: Twenty subjects (mean age: 30.8 ± 6.4 years; Male: 80%; mean BMI 24.5 kg/m^2) were enrolled in one of two dose cohorts (10 per cohort) and were randomized to either SAR438544 (N=6) with 50 μg in cohort 1 and 150 μg in cohort 2, placebo (N=2) or 1 mg glucagon control (N=2). Safety and tolerability were monitored. All subjects completed the study per protocol, and were included into safety, PK and PD analyses.
Results: There were no severe or serious TEAEs, and no AE of special interest. PK profiles showed a slight over dose-proportional increase in SAR438544 C_{max} (mean ± SD) of 1.82 ± 0.531 ng/mL (at 50 μg), 8.05 ± 0.749 ng/mL (at 150 μg) vs. 4.37 ± 1.47 ng/mL (at 1 mg glucagon). The t_{max} median (min - max) was 1.25 (1 - 1.5) h, 1 (0.75 - 2) h vs. 0.13 (0.08 - 0.17) h and $t_{1/2}$ (mean ± SD) was 1.34 ± 0.281 h, 1.22 ± 0.367 h vs. 0.795 ± 0.0676h for SAR438544 at 50 and 150 μg, and glucagon 1 mg, respectively. PD profiles showed a dose-dependent increase in mean BG-C_{max} (mmol/L) for SAR438544 at 50 and 150 μg with no BG response in the placebo group. The BG response for glucagon was greater than that for SAR438544. The mean values of BG-C_{max} (mean change from baseline) were 5.07 (0.19), 5.45 (0.49), 7.21 (2.37) vs. 9.26 (4.25) mmol/L for placebo, SAR438544 50 and 150 μg, and glucagon 1 mg, respectively.
Conclusion: In conclusion, this first-in-human study demonstrated that the stable formulation of this glucagon receptor agonist was well tolerated, safe and active at the doses tested.
Clinical Trial Registration Number: NCT02625636
Supported by: Sanofi
Disclosure: **M. Hompesch:** Stock/Shareholding; ProSciento, Inc.

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First-in-class PET tracer for the glucagon receptor

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Background and aims: The glucagon receptor (GCGR) is emerging as an important target in anti-diabetic therapy, especially as part of the pharmacology of dual glucagon/glucagon-like-peptide-1(GCG/GLP-1) receptor agonists. However, currently there are no suitable target engagement biomarkers for in vivo proof of binding mechanism and specificity for GCGR. For a GCG/GLP-1 receptor dual agonist, knowledge about the proportion of occupied receptors in vivo could promote the understanding of the physiological effect in terms of weight loss and glycemic control. Thus, it is imperative to develop target engagement markers of GCGR drug interactions in order to enable development of dual agonists. Here we describe a first-in-class GCG Positron Emission Tomography (PET) ligand, suitable for development as a tool for in vivo analysis of GCGR occupancy in the clinic.
Materials and methods: The GCGR selective agonist peptide S01-GCG was developed from analogues of modified glucagon. Potencies at rat, cynomolgus monkey and human GCGR were assessed by a functional cAMP assay in GCGR transfected HEK293 cells. Chelate conjugated peptide, S01-GCG, was radiolabeled with Gallium-68 (^{68}Ga) radionuclide. [^{68}Ga]DO3A-S01-GCG was evaluated for selectivity (co-incubation with 10 μM GCG, 10 μM unlabeled DO3A-S01-GCG or 1 μM GLP-1), affinity (n=5) and internalization (n=3) in GCGR transfected HEK293 cells as well as in frozen liver sections from rat, cynomolgus monkey and man by autoradiography (n=3). In vivo biodistribution, dosimetry as well as GCGR specificity studies (co-administration of 1 mg/kg DO3A-S01-GCG peptide) were evaluated in Sprague Dawley rats (n=24).
Results: S01-GCG displayed functional potency for the GCGR in the range of the natural ligand but limited potency for the GLP-1 receptor. ^{68}Ga-radiolabelling of DO3A-S01-GCG was highly reproducible with specific activity in excess of 50 MBq/nmol with high reproducibility and with radiochemical purity >95%. [^{68}Ga]DO3A-S01-GCG binding to transfected cells and liver sections of all studied species was mediated by the GCGR, with negligible cross-reactivity to GLP-1R. In cells, GCGR binding triggered internalization of [^{68}Ga]DO3A-S01-GCG. Affinity to the human GCGR in transfected cells was 17±8 nM. *In vivo*, [^{68}Ga]DO3A-S01-GCG displayed retention exclusively in liver, spleen and kidney of the rat. The binding in liver and spleen, but not kidney, was GCGR-selective as it could be competed away >80% by co-injection of unlabeled DO3A-S01-GCG. The human predicted absorbed doses (kidneys 0.54 mSv/MBq and effective dose 20 μSv/MBq) allows for repeated clinical scanning annually.
Conclusion: We present evidence of a first-in-class PET tracer targeting the GCG receptor, with suitable properties for clinical development. This tool has potential to provide direct quantitative evidence of GCG receptor occupancy in humans.
Supported by: Sanofi-Aventis
Disclosure: **O. Eriksson:** Employment/Consultancy; Antaros Medical AB. Grants; The study was funded by Sanofi.

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A novel long-acting glucagon analog (HM15136) offers favorable stability, PK/PD, and therapeutic potentials in congenital hyperinsulinism animal model

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Background and aims: Congenital hyperinsulinism (CHI) is a rare genetic disorder characterized by unregulated insulin secretion which leads to persistent and severe hypoglycemia. The available therapeutics have limited efficacy and lead to poor treatment adherence due to frequent dosing and/or need for intravenous infusion. Although glucagon has been considered as one of the most potent therapeutic options, its utilization is limited due to poor solubility, limited stability at physiological pH, and short duration of action. To overcome these limitations, we have developed a novel long-acting glucagon analog, HM15136. HM15136 consists

of a glucagon analog conjugated to the human aglycosylated Fc fragment via a short PEG linker. This study investigated the therapeutic potential of HM15136 by evaluating its 1) solubility and stability, 2) in vitro biological functions, and 3) PK/PD in rodent models.

Materials and methods: Solubility of HM15136 at pH7.0, and solution stability at 4 and 25°C were evaluated via LC (liquid chromatography) analysis. Intracellular signaling was measured as cAMP accumulation in CHO cells stably expressing human glucagon receptor (GCGR). The effects on glucose production were investigated in rat primary hepatocytes by assessing glycogenolysis and gluconeogenesis rates. For PK assessments, blood samples were collected after single subcutaneous or intravenous administration of HM15136 in SD rats, and HM15136 concentration was quantified using an in-house established ELISA. To test in vivo efficacy, rats made hypoglycemic by exogenous insulin were administrated with HM15136, and the blood glucose (BG) level was monitored. To evaluate long-term therapeutic efficacy, CHI mimetic rats were established by implanting an osmotic pump releasing insulin.

Results: First, HM15136 showed improved solubility at pH 7.0, compared to native glucagon (≥17 vs. 0.03 mg/mL), and displayed superior solution stability over time both at 4 and 25°C condition. HM15136 induced intracellular cAMP accumulation through GCGR (vs. glucagon, 12% and 21% for human and mouse receptor, respectively). Consistent with these results, HM15136 promoted glycogenolysis and gluconeogenesis in rat primary hepatocytes in a dose-dependent manner, validating the glucose producing ability of HM15136. The half-life of native glucagon and HM15136 in SD rats was determined as 5 min and 36 hr, respectively. Moreover, HM15136 showed high bioavailability (89.5%)in SD rats. Finally, in vivo efficacy studies indicated that HM15136 not only rapidly reversed acute hypoglycemia, but also sustainably increased BG in human CHI mimetic rats after single administration or when dosed mimicking human weekly administration.

Conclusion: Based on solubility/stability results, HM15136 possess more favorable physicochemical features than native glucagon. In addition, in vitro results confirmed the glucagon-like action of HM15136. Together with improved bioavailability and longer half-life, prolonged BG increasing ability in disease models suggests a once-weekly dosing potential of HM15136. HM15136 may represent a treatment for the unmet medical need of CHI.

Disclosure: **S. Jung:** None.

PS 069 SGLT2 inhibitors in the real world

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Characteristics of patients with type 2 diabetes initiating canagliflozin compared with other treatment regimens in the UK

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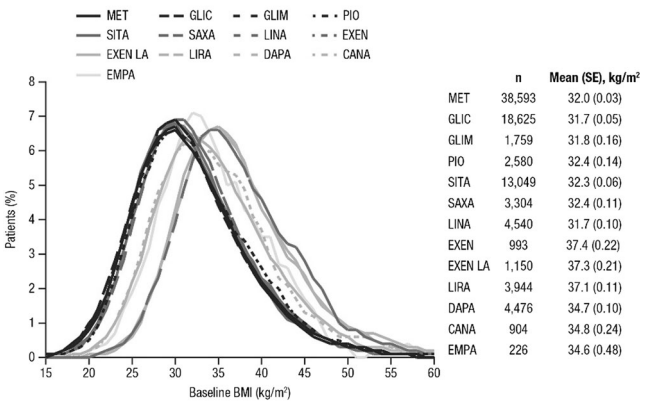
Background and aims: This study describes demographic and clinical characteristics of patients with type 2 diabetes mellitus (T2DM) initiating canagliflozin (CANA), a sodium glucose co-transporter 2 inhibitor (SGLT2i), compared with those who initiated other antihyperglycaemic agents (AHAs) in the United Kingdom.

Materials and methods: This analysis was based on retrospective data in the United Kingdom extracted from Clinical Practice Research Datalink between 2012 and 2015 (N = 149,767).

Results: Among patients initiating CANA, mean age was 59.5 years, HbA1c was 9.5%, body mass index (BMI) was 34.8 kg/m^2, and T2DM duration was 9.2 years. Patients initiating SGLT2i and glucagon-like peptide-1 receptor agonists (GLP-1 RA) tended to be younger, have higher HbA1c, and were more likely to be on background AHAs compared with patients initiating metformin (MET), sulphonylureas (SU), dipeptidyl peptidase-4 inhibitors (DPP-4i), and pioglitazone (PIO). Patients initiating GLP-1 RA tended to have the highest BMI (mean [standard error (SE)] = 37.2 [0.09] kg/m^2), followed by patients initiating SGLT2i (mean [SE] = 34.7 [0.09] kg/m^2; Figure). The time since T2DM diagnosis was similar across AHA classes, except for MET, which tends to be used as initial therapy.

Conclusion: These findings show that patients initiating CANA in the United Kingdom generally have characteristics similar to those initiating GLP-1 RA, but may differ compared with patients initiating MET, SU, DPP-4i, and PIO.

Figure. Distribution of baseline BMI with CANA and other AHAs.



	n	Mean (SE), kg/m^2
MET	38,593	32.0 (0.03)
GLIC	18,625	31.7 (0.05)
GLIM	1,759	31.8 (0.16)
PIO	2,580	32.4 (0.14)
SITA	13,049	32.3 (0.06)
SAXA	3,304	32.4 (0.11)
LINA	4,540	31.7 (0.10)
EXEN	993	37.4 (0.22)
EXEN LA	1,150	37.3 (0.21)
LIRA	3,944	37.1 (0.11)
DAPA	4,476	34.7 (0.10)
CANA	904	34.8 (0.24)
EMPA	226	34.6 (0.48)

GLIC, gliclazide; GLIM, glimepiride; SITA, sitagliptin; SAXA, saxagliptin; LINA, linagliptin; EXEN, exenatide; LA, long acting; LIRA, liraglutide; DAPA, dapagliflozin; EMPA, empagliflozin.

Supported by: Janssen Pharmaceutica NV
Disclosure: **G. Hamilton:** Employment/Consultancy; Janssen-Cilag Ltd.

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Dapagliflozin compared to DPP4i treatment is associated with lower risk of kidney disease, heart failure and all-cause death: CVD-REAL Nordic

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Background and aims: A main goal of T2D management is to prevent organ complications, including renal and cardiovascular disease (CVD). We aimed to investigate associations of hospitalization for kidney disease (HKD), hospitalization for heart failure (HHF) and all-cause death (ACD) with use of either dapagliflozin or DPP4 inhibitors (DPP4i) in T2D patients.
Materials and methods: Using nationwide registries in Norway and Sweden, all T2D patients dispensed with glucose lowering drugs during 2013—2016 were identified and clinical events were collected. All new users of dapagliflozin (essentially the only SGLT2 inhibitor used) and new users of DPP4i were identified. Then, propensity score matching, 1:3, was performed by using extensive data on patient characteristics, co-morbidities and drug treatment. HKD is defined as any hospital care (in- or outpatient visit) with the main diagnosis of chronic, acute and unspecified kidney disease. HHF is defined as any hospital care (in- or outpatient visit) with the main diagnosis of heart failure. Cox survival models estimated hazard ratio per country and weighted averages are presented.
Results: Out of a total of 77,074 new users of dapagliflozin and DPP4i, 34,328 T2D patients remained following propensity score matching (dapagliflozin, n=8582; DPP4i, n=25,746). These groups were well balanced at baseline; 61 years, 41% women, 21% CVD, 19% microvascular disease, 1% kidney disease, mean follow-up 0.98 years, a total of 33,612 patient-years. Dapagliflozin was associated with lower risk of HKD, HHF and ACD compared to the DPP4i group (Table).
Conclusion: In T2D patients, dapagliflozin was associated with lower risks of hospitalization for kidney disease, hospitalization for heart failure and all-cause death when compared to DPP4i treatment.

	Dapagliflozin N=8582		DPP4i N=25,746		Weighted average estimates Dapagliflozin vs DPP4i; N=34,328		
	No. events	Rate/100 PYR	No. events	Rate/100 PYR	Hazard ratio	95% CI	p-value
Hospitalization for kidney disease	52	0.64	417	1.64	**0.38**	(0.29-0.51)	<0.001
Hospitalization for heart failure	77	0.95	375	1.47	**0.63**	(0.50-0.81)	<0.001
All cause death	106	1.04	468	1.44	**0.73**	(0.59-0.91)	0.004

Supported by: AstraZeneca
Disclosure: **J.W. Eriksson:** Employment/Consultancy; Consultancy: Merck Sharp & Dohme Corp., AstraZeneca, Sanofi, Novo Nordisk A/S. Grants; AstraZeneca, Novo Nordisk A/S. Lecture/other fees; AstraZeneca, Novo Nordisk A/S.

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Real-world persistence and HbA_{1c} goal attainment in type 2 diabetes patients initiated on canagliflozin or a glucagon-like peptide-1

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Background and aims: This study compared real-world persistence and HbA1c goal attainment in patients with type 2 diabetes mellitus (T2DM) initiated on oral canagliflozin 300 mg (CANA) versus injectable glucagon-like peptide 1 (GLP-1).
Materials and methods: Adults with T2DM newly initiated on CANA 300 mg or a GLP-1 (i.e., albiglutide, dulaglutide, exenatide, or liraglutide; with date of initiation as the index date) were identified from the QuintilesIMS EMRs - US database (03/29/2012-04/30/2016). Inverse probability of treatment weighting accounted for differences in baseline characteristics. Outcomes were compared using weighted Cox models (hazard ratios [HRs] and confidence intervals [CIs]) and Kaplan Meier curves and included time to: HbA1c <8%, discontinuation (gap >90 days), add/switch to a new antihyperglycaemic agent (AHA), and the composite of failure to maintain HbA1c <8% or add/switch to a new AHA. Attaining an HbA1c goal of <8% was evaluated among patients with baseline HbA1c ≥8%. Failure to maintain HbA1c <8% was evaluated among patients starting at HbA1c <8% (from index date) or reaching HbA1c <8% (from first day HbA1c below goal).
Results: 11,435 CANA and 11,582 GLP-1 patients (62.6% liraglutide) formed the weighted study cohorts, with well-balanced baseline characteristics and HbA1c. Time to HbA1c <8% was comparable (HR [95% CI]: 0.98 [0.91, 1.06]; p = 0.642) as was failure to maintain HbA1c <8% (HR: 1.00 [0.90, 1.11]; p = 0.988). CANA patients were 30% less likely to discontinue than GLP-1 patients (HR [95% CI]: 0.70 [0.66, 0.74]; p <0.001; median time to discontinuation 12.4 vs. 8.6 months) and were 28% less likely to add/switch to a new AHA (HR: 0.72 [0.68, 0.77]; p <0.001; median 21.3 vs. 15.1 months). CANA patients were 17% less likely to either fail to maintain HbA1c <8% or add/switch to a new AHA (HR [95% CI]: 0.83 [0.77, 0.90]; p <0.001; median 15.4 vs. 12.6 months).
Conclusion: Reaching and maintaining HbA1c below 8% was comparable between CANA and GLP-1 patients; however, fewer CANA patients required the addition/switch to a new AHA vs. GLP-1 patients.
Supported by: Janssen Scientific Affairs, LLC
Disclosure: **M. Pfeifer:** Employment/Consultancy; Janssen Scientific Affairs, LLC.

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Glycaemic control, weight loss, and use of other antihyperglycaemics in patients with type 2 diabetes initiated on canagliflozin or sitagliptin: a real-world analysis

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Background and aims: Real-world (RW) data showing benefits of canagliflozin (CANA) over sitagliptin (SITA) are emerging. This study assessed glycaemic control, weight loss, durability of glycaemic control, and use of other antihyperglycaemic agents (AHA) in patients initiated on CANA versus SITA.
Materials and methods: Adults with type 2 diabetes mellitus (T2DM) initiated on CANA or SITA (i.e., index date) were identified from the QuintilesIMS Real-World Data Electronic Medical Records - US database (03/29/2012-04/30/2016). Inverse probability of treatment weighting accounted for differences in baseline characteristics between cohorts. Outcomes included HbA1c over time and time to: reaching HbA1c goal (<7%, <8%, <9%), weight loss ≥5%, failure to maintain HbA1c goal, add/switch to a new AHA, and the composite of failure to maintain HbA1c goal or add/switch to a new AHA. HbA1c over time was assessed using moving averages at 3-month intervals pre and post-index. HbA1c goals were evaluated among patients with baseline HbA1c above goal. Failure to maintain HbA1c goal was evaluated among patients with baseline HbA1c below goal or who reached goal following index treatment initiation. Time-to event outcomes were compared using weighted Cox models and Kaplan Meier curves.

Results: A total of 14,165 CANA patients and 15,528 SITA patients formed the study population. Baseline characteristics and HbA1c were well balanced between weighted cohorts. Post-index, mean HbA1c declined in both cohorts and was significantly lower ($p < 0.01$) in CANA versus SITA patients at each interval, up to 30 months (except 21 months, $p = 0.18$). CANA patients were 12 to 15% more likely to reach HbA1c goals of <7%, <8%, or <9% (all $p < 0.01$) and 47% more likely to lose ≥5% of body weight ($p < 0.01$), relative to SITA patients. CANA patients were 31% less likely to add/switch to a new AHA (median time to switch 20.3 vs. 13.1 months) and 10 to 15% less likely to fail to maintain their HbA1c <7%, <8%, or <9% (all $p < 0.01$). Combining these two endpoints as a proxy for treatment failure, CANA patients were 12 to 16% less likely to fail to maintain goal or add/switch to a new AHA (all $p < 0.01$).

Conclusion: In a RW analysis, CANA patients were more likely to reach HbA1c goals, weight loss goals, and to maintain HbA1c below goal compared to SITA patients. They were also less likely to add new AHAs and to either fail to maintain or add/switch to a new AHA. These findings suggest a higher durability of glycaemic control in patients initiated on CANA relative to SITA.

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Disclosure: **M. Ingham:** Employment/Consultancy; Janssen Scientific Affairs, LLC.

Conclusion: Canagliflozin reduced HbA1c, weight, BMI, systolic blood pressure and ALT by clinically and statistically significant amounts in a wide range of real-world UK patients with type 2 diabetes. Rates of genital infection and urinary tract infection were similar to those found in clinical trials. Hypoglycaemia only occurred in patients on insulin.

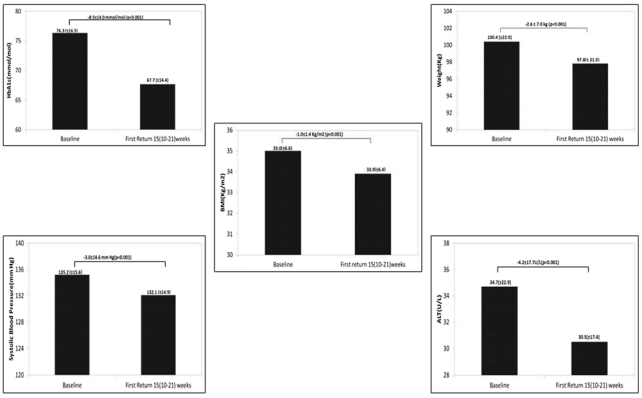

Figure: Mean (±SD) HbA1c (n=462) , weight (n=421), BMI (n=364), systolic blood pressure (n=444)and ALT(n=315), baseline vs first return (after median (interquartile range) weeks) to clinic following commencement of canagliflozin.

Disclosure: **M. Yadagiri:** None.

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One year metabolic outcomes in the ABCD nationwide canagliflozin audit

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Background and aims: The ABCD nationwide canagliflozin audit was launched in January 2016 to evaluate the efficacy and safety of canagliflozin in real clinical use in the UK.

Materials and methods: One year on, 40 contributors from 21 centres across the UK have submitted data on 687 canagliflozin-treated patients (61.8% males, mean (±SD) aged 59.8 ±10.8 years, weight 100.4±21.9 kg, BMI 34.9±6.6 kg/m^2 and HbA1c 76.3±16.6mmol/mol, median (range) duration of diabetes 7.0(2.7-12.0) years. Those with baseline and follow-up HbA1c within a median (range) of 14.8(10.2-21.0) weeks were included in the analysis.

Results: Mean (±SD) HbA1c fell by 8.5±14.0 mmol/mol from 76.3 ±16.5 mmol/mol to 67.7±14.4 mmol/mol (n=462, $p<0.001$) and weight fell by 2.6±7.0 kg from 100.4±22.0 kg to 97.8±21.5kg (n=421, $p<0.001$). BMI dropped by 1.0 ±1.4 Kg/m² from mean of 35.0±6.6 Kg/m² to 33.9±6.4 Kg/m² (n=364, $p<0.001$), systolic blood pressure fell by 3.0±14.6 mmHg from mean of 135.2±15.6 mm Hg to 132.1±14.9 mmHg (n=444, $p<0.001$) and alanine aminotransaminase (ALT) levels dropped by 4.2±17.7 U/L from 34.7±22.9 U/L to 30.5±17.4U/L (n=315, $p<0.001$) (figure). Out of 254 patients treated with canagliflozin where data was provided, 8.2% (n=21) had genital infections requiring treatment, 2.7% (n=7) urinary tract infection, 6.7% (n=17) minor, 1% (n=3) moderate and 1 %(n=3) severe hypoglycaemia respectively. All the hypoglycaemia cases were on insulin.

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Baseline characteristics and treatment patterns of Canadian CAnagliflozin REgistry (CanCARE): assessment of canagliflozin treatment in usual clinical practice in Canada

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Background and aims: Although extensively studied in randomized controlled clinical trials, there is limited information on the effects of canagliflozin (CANA) in a real-world practice setting in Canada.

Materials and methods: This prospective cohort study enrolled SGLT2 inhibitor naïve T2DM adult patients with A1c ≥7% and eGFR ≥ 60 mL/min/1.73m^2 who were on a stable anti-hyperglycemic agent (AHA) regimen and were initiated on CANA as part of their optimal treatment approach. The objectives of this baseline analysis are to evaluate patient profiles and treatment patterns at the time of CANA initiation.

Results: Table 1 summarizes baseline characteristics of the study. A total of 528 T2DM patients in 28 centers (58% general practitioners - GPs, 42% specialists) initiated CANA treatment, with 83% starting on 100 mg and 17% on 300 mg dose. CanCARE included a diverse study population (67.4% Caucasian; 17.0% South Asian; 5.3% East Asian, 4.9% African/African American; 3.4% Other/Not Reported) of males (60.6%) and females (39.4%). Metformin (89%), DPP-4 inhibitors (43%), sulfonylureas (40%) and insulin (24%) were the most prescribed AHAs at baseline. Of patients on insulin (mean dose 60 IU (±49), 69% were treated by specialists. More patients treated by specialists had an A1c >8.5% (44%) and had a T2DM diagnosis for >10 years (59%) while more of those treated by GPs had an A1c of 7.5 - 8.5% (46%) and had a diagnosis of T2DM for <5 years (39%).

Conclusion: CANA is being prescribed in a broad variety of patient populations in Canada.

Table 1. Baseline Characteristics and Initial Treatment Patterns of Canagliflozin Treated Patients

Patient Characteristic	N=528 Unless Otherwise Noted
Age, y	60.7 ± 10.78
Duration of Diabetes, y	9.8 (7.30)
Cardiac Disorders, n (%)	84 (15.9%)
A1c, mean (std)	8.4 (1.21)
Systolic Blood Pressure (mmHg)	130.9 ±12.89 (N=526)
Diastolic Blood Pressure (mmHg)	78.4 ± 9.33 (N=526)
Body Mass Index (kg/m^2)	32.1 ±6.41 (N=523)
eGFR (mL/min/1.73m^2)	86.8 ±23.95 (N=513)
Canagliflozin Initiation Treatment Patterns, n (%)	
Canagliflozin Only	13 (2.5%)
Canagliflozin + 1 non-insulin AHA	126 (23.9%)
Canagliflozin + 2 non-insulin AHAs	177 (33.5%)
Canagliflozin + 3 non-insulin AHAs	78 (14.8%)
Canagliflozin + Insulin +/- non-insulin AHA	127 (24.1%)
Antihypertensive medications	382 (72.3%)
Lipid lowering medications	428 (81.1%)

Notes: For categorical variables values are n (%) with % = 100*n/N with N = 528; for continuous variables values are mean ± std (N).

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Disclosure: **V. Woo:** Honorarium; Novo Nordisk A/S, Eli Lilly and Company, Merck, Boehringer Ingelheim, Bristol-Myers Squibb, Sanofi, AstraZeneca, Johnson & Johnson Diabetes, Roche, Abbott. Lecture/other fees; Novo Nordisk A/S, Eli Lilly and Company, Merck, Boehringer Ingelheim, Bristol-Myers Squibb, Sanofi, AstraZeneca, Johnson & Johnson Diabetes, Roche, Abbott.

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Predictors of response to SGLT2-inhibitors and DPP4-inhibitors

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Background and aims: Stratified medicine targets treatment according to patient characteristics. We have shown simple criteria of sex and obesity can predict differential response to thiazolidinediones and sulfonylureas. SGLT2 inhibitors and DPP4-inhibitors are also second-line therapies used to treat type 2 diabetes, with different mechanisms of action. We aimed to determine whether patient characteristics predicted differential response to these two therapies.
Materials and methods: Patients with prescription records and valid 6-month response (change in HbA1c from baseline) for DPP4-inhibitors (n=21117) or SGLT2-inhibitors (n=3071) were identified from the UK Primary Care Clinical Practice Research Datalink. Regression models were used to identify predictors of response, with βs presented as the change in HbA1c per 1 standard deviation increase in the predictor (negative βs representing a better response at higher values of the predictor).
Results: Higher eGFR was associated with a lesser response to DPP4-inhibitors but a better response to SGLT2-inhibitors (β[SE]= 0.22[0.09] v -1.81[0.26], p<0.0001). Higher BMI and triglycerides were associated with a lesser response to DPP4 inhibitors compared with SGLT2 inhibitors (BMI: β[SE]=0.99[0.1] v -0.13[0.24], triglycerides: 0.76[0.14] v -0.36[0.37], p<0.001 for both). For both DPP4-inhibitors and SGLT2-inhibitors, higher baseline HbA1c (DPP4: β[SE]=-8.46[0.11]; SGLT2: β[SE]=-9.36 [0.26], p<0.0001 for both), and shorter duration of diabetes (DPP4: β[SE]=0.45[0.08]; SGLT2: β[SE]=1.57[0.21], p<0.001 for both) were associated with a greater reduction in HbA1c at 6 months.
Conclusion: These preliminary analyses identify simple criteria that may partly explain variability in the response to SGLT2-inhibitors and DPP4-inhibitors. The associations between eGFR and 6-month response to SGLT2-inhibitors and DPP4-inhibitors go in opposite directions, indicating potential criteria to aid treatment decisions in Type 2 diabetes.
Supported by: MRC
Disclosure: **B.M. Shields:** None.

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Efficacy and safety of SGLT2 in Ramadan

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Background and aims: It is estimated that approximately 40-50 million people with diabetes worldwide fast during the holy month of Ramadan. During this fasting period, Muslims abstain from food and drinks (including oral medications) from dawn to dusk. The risk of hypoglycemia varies according to oral anti-diabetic agent used for treatment[1] ,which is of significant concern in insulin, sulfonylureas and/or non-sulfonylurea insulin secretagogues.however in consider safe to continue other anti-diabetic as lower risk of hypoglycemia . SGLT2 is a low-affinity, high capacity glucose transporter located in the proximal tubule in the kidneys, and it is responsible for 90% of glucose reabsorption. Inhibition of SGLT2 leads to the decrease in blood glucose as a consequence of increased renal glucose excretion it has insulin-independent action, are effective in reducing HbA1c by 0.5%-1.5%, promote weight loss, have a low incidence of hypoglycemia . To study the effectiveness and safety of SGLT2 in emarati population during Ramadan
Materials and methods: We screened all Muslim patients with type 2 diabetes who had been were on, or started on treatment with SGLT2 inhibitors by March 30th, 2016 for eligibility for the trial. All prescriptions were compliant with DHA protocols and recommendations hence none was pregnant, all were > 18 years of age, and none of the patients was having an e-GFR of <45ml/min/m2. We looked at demographic data as well as lab results before Ramadan. Another set of Data was collected after Ramadan.
Results: 417 patients, the majority were females (58.5%). The mean age was 54+ 11.6. At baseline, the mean duration of diabetes was 13.4+6.6 years, and the duration ranged between 3months to 37 years. The mean glycated hemoglobin level was 8.3 + 1.7%. Mean weight was 83.8+ 16.6kg.Out of 226 patients on SGLT2 inhibitors + oral hypoglycemic agents, 41 (18.3%) episodes of hypoglycemia were seen (36.3% of all hypoglycemic episodes seen during Ramadan), while 71 episodes (38.1%), (62.9% of all hypoglycemic episodes seen during Ramadan), were seen in those using insulin (N=191) the P=0.000 highly significant.The

mean glycated hemoglobin level was 8.3 + 1.7% before Ramadan that has significantly reduced to 7.8+ 1.3% (p=0.0001) after Ramadan.
Conclusion: SGLT2 found to be safe and effective in reducing HBA1C during Ramadan
Disclosure: **H.M. Omran:** None.

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Tolerability of canagliflozin in patients with type 2 diabetes fasting during Ramadan: results of the Canagliflozin in RAmadan Tolerance Observational Study (CRATOS)

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Background and aims: There is a large population of people with type 2 diabetes mellitus (T2DM) who are Muslims and fast during Ramadan. Changes in the pattern and amount of meal and fluid intake during Ramadan in addition to the long fasting hours, especially in Europe, may increase the risk of hypoglycaemia and dehydration in these individuals. The Canagliflozin in Ramadan Tolerance Observational Study (CRATOS) evaluated the tolerability of canagliflozin (CANA), an SGLT2 inhibitor, compared with sulphonylureas (SUs) among patients with T2DM who fast during Ramadan.
Materials and methods: This non-randomized, parallel-cohort, prospective, comparative, observational study was conducted in the Middle East (Lebanon, Kuwait, United Arab Emirates) during Ramadan (6 June-5 July 2016) and enrolled patients who were taking CANA (n = 162) or any SU (n = 159) added to metformin ± DPP-4 inhibitor for >12 weeks before enrolment and had HbA1c ≤8.5% within 8 weeks before Ramadan. The proportion of patients with hypoglycaemia events and volume depletion events were assessed; propensity score analysis was used to calculate adjusted odds ratios (ORs) and 95% confidence intervals (CIs). Other assessments included medication and fasting adherence and adverse events (AEs).
Results: During Ramadan, fewer fasting patients experienced hypoglycaemia events with CANA versus SU (adjusted OR [95% CI]: 0.273 [0.104, 0.719]). Of hypoglycaemia events where blood glucose was measured at the time of the event, 2 of 6 with CANA and 27 of 37 with SU were confirmed by blood glucose <3.9 mmol/L. No severe hypoglycaemia events were reported in either group. More patients experienced volume depletion events (mostly dehydration) with CANA versus SU (adjusted OR [95% CI]: 3.5 [1.3, 9.2]). 82.1% and 78.0% of patients reported completing 30 days of fasting with CANA and SU, respectively. The most common reason for breaking fast among CANA patients (n = 29) was symptoms of excessive dehydration (24.1%) and among SU patients (n = 35) was symptoms of low blood sugar (34.3%). 98.8% and 96.2% of patients reported no missed doses of CANA and SU, respectively. One dose of CANA was missed due to excessive dehydration and 1 because the patient forgot; 4 doses of SU were missed to prevent hypoglycaemia, 1 as a precaution, and 1 because the patient forgot. CANA and SU were generally well tolerated, with low rates of AEs and no serious AEs (including hypoglycaemia and volume depletion-related AEs).
Conclusion: Among patients with T2DM fasting for Ramadan, the risk of a hypoglycaemia event was lower and the risk of a volume depletion event was higher with CANA versus SU. Overall, these findings support the use of CANA for the treatment of adults with T2DM who fast during Ramadan.
Clinical Trial Registration Number: NCT02737657
Supported by: Janssen Pharmaceutica NV
Disclosure: **M. Hasanein:** Lecture/other fees; Novo Nordisk, Lilly, Sanofi, Merck Sharp & Dohme, Janssen, AstraZeneca, Servier, Novartis, Takeda, Abbott, Johnson & Johnson.

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Complications of type 2 diabetes after SGLT2 inhibitor administration for 18 months: examination using two risk engines

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Background and aims: Sodium-glucose cotransporter-2 (SGLT2) inhibitors became available in Japan in April 2014. In recent years, several studies have shown SGLT2 inhibitors' preventive effects on diabetic complications, but the effects of long-term administration remain unknown. To examine the risk of diabetic complications among patients with type 2 diabetes mellitus (T2DM) 5 and 10 years after 18-month administration of an SGLT2 inhibitor, we used the Japan Diabetes Complications Study /the Japanese Elderly Diabetes Intervention Trial risk engine (JJ risk engine) and examined retrospectively. The risk engine uses an algorithm to calculate the risk of complications based on Japanese patient data , a large clinical study involving 59 diabetes centers. We also used the UK Prospective Diabetes Study (UKPDS) risk engine.
Materials and methods: Participants were 205 Japanese T2DM outpatients (age, 53.8±11.3years; BMI, 30.77±5.44 kg/m^2; DM duration, 8.1 ±5.7 years; HbA1c, 8.63±1.46%) who started to receive an SGLT2 inhibitor between April 2014 and September 2015 from our Medical University Hospital. Taking into account ethnical and racial differences, we used the JJ risk engine to examine the risk of CHD, stroke, death from non-cardiovascular disease, overt nephropathy, and progression of retinopathy 5 and 10 years after an 18-month administration of an SGLT2 inhibitor. We also used the UKPDS risk engine to examine the risk of fatal and non-fatal CHD, and fatal and non-fatal stroke 5 and 10 years after an 18-month administration of an SGLT2 inhibitor. Where applicable, we compared the two engines' estimates between pre- and post-18-month administrations of SGLT2 inhibitors.
Results: After the 18-month administration of an SGLT2 inhibitor, HbA1c, BMI, systolic blood pressure, and cholinesterase significantly decreased (8.63±1.46 to 7.57±1.20, 30.77±5.44 to 29.6±4.80, 136.7±14.6 to 130.2 ±15.2, 402.3±82.9 to 384.0±91.9, respectively), but HDL cholesterol significantly increased (46.5±10.6 to 48.7±11.5). Total cholesterol showed no significant differences. Compared with pre-administration risk, post-administration risk estimated with the JJ risk engine was significantly lower in CHD (5 years post treatment: 2.00±2.92 to 1.40±1.29, p<0.01; 10 years post treatment: 5.55±3.90 to 4.59±2.61, p<0.001), stroke (5 years: 2.82±2.87 to 2.25±2.30, p<0.01; 10 years: 4.41±3.72 to 3.61±3.25, p<0.01), and overt nephropathy (5 years: 5.20±3.56 to 3.88±2.42, p<0.001; 10 years: 9.53±4.82 to 7.60±4.27, p<0.001). The risk was significantly higher for death from non-cardiovascular disease (5 years: 1.13±1.53 to 1.30±1.94, p<0.05; 10 years: 2.33±3.53 to 2.65±4.04, p=0.001), and no significant differences in the risk of progression of retinopathy were detected. Thirty-six patients discontinued receiving an SGLT2 inhibitor due to side effects or other reasons including: discontinuation of hospital visits (14 patients); polyuria (3 patients); genital infection (2 patients); rash, hypoglycemia, dizziness, muscle pain, transient ischemic attack (1 patient each); and others (12 patients).
Conclusion: This study shows that SGLT2 inhibitors potentially decrease the risk of CHD, stroke, and overt nephropathy 5 and 10 years after 18-month administration. We found that some estimates were different between the JJ and UKPDS risk engines, which may be attributed to Japanese people's higher morbidity of stroke and lower morbidity of CHD than Europeans.
Disclosure: **A. Teshima:** None.

PS 070 SGLT2 inhibitors: new class members and combinations

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Sustained 52 week efficacy and safety of triple therapy with dapagliflozin + saxagliptin vs dual therapy with sitagliptin as add-on to metformin in uncontrolled type 2 diabetes

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Background and aims: Adding a single antidiabetes agent to metformin (MET) often fails to improve glycaemic control meaningfully in type 2 diabetes mellitus (T2DM) patients with high glycated haemoglobin (HbA_{1c}) levels. The efficacy and safety of triple therapy using dapagliflozin (DAPA) plus saxagliptin (SAXA) added to MET vs dual therapy with sitagliptin (SITA) added to MET were evaluated in a randomized, double-blind, double-dummy, 26-week trial in patients with T2DM. Safety and tolerability profiles were assessed in an extension to 52 weeks.

Materials and methods: Patients with HbA_{1c} 8.0%—10.5% on stable MET (≥1500 mg/day) were randomized (1:1) to once-daily add-on oral therapy with DAPA 10 mg/day plus SAXA 5 mg/day or SITA 100 mg/day. The primary end point was change in HbA_{1c} from baseline to week 26. Exploratory end points assessed at 52 weeks included change in HbA_{1c} from baseline, proportion of patients achieving a therapeutic glycaemic response (HbA_{1c} <7.0%), changes in body weight and fasting plasma glucose (FPG), and proportion of patients requiring rescue therapy or discontinuation for lack of glycaemic control. Incidences of adverse events (AEs) and hypoglycaemia were also assessed.

Results: Of 461 randomized patients, 411 (89%) completed the 26-week study, and 402 (87%) entered the extension phase. Mean±SD baseline data at randomization were similar between groups (age, 56 ±9.2 years; HbA_{1c}, 8.8±0.9%; T2DM duration, 8.0±5.4 years; BMI, 33.1±6.2 kg/m^2). Overall, efficacy outcomes achieved at week 26 were maintained up to week 52. Reduction from baseline to week 52 in HbA_{1c} was greater with DAPA+SAXA+MET vs SITA+MET (−1.3% vs −0.8%) (Table). Reductions in body weight (−2.3 kg vs −0.8 kg) and FPG (−25.5 mg/dL vs −3.6 mg/dL) were greater, and more patients achieved HbA_{1c} <7.0% (33.0% vs 19.5%) with DAPA+SAXA+MET vs SITA+MET. Fewer patients required rescue or discontinuation with triple vs dual therapy (19% vs 32%). Incidences of AEs of interest with DAPA+SAXA+MET vs SITA+MET were confirmed hypoglycaemia, 5.2% vs 3.9%; urinary tract infections, 6.5% vs 3.5%; and genital infections, 3.4% vs 2.2%. Fewer patients discontinued owing to AEs with triple vs dual therapy (1.7% vs 4.4%). Safety and tolerability profiles were consistent with 26-week data.

Conclusion: After 52 weeks of treatment, adding DAPA+SAXA to MET provided sustained greater improvements in glycaemic control, greater weight reductions, no increased hypoglycaemic risk, and a reduced need for rescue/discontinuation compared with SITA+MET, consistent with week 26 results. Safety and tolerability of both regimens were similar to previous findings.

Table. Results for efficacy end points at week 52

Study end point	DAPA+SAXA+MET N=232	SITA+MET N=229	Difference (95% CI)
Mean±SD HbA_{1c} (%) at baseline	n=224 8.8±0.8	n=219 8.9±0.9	–
Change in HbA_{1c} from baseline (%)			
Week 26	n=206 −1.4±0.1	n=184 −1.1±0.1	−0.3 (−0.5, −0.1)
Week 52	n=156 −1.3±0.1	n=112 −0.8±0.1	−0.5 (−0.7, −0.3)
Proportion of patients with HbA_{1c} <7.0% (%)	n=224 33.0±3.1	n=219 19.5±2.6	13.5 (5.6, 21.4)
Change in body weight from baseline (kg)	n=157 −2.3±0.3	n=112 −0.8±0.3	−1.6 (−2.4, −0.8)
Change in FPG from baseline (mg/dL)	n=156 −25.5±3.3	n=111 −3.6±3.7	−22.0 (−31.7, −12.3)
Proportion of patients discontinued owing to lack of glycaemic control or rescued for not achieving pre-specified glycaemic targets (%)	n=42* 18.6±2.5	n=75* 32.3±3.1	−13.8 (−21.4, −6.1)

All data are adjusted mean±SE unless otherwise specified.
A mixed statistical model was used to analyse between-group differences.
n is the number of randomized patients with non-missing baseline and week 26 or week 52 values (last observation carried forward), except for mean baseline and week 26 or week 52 HbA_{1c}, where n is the number of randomized patients with non-missing baseline value and at least one post-baseline value.
*number of responders.
CI, confidence interval; DAPA, dapagliflozin; FPG, fasting plasma glucose; HbA_{1c}, glycated haemoglobin; MET, metformin; SAXA, saxagliptin; SITA, sitagliptin.

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Efficacy and safety of ertugliflozin in patients with type 2 diabetes inadequately controlled with metformin monotherapy: VERTIS MET trial

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Background and aims: Ertugliflozin (ERTU) is an oral sodium-glucose cotransporter 2 inhibitor in development for treatment of patients with T2DM. This Phase 3, randomised, double-blind, 26-wk, multicenter study with an ongoing 78-wk extension evaluated the efficacy and safety of ERTU vs placebo (PBO) in adults with T2DM inadequately controlled (HbA1c 7.0-10.5%) on metformin (MET) monotherapy (≥1500 mg/day for ≥8 wks).

Materials and methods: Patients (N=621) with baseline mean ± SD age 56.6 ± 8.8 y, T2DM duration 8.0 ± 6.0 y, BMI 30.9 ± 4.7 kg/m^2 and HbA1c 8.1 ± 0.9% received PBO, ERTU 5 mg/day or ERTU 15 mg/day in a 1:1:1 ratio. Changes from baseline in efficacy endpoints (HbA1c [primary endpoint], fasting plasma glucose [FPG], body weight and BP), and proportion of subjects with HbA1c <7% at Wk 26 were tested for each ERTU group vs PBO. All efficacy analyses were based on the full analysis set, excluding data after initiation of glycaemic rescue therapy. A constrained longitudinal data analysis model with fixed effects for treatment, time, prior

antihyperglycaemic agent, baseline estimated glomerular filtration rate, menopausal status (men, premenopausal women, women perimenopausal or <3 y postmenopausal, women ≥3 y postmenopausal) and interaction of time by treatment was used to assess the endpoints. To control the overall Type I error rate at 0.05, a sequential testing approach was used across efficacy endpoints. Pre-specified adverse events (AEs) of special interest were subject to inferential testing without multiplicity control (p-values provided if <0.05). Percent changes from baseline in bone mineral density (BMD) were also assessed for ERTU vs PBO at Wk 26.

Results: At Wk 26, ERTU groups had significantly reduced HbA1c, FPG, body weight and BP vs PBO (Table). Patients in ERTU groups were significantly more likely to have HbA1c <7% at Wk 26 vs PBO (5 mg, 35%; 15 mg, 40%; vs PBO, 16%; p<0.001 for both ERTU groups). The incidence of AEs was 45.0%, 42.5%, and 50.2% in the PBO, ERTU 5 mg and ERTU 15 mg groups, respectively. The incidence of genital mycotic infections increased in ERTU groups vs PBO (females: PBO, 0.9%; ERTU 5 mg, 5.5%; ERTU 15 mg, 6.3% [p=0.032 vs PBO]; males: PBO, 0; ERTU 5 mg, 3.1%; ERTU 15 mg, 3.2%). The incidence of urinary tract infections was 1.0%, 2.9% and 3.4% in the PBO, ERTU 5 mg and ERTU 15 mg groups, respectively. The incidence of symptomatic hypoglycaemia and hypovolaemia AEs was similar between groups. ERTU had no adverse impact on BMD at Wk 26 (Table).

Conclusion: ERTU added to MET in patients with inadequately controlled T2DM improved glycaemic control, reduced body weight and BP without impacting BMD, but with an increased incidence of GMIs.

		PBO		ERTU 5 mg	ERTU 15 mg
HbA1c (%), mean ± SD	Baseline / Wk 26	8.2 ± 0.9 / 7.8 ± 1.1		8.1 ± 0.9 / 7.3 ± 0.8	8.1 ± 0.9 / 7.2 ± 0.8
		ERTU 5 mg vs PBO		**ERTU 15 mg vs PBO**	
		Estimate (95% CI)	p-value	Estimate (95% CI)	p-value
Efficacy: Change from baseline at Wk 26 (difference in least squares mean)*	HbA1c (%)	-0.70 (-0.87, -0.53)	<0.001	-0.88 (-1.05, -0.71)	<0.001
	FPG (mmol/L)	-1.48 (-1.83, -1.14)	<0.001	-2.12 (-2.47, -1.78)	<0.001
*p-values provided for efficacy endpoints only	Body weight (kg)	-1.67 (-2.24, -1.11)	<0.001	-1.60 (-2.16, -1.03)	<0.001
	Systolic blood pressure (mmHg)	-3.68 (-5.96, -1.39)	0.002	-4.50 (-6.81, -2.19)	<0.001
	Diastolic blood pressure (mmHg)	-1.82 (-3.24, -0.39)	0.013	-2.42 (-3.86, -0.98)	0.001
BMD: Change from baseline at Wk 26 (%) in BMD (difference in least squares mean)	Lumbar spine	-0.23 (-0.83, 0.37)	—	-0.10 (-0.71, 0.50)	—
	Femoral neck	0.30 (-0.38, 0.99)	—	0.70 (0.00, 1.39)	—
	Total hip	0.08 (-0.33, 0.48)	—	0.27 (-0.15, 0.68)	—
	Distal forearm	-0.21 (-0.78, 0.35)	—	-0.19 (-0.76, 0.39)	—

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Disclosure: **B. Charbonnel:** None.

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Safety and efficacy of ertugliflozin plus sitagliptin vs either treatment alone after 52 wks in patients with type 2 diabetes poorly controlled on metformin: VERTIS FACTORIAL extension

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Background and aims: Ertugliflozin (ERTU) is an oral sodium/glucose cotransporter 2 (SGLT2) inhibitor in development for treatment of type 2 diabetes mellitus (T2DM). This study compared the safety and efficacy of co-administration of ERTU 5 mg or 15 mg plus sitagliptin (SITA) 100 mg compared with either treatment alone over 52 weeks.

Materials and methods: In a double-blind Phase 3 trial, 1233 patients with HbA1c 7.5-11.0% on stable metformin monotherapy ≥1500 mg/day were randomised into 5 groups (Table). ERTU + SITA combinations were compared with corresponding ERTU doses (5 or 15 mg) or SITA alone. The primary outcome was at Week 26; treatment was continued in a double-blind 26-week extension phase.

Results: Mean HbA1c at baseline was 8.6%. After 52 weeks in the ERTU+SITA groups, greater reductions in HbA1c, fasting plasma glucose (FPG) (vs ERTU or SITA alone), body weight and systolic BP (vs SITA alone) were observed (Table). The odds of having an HbA1c <7.0% were greater for ERTU+SITA versus ERTU or SITA alone. Administration of ERTU alone or with SITA was well tolerated overall. Rates of genital mycotic infections with ERTU+SITA were similar to those observed with ERTU alone, and significantly higher than those observed with SITA alone (p<0.05, except ERTU 5 mg+SITA in females). Symptomatic hypoglycaemia rates were not significantly different among groups but were highest in the ERTU 15 mg+SITA group. Overall the incidences of urinary tract infection and hypovolaemia were similar across groups.

Conclusion: Co-administration of ERTU+SITA resulted in effective glycaemic control sustained over 52 weeks and was generally well-tolerated.

Table. Summary of key efficacy endpoints at Week 52 (excluding rescue approach)

		ERTU 5 mg (n=250)	ERTU 15 mg (n=248)	SITA 100 mg (n=247)	ERTU 5 mg + SITA 100 mg (n=243)	ERTU 15 mg + SITA 100 mg (n=244)
Change from baseline, LS mean (95% CI)†	**HbA1c, %**	−1.0 (−1.1, −0.8)	−0.9 (−1.1, −0.8)	−0.8 (−1.0, −0.7)	−1.4 (−1.5, −1.2)	−1.4 (−1.5, −1.3)
	FPG, mmol/L	−1.6 (−1.9, −1.3)	−1.7 (−2.0, −1.4)	−0.8 (−1.1, −0.5)	−2.2 (−2.5, −1.9)	−2.3 (−2.6, −2.0)
	mg/dL	*−28.7 (−33.7, −23.6)*	*−30.8 (−36.1, −25.5)*	*−15.2 (−20.6, −9.8)*	*−39.3 (−44.3, −34.2)*	*−41.8 (−46.8, −36.8)*
	Body weight, kg	−2.4 (−2.9, −1.8)	−3.2 (−3.8, −2.7)	−0.1 (−0.7, 0.5)	−2.4 (−3.0, −1.8)	−2.8 (−3.4, −2.2)
	Systolic BP, mmHg	−2.7 (−4.2, −1.2)	−1.6 (−3.1, 0.0)	−0.2 (−1.8, 1.5)	−2.3 (−3.8, −0.8)	−2.2 (−3.7, −0.7)
Patients with an HbA1c <7.0%, n (%)		64 (25.6)	56 (22.6)	66 (26.7)	99 (40.7)	97 (39.8)

†Constrained longitudinal (cLDA) model with fixed effects for treatment, time, baseline eGFR (continuous), and the interaction of time by treatment. As the primary outcome was at Week 26, no formal statistical inference was performed for efficacy endpoints at Week 52 to compare treatment groups
LS, least squares

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Safety and efficacy of ertugliflozin in combination with sitagliptin in patients with type 2 diabetes inadequately controlled on diet and exercise: the VERTIS SITA trial

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Background and aims: Ertugliflozin (ERTU) is an oral sodium/glucose cotransporter 2 (SGLT2) inhibitor in development for the treatment of type 2 diabetes mellitus (T2DM). The efficacy and safety of initial combination therapy of ERTU and sitagliptin (SITA) was compared to that of placebo (PBO) in a 26-week, double-blind, randomised Phase 3 trial.

Materials and methods: Patients (n=291) with HbA1c 8.0-10.5% on diet and exercise were randomised 1:1:1 to ERTU 5 mg + SITA 100 mg (E5/S100), ERTU 15 mg + SITA 100 mg (E15/S100), or PBO.

Results: Baseline characteristics were generally comparable among groups (mean age 55.6 years, HbA1c 8.9%, T2DM duration 6.3 years, eGFR 90.7 mL/min/1.73m^2). After 26 weeks, significantly greater reductions from baseline were observed in HbA1c, fasting plasma glucose (FPG), 2-hour post meal glucose (PMG), body weight, and systolic blood pressure (SBP) in patients receiving E5/S100 and E15/S100 compared with PBO (Table). Observed reductions in diastolic BP were not significant (p>0.05). The odds of having an HbA1c <7.0% were significantly higher in E5/S100 and E15/S100 groups than PBO. The incidence of adverse events (AEs) was generally similar across groups. Pre-specified AEs of urinary tract infections, genital mycotic infections by gender, symptomatic hypoglycaemia and hypovolaemia were subjected to inferential testing: incidence rates were low and not significantly different across groups.

Conclusion: Initial co-administration of ERTU with SITA provided effective glycaemic control over 26 weeks and was generally well-tolerated.

Table. Summary of key efficacy endpoints at Week 26 (excluding rescue approach)

		PBO (n=96)	E5/S100 (n=98)	E15/S100 (n=96)	Pairwise comparison: E5/S100 vs PBO	Pairwise comparison: E15/S100 vs PBO
LS mean change from baseline (95% CI)[1]	A1C, %	−0.4 (−0.7, −0.2)	−1.6 (−1.8, −1.4)	−1.7 (−1.9, −1.5)	−1.2 (−1.5, −0.8)*	−1.2 (−1.6, −0.9)*
	FPG, mmol/L	−0.5 (−1.0, −0.0)	−2.7 (−3.1, −2.2)	−3.1 (−3.5, −2.6)	−2.2 (−2.8, −1.6)*	−2.6 (−3.2, −1.9)*
	mg/dL	*−9.3 (−18.6, −0.02)*	*−48.3 (−56.1, −40.4)*	*−55.4 (−63.3, −47.4)*	*−38.9 (−49.9, −28.0)**	*−46.1 (−57.1, −35.0)**
	2-hour PMG, mmol/L	−1.1 (−2.0, −0.3)	−4.6 (−5.3, −3.9)	−5.0 (−5.7, −4.3)	−3.5 (−4.5, −2.5)*	−3.9 (−4.9, −2.9)*
	mg/dL	*−20.4 (−35.6, −5.1)*	*−82.8 (−96.0, −69.6)*	*−90.0 (−103.3, −76.7)*	*−62.4 (−80.5, −44.4)**	*−69.7 (−87.8, −51.5)**
	Body weight, kg	−0.9 (−1.7, −0.2)	−2.9 (−3.6, −2.3)	−3.0 (−3.7, −2.4)	−2.0 (−3.0, −1.0)*	−2.1 (−3.1, −1.1)*
	SBP, mmHg	2.4 (−0.3, 5.2)	−2.0 (−4.2, 0.2)	−4.0 (−6.2, −1.8)	−4.4 (−7.9, −1.0)**	−6.4 (−9.8, −3.0)*
Patients with an HbA1c <7.0%, n (%)		8 (8.3)	35 (35.7)	30 (31.3)	OR:[3] 6.9 (2.8, 16.8)*	OR:[3] 7.4 (3.0, 18.3)*

*p<0.001 vs PBO; **p=0.011 vs PBO
[1]Constrained longitudinal (cLDA) model with fixed effects for treatment, time, antihyperglycaemic medication wash-off status, baseline eGFR (continuous) and the interaction of time by treatment
[3]Adjusted OR (95% CI) vs PBO based on a logistic regression model fitted with fixed effects for treatment, antihyperglycaemic medication wash-off status, baseline A1C and baseline eGFR (continuous), with missing data imputed using the cLDA model
LS, least squares

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Long-term efficacy and safety of ertugliflozin monotherapy in patients with inadequately controlled type 2 diabetes despite diet and exercise: the 52-week VERTIS MONO study

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Background and aims: Ertugliflozin (ERTU) is an oral sodium-glucose cotransporter 2 inhibitor that is in development for the treatment of patients with T2DM. This Phase 3, multicenter, randomised study in adults with T2DM and HbA1c 7.0-10.5% despite diet and exercise comprised a 26-week, double-blind, placebo (PBO)-controlled period followed by a 26-week, active-controlled period in which patients in the PBO arm who had not received glycaemic rescue therapy had metformin (MET) added. Efficacy and safety of ERTU through Week 52 are reported.
Materials and methods: Patients (N=461) with baseline mean (± SD) age 56.4 (± 11.0) years, T2DM duration 5.0 (± 5.1) years, BMI 33.0 (± 6.7) kg/m^2 and HbA1c 8.2 (± 1.0)% were randomized to PBO, ERTU 5 mg/day or ERTU 15 mg/day in a 1:1:1 ratio. Least square (LS) mean (95% CI) changes from baseline in efficacy endpoints, and the proportion of subjects with HbA1c <7% at Week 52 were assessed for the ERTU groups. Genital mycotic infection (GMI), urinary tract infection (UTI), symptomatic hypoglycaemia and hypovolaemia adverse events (AEs) were subject to inferential testing without multiplicity control (p-values provided if <0.05).
Results: At Week 52, clinically meaningful reductions from baseline in HbA1c, fasting plasma glucose (FPG), body weight and systolic blood pressure (SBP) were observed in both ERTU groups. Approximately one-third of patients who received ERTU had HbA1c at goal (Table). Incidence of GMIs in females was significantly higher for ERTU 5 mg (26.9%; p=0.010) and ERTU 15 mg (29.0%; p=0.005) vs PBO/MET (9.9%) and in males, was higher for ERTU 5 mg (3.4%) and significantly higher for ERTU 15 mg (7.8%; p=0.042) vs PBO/MET (1.2%). The majority of GMIs occurred in the first 6 months of treatment. Incidence of UTIs was lower for ERTU 5 mg (10.9%) and ERTU 15 mg (6.6%; p=0.039) vs PBO/MET (13.7%). ERTU did not increase the incidence of symptomatic hypoglycaemia or hypovolaemia compared with PBO/MET.
Conclusion: ERTU, given over 52 weeks to adults with inadequately controlled T2DM despite diet and exercise, improved glycaemic control, reduced body weight and SBP, but increased the incidence of GMIs.

	Endpoint	ERTU 5 mg	ERTU 15 mg
Change from baseline at Week 52 [LS mean (95% CI)][a]	HbA1c (%)	-0.74 (-0.89, -0.58)	-0.89 (-1.05, -0.74)
	FPG (mmol/L)	-1.67 (-2.06, -1.28)	-2.08 (-2.46, -1.71)
	Body weight (kg)	-3.23 (-3.91, -2.55)	-3.38 (-4.06, -2.71)
	SBP (mmHg)	-3.27 (-5.57, -0.97)	-2.24 (-4.42, -0.05)
	DBP (mmHg)	-0.73 (-2.14, 0.67)	0.18 (-1.15, 1.51)
Percentage of patients with HbA1c <7% at Week 52[b]		29.5	33.1

All efficacy analyses are based on the full analysis set, excluding data after initiation of glycaemic rescue therapy. Patients initially randomised to PBO and not rescued during the first 26-week period switched to MET in the active-controlled period; therefore, there were no formal comparisons for efficacy between the PBO/MET and ERTU treatment arms at Week 52.
[a]LS means obtained from a cLDA model with fixed effects for treatment, time, prior antihyperglycaemic medication (yes, no), baseline eGFR (continuous) and the interaction of time (categorical variable) by treatment. Time was treated as a categorical variable.
[b]Missing data at Week 52 imputed using the cLDA model fitted with fixed effects as in the primary analysis.
cLDA, constrained longitudinal data analysis; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate.

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Disclosure: **S. Terra:** Employment/Consultancy; Pfizer, Inc. Stock/Shareholding; Pfizer, Inc.

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Glycaemic control and beta cell function in type 2 diabetes on SGLT2i and GLP-1RA combination therapy

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Background and aims: To examine whether SGLT2 inhibition plus GLP-1RA combination therapy provides superior clinical and metabolic benefit compared to monotherapy with each agent,
Materials and methods: We randomized 24 inadequately controlled (A1c=8.3+/-0.35) T2D patients treated with met +/- SU to receive either canagliflozin (300 mg/d; n=8, CANA), liraglutide (1.8 mg/d; n=8, LIRA) or both (n=8, COMBO). Baseline characteristics were similar across all groups. After a baseline OGTT and measurements of systolic blood pressure [SBP], body weight [BW], A1c & fasting plasma glucose [FPG], a 16-wk treatment period was started. Clinical parameters & OGTT were repeated at study end. Matsuda [MI]* for insulin sensitivity, insulinogenic index [ΔI/ΔG] & insulin secretion/insulin resistance [disposition] index [IS/IR=ΔI/ΔG **X** MI*] were calculated.
Conclusion: Combination therapy with canagliflozin and liraglutide is accompanied by a greater than additive effect on body weight and systolic blood pressure, and an additive effect on glycemic control and beta cell function. These findings provide strong rationale for the use of SGLT2i and GLP-1RA in poorly controlled T2D patients to decrease A1c, improve beta-cell function, and promote body weight reduction.

VARIABLES	LIRA	CANA	COMBO	p-value
Body Weight (kg)**	-2.6 ±1.4	-3.4 ±0.6	-7.2 ±1.2	0.01
SBP (mmHg)**	0 ±7	-6 ±3	-16 ±3	0.04
HbA1c (%)	-1.59 ±0.54	-1.10 ±0.32	-1.94 ±0.49	0.39
FPG (mg/dl)@	- 44 ±15	- 32 ±13	- 76 ±24	0.18
Mean OGTT PG (mg/dl)@	-84 ±15	-75 ±14	-134 ±23	0.04
Insulinogenic Index [ΔI/ΔG]@	+0.58 ±0.18	+0.17 ±0.11	+0.93 ±0.40	0.05
Ins. Sec./Ins. Resist. [ΔI/ΔG X MI*]@	+0.73 ±0.20	+0.67 ±0.25	+1.26 ±0.26	0.05

*Corrected for glycosuria;**COMBO is more than additive;@COMBO is additive; FPG=fasting plasma glucose; OGTT=oral glucose tolerance test; PG=plasma glucose; SBP=systolic blood pressure; ANOVA method used to calculate p-value;

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Influence of physical activity on soft lean mass reduction and carbohydrate intake on glucose-lowering effect of tofogliflozin in Japanese type 2 diabetes subjects

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Background and aims: Recent clinical studies have indicated that sodium-glucose cotransporter 2 (SGLT2) inhibitors may reduce not only fat mass but also lean body mass. It is important to elucidate the factors contribute to change in body composition and glucose-lowering effect of SGLT2 inhibitors. Our aim was to evaluate the changes in soft lean mass (SLM), representative of muscle volume, and metabolic parameters in relation with food intake and physical activity during tofogliflozin treatment in Japanese patients with type 2 diabetes (T2DM).
Materials and methods: This was a prospective observational study, enrolled 20 obese patients with T2DM (10 males, 10 females, age 58.6 ± 9.8 years, body weight 72.9 ± 14.1 kg, HbA1c 8.0 ± 0.4%). Patients received tofogliflozin 20 mg once daily for 24 weeks. Body composition assessed by bioelectrical impedance analysis, metabolic parameters, dietary intake, and physical activity score were evaluated at baseline, 4, 12, and 24 weeks. The primary endpoint was change in SLM from baseline to week 24. To examine the influence of dietary intakes, we examined the correlation between changes in HbA1c or loss of SLM and change in food intake.
Results: SLM significantly decreased by -1.46 kg (95% confidence interval (CI): -1.96, -0.98, $p < 0.001$) at 24 weeks with the change in body weight by -1.89 kg (95% CI: -2.50, -1.22, $p < 0.001$). Fasting plasma glucose and HbA1c decreased significantly at 24 weeks (167.6 ± 38.6 to 128.1 ± 25.2 mg/dl, $p<0.001$, and 8.11 ± 0.38 to 7.30 ± 0.44%, $p<0.001$, respectively). Daily total energy and carbohydrate intake did not differ significantly at 12, 24weeks compared with baseline. However, changes in HbA1c showed significant positive correlation with changes in carbohydrate intake ($r = 0.70$, $p = 0.004$), while changes in total energy intake did not show correlation with changes in HbA1c ($r = 0.35$, $p = 0.18$). Physical activity score at baseline and 24weeks showed negative correlation with SLM reduction ($r=0.65$, $p=0.016$, and $r=0.56$, $p=0.045$, respectively). Fasting plasma C-peptide (CPR) decreased significantly after 24 weeks of treatment (2.56 ± 1.21 to 2.24 ± 0.86 ng/ml, $p=0.016$), and the decrease in CPR was correlated with SLM reduction ($r = 0.62$, $p = 0.026$).
Conclusion: Tofogliflozin significantly decreased SLM, and the loss of SLM might be caused by increased energy loss through enhanced urinary glucose excretion with decrease in insulin secretion and low physical activity. In addition, increase in carbohydrate intake attenuated HbA1c reduction suggesting that adequate diet therapy is necessary to retain glucose-lowering effect of SGLT2 inhibitors.
Clinical Trial Registration Number: UMIN000015402
Disclosure: **T. Iwakura:** None.

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A 12-week dose-ranging study of sotagliflozin, a dual SGLT1 and SGLT2 inhibitor, as adjunct therapy to insulin in type 1 diabetes (inTandem4)

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Background and aims: Sotagliflozin (SOTA) is a dual SGLT1 and SGLT2 inhibitor in Phase 3 development for type 2 diabetes (T2D) and as adjunct to insulin in type 1 diabetes (T1D). Inhibition of SGLT1 delays and reduces glucose absorption in the proximal intestine, improving postprandial glycemic control. SGLT2 inhibition reduces renal glucose reabsorption.
Materials and methods: In a double-blind Phase 2 dose-ranging trial, 141 adults with T1D treated with MDI or pump and A1C 7.0-10.0% at screening were randomly assigned 1:1:1:1 to once-daily SOTA (75, 200, 400 mg) or placebo, and stable insulin dosing for 12 weeks.
Results: Baseline characteristics (expressed as range of means for the arms) were comparable among groups; age: 42-48 yrs, duration of T1D: 22-27 yrs, BMI: 27-32 kg/m^2, total daily insulin: 0.65 -0.77 U/kg, A1C at randomization: 7.95-8.07%. After 12 weeks, adjunctive SOTA 200 and 400 mg were more effective than placebo in reducing A1C. All dosages of SOTA reduced body weight and increased UGE vs. placebo. SOTA 400 mg was more effective than placebo in decreasing 2-hour PPG, and decreasing SBP in those with SBP ≥130 mm Hg at baseline. The overall incidence of TEAEs was lower in the SOTA arms. Incidences of DKA, SH, nausea, diarrhea and genital mycotic infections, were low.
Conclusion: These results support the use of SOTA 200 mg and 400 mg as Phase 3 doses in the T1D development program.

Efficacy and Safety from Randomization to Week 12				
Results	Placebo n = 36	SOTA 75 mg n = 35	SOTA 200 mg n = 35	SOTA 400 mg n = 35
Efficacy				
Baseline A1C	7.95	8.00	8.07	8.05
A1C LSMD vs. placebo[1], % (*p*-value)	N/A	-0.25 (0.07)	-0.48 (<0.001)	-0.38 (0.006)
2-hr PPG LSMD vs. placebo[1], mmol/L (*p*-value)	N/A	-1.1 (0.28)	-1.5 (0.15)	-2.7 (0.006)
FPG LSMD vs. placebo[1], mmol/L (*p*-value)	N/A	-0.5 (0.50)	-0.5 (0.48)	-1.2 (0.09)
Body weight LSMD vs. placebo[1], kg (*p*-value)	N/A	-1.3 (0.038)	-2.4 (<0.001)	-2.6 (<0.001)
24-hr UGE LSMD vs. placebo[1], g (*p*-value)	N/A	+42 (0.006)	+58 (<0.001)	+70 (<0.001)
SBP LSMD vs. placebo in those with Baseline SBP ≥130 mm Hg[2] (*p*-value)	N/A	-8.4 (0.26)	-6.8 (0.28)	-14.3 (0.013)
Patients with Safety Events				
Any treatment-emergent adverse event, n (%)	18 (50.0)	17 (48.6)	10 (28.6)	12 (34.3)
AE as primary reason for early discontinuation of core treatment period, n	1	1	0	0
Serious adverse event, n	1	1	1	1
Death, n	0	0	0	0
DKA, n	0	0	0	1
Severe hypoglycemia, n	0	1	1	1
Nausea, n	0	1	0	0
Diarrhea, n	3	0	1	1
Genital mycotic infection, n	0	1	1	1

LSMD: least squares mean difference, PPG: postprandial glucose, FPG: fasting plasma glucose, UGE: urinary glucose excretion, SBP: systolic blood pressure. TEAE: treatment-emergent adverse event

[1]Statistical comparisons of each SOTA arm to placebo were preplanned and performed using a generalized linear model with repeated measures statistics. [2]The LSMD analysis for SBP was post hoc. n for SBP analysis: placebo n=6, SOTA 75 mg n=3, SOTA 200 mg n=8, and SOTA 400 mg n=11.

Clinical Trial Registration Number: NCT02459899
Disclosure: **C. Baker:** None.

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24-week efficacy and safety of sotagliflozin, a dual SGLT1 and SGLT2 inhibitor, as adjunct therapy to insulin in type 1 diabetes (inTandem1)

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Background and aims: Sotagliflozin (SOTA) is a dual SGLT1 and SGLT2 inhibitor in Phase 3 development for type 2 diabetes (T2D) and as adjunct to insulin in type 1 diabetes (T1D). Inhibition of SGLT1 delays and reduces glucose absorption in the proximal intestine, improving postprandial glycemic control. SGLT2 inhibition reduces renal glucose reabsorption.
Materials and methods: In a double-blind Phase 3 trial, 793 adults with T1D, treated with multiple daily injection or pump therapy, with A1C 7.0-11.0% at Screening were randomized 1:1:1 to placebo or SOTA 200 or 400 mg after a 6-week insulin optimization period.
Results: Baseline characteristics were comparable among groups. Total mean (SD); age: 46.1 (13.1) yrs, T1D duration: 24.4 (12.8) yrs, BMI: 29.7 (5.4) kg/m^2, Total daily insulin dose: 0.73 (0.36) U/kg, Baseline A1C: 7.58 (0.73) %. After 24 weeks, adjunctive SOTA 200 and 400 mg resulted in greater A1C reduction than placebo with more patients achieving the prespecified "net benefit" composite endpoint of A1C <7.0% and no severe hypoglycemia (SH) and no DKA (Table). Incidences of treatment-emergent adverse events were similar across groups. There was more SH in the placebo arm and more genital mycotic infections, diarrhea, and DKA in the SOTA arms.
Conclusion: In this study, SOTA adjunct therapy to insulin in T1D, improved glycemic control with a safety profile supporting further clinical development.

Efficacy and Safety from Randomization to Week 24			
Results	Placebo n = 268	SOTA 200 mg n=263	SOTA 400 mg n =262
Efficacy			
A1C at Screening, %	8.21	8.26	8.20
A1C at Baseline, after 6-week insulin optimization, %	7.55	7.61	7.57
A1C at Week 24, %	7.50	7.17	7.08
A1C at Week 24 LSM change from Baseline[1], % (*p*-value)	-0.08 (0.027)	-0.43 (<0.001)	-0.49 (<0.001)
A1C at Week 24 LSMD vs. placebo[1], % (*p*-value)	N/A	-0.35 (<0.001)	-0.41 (<0.001)
Patients With Safety Event			
Any treatment-emergent adverse event, n (%)	181 (67.5)	177 (67.3)	186 (71.0)
AE as primary reason for early discontinuation of core treatment period, n (%)	4 (1.5)	3 (1.1)	10 (3.8)
Serious adverse events, n (%)	9 (3.4)	10 (3.8)	18 (6.9)
Death, n (%)	0 (0)	0 (0)	0 (0)
DKA, n (%)	0 (0)	3 (1.1)	8 (3.1)
Severe hypoglycemia, n (%)	18 (6.7)	11 (4.2)	12 (4.6)
Diarrhea[2], n (%)	15 (5.6)	19 (7.2)	26 (9.9)
Genital mycotic infection, n (%)	9 (3.4)	16 (6.1)	27 (10.3)
Efficacy and Safety			
Net benefit (proportion with A1C <7.0% at Week 24 and no SH and no DKA, randomization to Week 24), n (%)	58 (21.6)	88 (33.5)	114 (43.5)
Net benefit difference vs. placebo[1], % (*p*-value)	N/A	12 (0.002)	22 (<0.001)
LSM: least squares mean. LSMD: least squares mean difference. [1]Statistical comparisons of each SOTA arm to placebo were preplanned and performed using a generalized linear model with repeated measures statistics. [2]Discontinuation of drug due to diarrhea was: 0.4% placebo, 0% SOTA 200 mg, and 0.4% SOTA 400 mg.			

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Disclosure: **J.B. Buse:** Employment/Consultancy; Profil Institute for Clinical Research, Inc., PhaseBio Pharmaceuticals, Inc. Stock/Shareholding; PhaseBio Pharmaceuticals, Inc. Other; AstraZeneca, Eli Lilly and Company, GI Dynamics, Inc., Lexicon Pharmaceuticals, Inc., Novo Nordisk Inc., Orexigen Therapeutics, Inc., Dance Biopharm, ADOCIA, Metavention, Elcelyx Therapeutics, Inc., VtV Therapeutics, VA Medical Center, Boehringer Ingelheim Pharmaceuticals, Inc., Bristol-Myers Squibb Company, Johnson & Johnson Services, Inc.

PS 071 SGLT2 inhibitors: glucagon and liver issues

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Glucagon levels during short-term SGLT2 inhibition are largely regulated by plasma glucose changes

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Background and aims: Sodium glucose cotransporter 2 (SGLT2) inhibitors have been reported to increase glucagon (glg) secretion, but it is not known to what extent this is mediated via direct islet effects or plasma glucose (PG) lowering. We assessed the impact of PG changes following acute SGLT2 inhibition on circulating levels of glg and lipid-derived energy substrates.
Materials and methods: In a randomized 3-way crossover open-label study, 15 metformin-treated T2D patients (12 M, age 67±6y, BMI 27.1 ±2.9 kg/m^2, HbA1c 56±6 mmol/L; mean±SD) were investigated after overnight fast. A single oral dose of dapagliflozin (Dapa) 10mg was given at the start of all investigations, directly followed by a 5h variable infusion of 10% glucose to maintain glucose at the ambient baseline level (ΔAUC for PG 0 ±1%; mean±SEM; **visit G**) or isotonic NaCl allowing PG to drop (-16±1%; **visit N**). Glg, insulin (ins) and energy substrates were measured repeatedly during infusion periods and urinary glucose excretion was assessed. The 5h infusions were followed by 2h OGTTs, and in addition oral saxagliptin 5mg was co-administered with Dapa at one visit (not reported here).
Results: Plasma glucagon, the primary endpoint measure, was significantly lower during visit **G** vs **N**, but fell from baseline in both cases. Insulin and C-peptide were decreased in visit **N** but not in **G**. Glg/ins ratio markedly rose in visit **N** and fell during visit **G**. NEFA, glycerol and β-OH-ketones increased during visit **N** and levels were higher than in visit **G**. UGE correlated to glucose infusion rate during visit **G** (R^2 0.60, $p<0.05$), but it did not differ significantly between visit **G** and **N** (17±2.6 vs 13±1.1 g/5h). In multivariate regression analyses, change in PG during **G** and **N** visits was the only significant predictor of change in glg (R^2 0.28, $p<0.01$) following adjustments for age, sex, BMI and HbA1c. Fasting PG, ins and glg as well as ins changes and UGE were not significantly associated with glg changes. Glycerol, NEFA and β-OH-ketones were all lower during **G** vs **N**.
Conclusion: In T2D patients given a single dose Dapa, short-term glucagon regulation is largely mediated by glycemic changes. Direct α-cell effects by SGLT2 inhibition may be of less importance. In this study, no short-term rise in glucagon was seen after Dapa administration. Glucose lowering can also contribute to increased lipolysis, lipid oxidation and ketogenesis following SGLT2 inhibition.

Percent change from baseline with dapa 10 mg; 5 h glucose vs NaCl infusion			
	Glucose	NaCl	P-value, N vs G
Glucagon	-22 ± 4	-8 ± 3	<0.01
Insulin	1 ± 7	-31 ± 3	<0.01
Glucagon / insulin ratio	-17 ± 8	38 ± 7	<0.01
β-OH-ketones	7 ± 13	77 ± 16	<0.05
Glycerol	-20 ± 6	6 ± 8	<0.01
NEFA	-16 ± 4	14 ± 7	<0.01
N=13 completers. Data are Δ AUC of plasma conc. %, mean±SEM			

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Supported by: AstraZeneca
Disclosure: **P. Lundkvist:** Employment/Consultancy; Merck Sharp & Dohme Corp. Grants; AstraZeneca travel grant.

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Dapagliflozin increases glucagon secretion via direct effect on electrical activity in pancreatic A cells

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Background and aims: Glucagon (gcg) is one of the major regulators of blood glucose levels and its physiological role is to protect against hypoglycemia via stimulation of hepatic glucose output. Recently it has been shown that Na^+/glucose co-transporter2 inhibitors (SGLT2i) used for the treatment of diabetes increase glucagon levels in man. As for most neuroendocrine cells glucagon release from A-cells is regulated by electrical activity and we thus investigate if SGLT2i affect glucagon release via its electrogenic effects. Basal glucagon levels are elevated in all types of diabetes which contributes to diabetic hyperglycemia. In addition, diabetics lack proper glucagon response at low glucose, increasing the risk for dangerous hypoglycemia. Therefore it is important to understand the regulation of glucagon release and how it is affected by Dapagliflozin.
Materials and methods: Glucagon secretion was measured by ELISA assays. Dapagliflozin sensitive currents were recorded from A-cells by using the standard whole cell patch clamp configuration.
Results: Pancreatic A-cells were functionally identified by the inactivation properties of a TEA-resistant K+- and voltage sensitive Na+-currents. Resting conductance was measured by applying 10 mV pulses before and after addition of 10 nM Dapagliflozin. In A-cells Dapagliflozin reduced the resting conductance by 57.5 ± 24.9 pS (n = 7) but only by 8.6 ± 11.3 pS in non A-cells (n = 16), $p < 0.05$. We and other have previously shown that application of increasing doses of the KATP-channel opener diazoxide at high (11 mM) glucose results in a bell shaped dose response curve in glucagon secretion. SGLT2 transporters can be viewed as a background conductance giving rise to an inward depolarizing current. Blocking SGLT2 with dapagliflozin should thus be equivalent to activating outward, hyperpolarizing KATP-currents with diazoxide. Dapagliflozin should therefore produce a leftward shift of the diazoxide dose response curve. Our experimental data of secretion measurements confirmed this hypothesis. Dapagliflozin shifted the peak of the diazoxide dose response curve from 10 to 3 μM. whereas the fold stimulation was not affected (1.9 ± 0.3 at 3uM diazoxide + dapagliflozin ($p < 0.05$, n = 13) and 2.1 ± 0.3 at 10uM diazoxide, ($p < 0.05$, n = 13) compared to 11 mM glucose alone. Dapagliflozin did not affect insulin secretion making it unlikely that SGLT2 inhibition affects glucagon release via a paracrine mechanism.
Conclusion: Dapagliflozin reduces inward currents and increases glucagon secretion at high glucose via a direct effect on pancreatic A-cell electrical activity. The absence of any effect on insulin secretion makes it unlikely that dapagliflozin affects glucagon release paracrine via modulation of insulin or somatostatin since somatostatin inhibits both insulin and glucagon secretion. Blood glucose reduction achieved with SGLT2 inhibitors is similar to other treatments. It is thus unlikely that the increase in glucagon levels seen in patients treated with SGLT2 inhibitors is due to reduced blood sugar but rather due to a direct interference with A-cell electrical activity.
Disclosure: **M.F. El Hachmane:** None.

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The GLP1R agonist liraglutide inhibits hyperglucagonaemia induced by the SGLT2 inhibitor dapagliflozin

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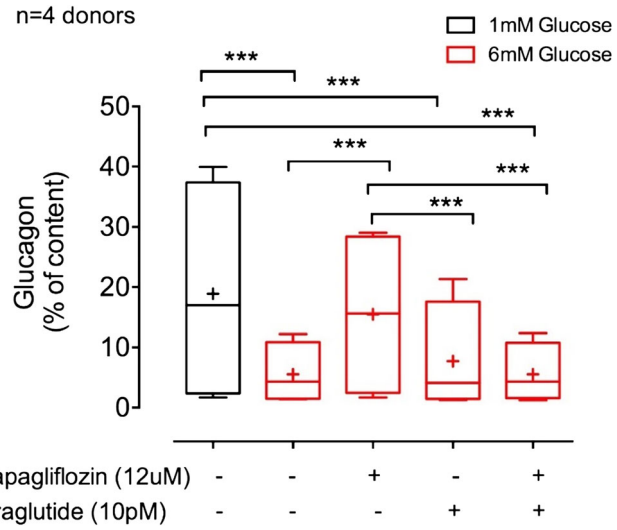
Background and aims: The newest pharmacologic treatments for type 2 diabetes (T2D) include glucagon-like peptide-1 receptor agonists (GLP1R-A) and sodium-glucose cotransporter 2 inhibitors (SGLT2-I). A recent clinical study in patients with T2D has demonstrated that the dual therapy with GLP1R-A and SGLT2-I reduced HbA1c levels more effectively than therapy with a GLP1R-A alone. However, there are currently no studies evaluating GLP1R-A, SGLT2-I, or the combination therapy, simultaneously. The aim of the current study was to determine the efficacy and the mechanism of action of the SGLT2-I, dapagliflozin, and GLP1R-A liraglutide, as mono-therapies or in combination for the treatment of T2D.
Materials and methods: Human pancreatic tissue, islets and plasma from healthy and diabetic mice were studied. GLP1R expression in the human pancreas was assessed by histology and qPCR analysis. Drug treatments with dapagliflozin and liraglutide were assessed for glycemia and pancreatic hormone secretion.
Results: Here we demonstrate that GLP1R is localized in alpha, beta and delta cells of human pancreatic islets. We also found that GLP1R gene expression was induced in the islets of obese normoglycemic and T2D individuals. Consistent with our previous data, SGLT2 inhibition by dapagliflozin induces glucagon secretion in human islet cultures, an effect that was markedly reduced by co-treatment with liraglutide. In healthy mice, we found that liraglutide alone or in the combination therapy significantly reduced glycemia and glucagon induced by dapagliflozin after an intra-peritoneal (IP) injection of glucose. However, acute (single administration) and chronic treatment (45 days) of db/db (diabetic) mice with the combination therapy markedly improved glycemic control compared to dapagliflozin or liraglutide treatments alone. Moreover, liraglutide significantly reduced hyperglucagonemia induced by dapagliflozin during fasting and after an IP injection of glucose.
Conclusion: Our results suggest that the combination of dapagliflozin and liraglutide needs to be studied in a prospective clinical trial because the effect of each of the molecules is synergistically enhanced by the addition of the partner drug.



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Disclosure: **C. Bonner:** None.

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Enlarged islet size, altered insulin$^+$ and glucagon$^+$ cell frequencies and modified blood-values for GLP-1, insulin and glucagon in Sglt1 knockout mice

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Background and aims: Inhibition of the sodium dependent glucose transporter (Sglt) 2 is a promising strategy in the treatment of diabetes leading to diminished glucose reabsorption from the kidney thereby contributing to glycemic control. Recently, also Sglt1 gained much attention in this context. Sglt1 is predominantly expressed in enterocytes of the small intestine regulating intestinal glucose uptake. So far, it was shown that Sglt1 inhibition contributes to the regulation of glucose homeostasis by reducing intestinal glucose absorption and upregulation of integrin secretion. However, if Sglt1 inhibition has additional effects of on other organs in terms of structural or functional changes still needs to be further investigated. Therefore, the aim of this study was to investigate if the loss of Sglt1 function in mice impacts on structure, cellular composition or function of pancreatic islets.
Materials and methods: Pancreatic islets were analyzed in Sglt1 knockout mice fed with a sugar-free, high fat diet ($Sglt1^{-/-}$). C57Bl/6 wildtype mice fed with standard chow (WTND) or fed with sugar-free, high fat diet (WTSD) served as controls. *Sglt1* expression was determined by qRT-PCR. Quantification of islet size was performed on hematoxylin & eosin (H&E)-stained pancreas sections. Immunohistochemical (IHC) stainings for insulin (INS) and glucagon (GCG) were used to investigate cellular composition of islets regarding the two most frequent cell types. Glucose-stimulated insulin secretion was performed on pancreatic islets *in vitro.* Furthermore, effects on INS, GCG or glucagon-like peptide 1 (GLP-1) levels in blood were analyzed by enzyme-linked immunosorbent assays (ELISA).
Results: In comparison to WTND and WTSD controls, pancreatic islets of Sglt1-/- mice displayed a significantly enlarged mean islet area. Additionally, Sglt1-/- islets showed increased frequencies for GCG+ cells while INS+ cells are less frequent in comparison to WTND or WTSD controls. Further, data obtained by glucose-stimulated insulin secretion assay revealed no response of Sglt1-/- islets and a delayed response of islets isolated from WTSD mice in comparison to the WTND control. Finally, basal blood-values for GLP-1 were increased in WTSD mice compared to the WTND or Sglt1-/- group while INS or GCG levels were similar in all three groups.
Conclusion: Our data show that Sglt1 knockout in mice impacts on size, cellular composition and function of pancreatic islets. Taken together, these findings suggest that further intense research is necessary to understand short and long term associated impacts of pharmacological Sglt1 inhibition on other organs to define its role as therapeutic option for the treatment of diabetes.
Disclosure: **M. Muehlemann:** None.

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Effect of dapagliflozin, saxagliptin, and the combination of both on glucagon, endogenous glucose production (EGP) and glycerol in patients with type 2 diabetes

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Background and aims: In recent investigations, the use of the SGLT-2 inhibitors has been associated with an increased risk of glucagon-induced diabetic ketoacidosis (DKA). The aim of this trial was to investigate the role of glucagon on EGP and glycerol rate of appearance (Ra glycerol) during treatment with either the SGLT-2i dapagliflozin and/or the DPP-4i saxagliptin in metformin only treated T2DM.
Materials and methods: In this randomized, double-blind crossover trial, 17 patients (age 53 ± 6 ys, diabetes duration 7.6 ± 5.8 ys, BMI 29.4 ± 3.5 kg/m², HbA1c 60.6 ± 10.3 mmol/mol (7.7 ± 3.1 %)) underwent a stepwise eu- (5.5 mmol/L, ambient insulin level), hyper- (11.1 mmol/L, no insulin), and high insulin (2.5mU/kg/min) hypoglycaemic clamp (5.5, 3.5, 2.5 mmol/L) with primed tracer infusion of [6,6-$2H_2$]glucose (prime: 9.6mg/kg, constant: 0.08mg/kg/min) and [1,1,2,3,3-$2H_5$]glycerol (prime: 1.5μmol/kg, constant: 0.1μmol/kg/min) at baseline and on day 7 of once daily treatment with dapagliflozin, or saxagliptin, or dapagliflozin+ saxagliptin. Glucagon was measured by specific double sandwich ELISA (Mercodia AB, Sweden). Isotopic glucose and glycerol were measured with gas-chromatography mass spectrometry.
Results: Dapagliflozin (with and without saxagliptin) did not increase glucagon during eu-, hyper-, and hypoglycaemia compared to no treatment. All 3 treatments significantly reduced EGP during hyper- (p=0.003-0.044), but not during hypoglycaemia. Compared to no treatment or saxagliptin, dapagliflozin did not increase Ra glycerol during eu-, hyper-, and hypoglycaemia. NEFA concentrations were significantly reduced during hyperglycaemia in saxagliptin (p=0.0041) and saxagliptin+dapagliflozin (p=0.046) treatment compared to no treatment. Ketone bodies were not significantly different. Insulin level was comparable for all treatment groups. C-Peptide was significantly higher during hyper- and hypoglycaemia (p=0.0084-0.0004) for dapagliflozin+saxagliptin compared to no treatment. No significant differences were observed for counterregulatory hormones adrenalin, human growth hormone (HGH) and cortisol. Saxagliptin reduced noradrenalin concentration significantly during euglycaemia at ambient insulin levels compared to no treatment (p=0.0497) and dapagliflozin (p=0.0126) and euglycaemia at high insulin levels compared to dapagliflozin (p=0.0135).
Conclusion: Dapagliflozin did not increase overall glucagon levels, Ra glycerol and EGP in metformin treated T2DM patients. Our data suggest that the risk of DKA during treatment with SGLT-2 inhibitor dapagliflozin is not mediated via glucagon associated fuel shift.

Variable	Treatment	Plasma glucose concentrations				
		5.5 mmol/L low insulin	11.1 mmol/L	5.5 mmol/L high insulin	3.5 mmol/L	2.5 mmol/L
Glucagon (pmol/L)	No treatment;	9.4 ± 4.1	6.2 ± 3.6	3.3 ± 3.4	6.6 ± 5.0	18.9 ± 14.0
	Dapagliflozin;	11.5 ± 6.0	7.0 ± 4.8	4.4 ± 3.6	6.5 ± 4.5	21.2 ± 12.6
	Saxagliptin;	11.1 ±6.2	6.3 ± 4.8	4.4 ± 4.1	7.2 ± 7.3	24.5 ± 18.9
	Dapagliflozin + Saxagliptin	11.6 ± 5.3	5.9 ± 4.9	4.1 ± 3.2	7.9 ± 10.3	16.5 ± 12.0
EGP (mg/kg/min)	No treatment;	2.2 ± 0.5	1.8 ± 0.4	0.8 ± 0.8	0.8 ± 0.5	0.9 ± 0.4
	Dapagliflozin;	2.5 ± 0.5**[#]	1.5 ± 0.6*	0.4 ± 0.4	0.6 ± 0.3	0.9 ± 0.4
	Saxagliptin;	2.2 ± 0.5	1.5 ± 0.4*	0.6 ± 0.4	0.6 ± 0.2	0.9 ± 0.2
	Dapagliflozin + Saxagliptin	2.4 ± 0.4	1.4 ± 0.6*	0.4 ± 0.3	0.5 ± 0.3	0.8 ± 0.3
Ra glycerol (μmol/kg/min)	No treatment;	2.0 ± 0.9	2.3 ± 0.9	1.2 ± 0.4	1.3 ± 0.4	1.8 ± 0.8
	Dapagliflozin;	1.7 ± 0.5[#]	1.8 ± 0.9*	0.8 ± 0.3**	0.8 ± 0.4**	1.4 ± 0.9*
	Saxagliptin;	1.4 ± 0.4*[$]	1.6 ± 0.6**	0.7 ± 0.2**	0.7 ± 0.2**	1.1 ± 0.5**
	Dapagliflozin + Saxagliptin	1.7 ± 0.7	1.6 ± 0.5**	0.8 ± 0.4**	0.8 ± 0.4**	1.4 ± 1.0*
NEFA (mmol/L)	No treatment;	0.4 ± 0.2	0.5 ± 0.3	0.02 ± 0.04	0.02 ± 0.1	0.1 ± 0.2
	Dapagliflozin;	0.4 ± 0.2	0.5 ± 0.3	0.02 ± 0.03	0.02 ± 0.05	0.1 ± 0.1
	Saxagliptin;	0.3 ± 0.2	0.4 ± 0.2*	0.02 ± 0.03	0.02 ± 0.03	0.1 ± 0.2
	Dapagliflozin + Saxagliptin	0.4 ± 0.3	0.4 ± 0.2*	0.01 ± 0.03	0.02 ± 0.04	0.1 ± 0.1

Numbers are means ± SD; EGP: endogenous glucose production; Ra glycerol: Rate of appearance glycerol;
*: p<0.05, **: p<0.001 for treatment vs. no treatment; [#]: p<0.05 Dapagliflozin vs. Saxagliptin;
[$]: p<0.05 Saxagliptin vs. Dapagliflozin+Saxagliptin

Supported by: AstraZeneca
Disclosure: **S. Sach-Friedl:** Grants; AstraZeneca.

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Effect of canagliflozin on liver function tests in patients with type 2 diabetes and presumed liver fibrosis suggestive of non-alcoholic steatohepatitis

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Background and aims: Type 2 diabetes mellitus (T2DM) and obesity are risk factors for non-alcoholic fatty liver disease (NAFLD), including its more severe form, non-alcoholic steatohepatitis (NASH). Non-invasive liver fibrosis risk scores, such as Fibrosis (FIB)-4, have been used in lieu of liver biopsy to identify patients with presumed liver fibrosis suggestive

of NASH. Canagliflozin (CANA), a sodium glucose co-transporter 2 (SGLT2) inhibitor, has been previously shown to improve glycaemic control, body weight (BW), and liver function tests (LFTs; eg, alanine aminotransferase [ALT], aspartate aminotransferase [AST], gamma glutamyl transferase [GGT]) across a broad array of T2DM patients in Phase 3 studies. Whether CANA improves LFTs in patients with T2DM and presumed liver fibrosis suggestive of NASH is unknown.

Materials and methods: This post hoc analysis used pooled data from the 4 placebo (PBO)-controlled, Phase 3 studies in a general population of patients with T2DM with an elevated FIB-4 score (≥1.3) consistent with liver fibrosis (~20% of the total population, N = 501; mean (SD) baseline BW = 89 (21) kg, HbA1C = 7.9 (0.9) %, ALT = 31 (20) U/L, AST = 30 (18) U/L, GGT = 49 (63) U/L, total bilirubin = 10 (5) μmol/L).

Results: At 26 weeks, both ALT and GGT were reduced with CANA 100 and 300 mg compared with PBO (Table).

Conclusion: In summary, in patients with T2DM and presumed liver fibrosis suggestive of NASH, both CANA 100 and 300 mg reduced liver enzymes compared with PBO.

Table. Changes in LFTs with CANA at Week 26.

	PBO	CANA 100 mg	CANA 300 mg
N	142	182	177
ΔALT, LSM (SE), %	4 (3)	–8 (2)	–11 (3)
PBO-subtracted ΔLSM (95% CI)	–	–11 (–20;–3)	–14 (–22;–5)
ΔAST, LSM (SE), %	–2 (2)	–8 (2)	–9 (2)
PBO-subtracted ΔLSM (95% CI)	–	–6 (–12;1)	–6 (–12;1)
ΔGGT, LSM (SE), %	12 (7)	–6 (3)	–5 (4)
PBO-subtracted ΔLSM (95% CI)	–	–18 (–35;–2)	–18 (–34;–1)
ΔTotal bilirubin, LSM (SE), %	1 (2)	4 (3)	5 (3)
PBO-subtracted ΔLSM (95% CI)	–	3 (–5;12)	5 (–4;14)

LSM, least squares mean; SE, standard error; CI, confidence interval.

Clinical Trial Registration Number: NCT01081834; NCT01106625; NCT01106677; NCT01106690

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Disclosure: **G. Meininger:** Employment/Consultancy; Janssen Research & Development, LLC.

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Intra- and inter-subject variability for increases in plasma ketone bodies in patients with type 2 diabetes treated with the SGLT2 inhibitor canagliflozin

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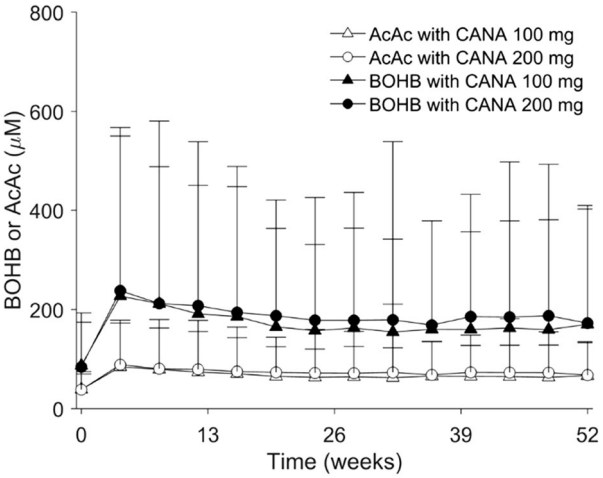
Background and aims: Overnight-fasted plasma ketone body concentrations are typically ~50-200 μM. Dramatic increases occurring during prolonged fasting or diabetic ketoacidosis have been well characterized, but the effects of modest changes in ketones occurring under different conditions are less understood. Recent findings indicate that failing hearts increasingly rely on ketones for energy, and that sodium glucose co-transporter 2 inhibitors (SGLT2i) modestly increase plasma ketones in most patients. These observations have stimulated a hypothesis that increases in ketones may contribute to SGLT2i-associated reductions in adverse cardiovascular and renal outcomes.

Materials and methods: Data from SGLT2i trials indicate that mean plasma ketone concentrations approximately double, with high between-subject variability. Here, we analyse data from a Phase 3 study with the SGLT2i canagliflozin. Overnight-fasted ketone concentrations were measured every 4 weeks for 52 weeks in 1,278 Japanese subjects treated with canagliflozin 100 or 200 mg.

Results: Mean plasma acetoacetate and β-hydroxybutyrate concentrations were increased at all post baseline visits, with similar increases seen with both canagliflozin doses (Figure). Median (interquartile range) percent change from baseline was 62% (180) for acetoacetate and 78% (234) for β-hydroxybutyrate. Approximately 2/3 of the variability in each measure was attributed to within-subject variability. Within-subject variability for plasma ketones is higher than that for other metabolites, and accounted for only 9%-17% of total variability for plasma glucose and HbA1c. Subjects in the lowest-response tertile exhibited no mean increase in ketones at any visit. Those in the highest-response tertile tended to be male and have the largest decrease in fasting glucose. Moreover, changes in plasma ketones were not fully explained by changes in plasma fatty acids, suggesting downstream effects of SGLT2i on hepatic metabolism that favour ketogenesis.

Conclusion: Canagliflozin treatment increases plasma ketone bodies in Japanese patients with T2DM, with high intra-patient variability. The increase in plasma ketone bodies is greater in magnitude and more variable than changes in other metabolic measures.



AcAc, acetoacetate; CANA, canagliflozin; BOHB, β-hydroxybutyrate.

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Supported by: Mitsubishi Tanabe Pharma Corporation; Janssen Research & Development, LLC

Disclosure: **D. Polidori:** Employment/Consultancy; Janssen Research & Development, LLC.

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Effects of canagliflozin versus glimepiride on adipokines, inflammatory biomarkers, and chemokines in patients with type 2 diabetes

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Background and aims: Type 2 diabetes mellitus (T2DM) and obesity are pro-inflammatory states that are associated with an increased risk of cardiovascular disease (CVD). Canagliflozin (CANA), a sodium glucose co-

transporter 2 (SGLT2) inhibitor, demonstrated superiority in lowering HbA1c versus glimepiride (GLIM) and greater body weight reduction via loss of fat mass in a 52-week, Phase 3 trial of patients with T2DM on metformin. This post hoc analysis assessed the effects of CANA versus GLIM on select adipokines, inflammatory markers, and chemokines that have been associated with impaired adipose tissue function, insulin resistance, and CVD.

Materials and methods: Serum samples were from randomly selected patients receiving CANA 300 mg (n = 100) or GLIM (n = 100) in the overall study. Change from baseline to Week 52 in serum leptin, adiponectin, CRP, PAI-1, VCAM-1, and MCP-1 was measured using a multiplex assay (Myriad RBM). Change in serum IL-6 and TNFα was measured with ultra high-sensitivity assays (Simoa-Quanterix). Change in leptin, adiponectin, and IL-6 was correlated with change in HbA1c, body weight, and lipids (HDL-C, LDL-C, triglycerides) within each group.

Results: At Week 52, the least squares mean (LSM) change in HbA1c was -0.99% with CANA and -0.91% with GLIM (baseline, 7.7-7.8%). LSM change in body weight was -4.1 kg with CANA and 0.7 kg with GLIM (baseline, 90-91 kg). At Week 52, median serum leptin was decreased 25% with CANA versus GLIM (-1.1 vs 1.0 ng/mL; difference [95% CI] of -2.8 ng/mL [-4.0, -1.6]). Median serum adiponectin was increased 17% with CANA versus GLIM (0.6 vs 0.0 μg/mL; difference [95% CI] of 0.5 μg/mL [0.3, 0.7]). There was a 22% reduction in median serum IL-6 (-0.3 vs 0.2 pg/mL; difference [95% CI] of -0.5 [-0.7, -0.2]) and a 7% increase in median serum TNFα (0.1 vs -0.1 pg/mL; difference [95% CI] of 0.2 pg/mL [0.0, 0.3]) with CANA versus GLIM. No between-group differences were observed with CRP, PAI-1, VCAM-1, or MCP-1. Change in leptin was correlated with change in body weight ($r \geq 0.35$) only; change in adiponectin and IL-6 was not correlated with change in HbA1c, body weight, or lipids.

Conclusion: CANA 300 mg demonstrated reductions in serum leptin and IL-6, and an increase in adiponectin versus GLIM in patients with T2DM; CANA was also associated with a small increase in TNFα and had neutral effects on other biomarkers. The CANA-related changes in leptin, adiponectin, and IL-6 were independent of glycaemic benefit, and the changes in adiponectin and IL-6 were independent of weight loss in this analysis. These collective results suggest that CANA may improve adipose tissue function, which may have positive effects on cardiometabolic health.

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Supported by: Janssen Global Services, LLC

Disclosure: **L. Van Gaal:** Grants; EU (Hepadip & Resolve consortium) and National Research Funds, Belgium. Other; Member of the Advisory Board and Speakers Bureau of Astra Zeneca, Boehringer Ingelheim, Eli Lilly, Janssen J & J, Merck MSD, Novo Nordisk and Sanofi (period 2013-2016).

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SGLT2 inhibition by empagliflozin attenuates ectopic fat accumulation and improves cardiac index in parallel to ketone bodies production: the EMPAFAT study

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Background and aims: Ectopic fat deposition including myocardial, hepatic and skeletal muscle steatoses leads to insulin-resistance, cardiomyocytes dysfunction through lipotoxicity. Empagliflozin (EMPA) a specific inhibitor of sodium-glucose co-transporter 2 (SGLT-2), is a novel antihypergylcemic agent which reduced cardiovascular deaths in type 2 diabetic patients. The mechanisms underlying this improvement are yet unknown. Therefore, we set-up in mice a multimodal MR protocol to analyze the early effect of EMPA on cardiac function and ectopic fat deposition.

Materials and methods: C57BL6J/R mice were fed with high-fat (35%)-high-sucrose (34%) diet (HFHS) or standard diet (SD). After 1 month (M0), 30mg/kg EMPA or placebo treatment was initiated orally in the HFHS group. 4.7T MRI examinations were performed at baseline (M0) and after 1month (M1) and 3 month (M3) of treatment to assess cardiac function with cine cardiac MR (CMR) according to the hemisphere cylinder model and myocardial (MTGC), hepatic (HTGC) and muscular (MuTGC) triglyceride contents using ^{1}H-spectroscopy. Intraperitoneal glucose tolerance test (IPGTT) and fasting ketone bodies were assessed at M1 and M3.

Results: EMPA attenuated weight gain, ameliorated glucose homeostasis in mice under HFHS. EMPA decreased significantly ($p<0.05$) glucose AUC during IPGTT (EMPA 332 ± 28 vs HFHS 565± 30; SD 268± 13), HOMA-IR (EMPA 51.5 ± 8.01 vs HFHS 101.3 ± 16.8; SD 10.6 ± 2.7), insulin/glucagon ratio (EMPA 62.3 ± 10.4 vs HFHS 223.4 ± 63.5; SD 35.6 ± 12.7) and EMPA increased ketone bodies levels (β-hydroxybutyrate: 0.14 ±0.01 mM vs 0.09±0.01 mM, p=0.02). HFHS diet increased the three ectopic fat, Muscular triglyceride content (MuTGC), hepatic triglyceride content (HTGC), and myocardial triglyceride content (MTGC), After 1 month of treatment (M1), Empa greatly decreased MuTGC (EMPA 2.09±1.08%, vs HFHS 4.88±1.49%, p=0.004; SD 1.09±0.19%), and HTGC (EMPA 7.6 ±2.4% vs HFHS 33.2±8.5%, p=0.002; SD 2.3±0.7%). A similar effect of EMPA was found at M3. By contrast, the increase in MTGC with HFHS was not prevented by EMPA (EMPA 0.6±0.13% vs HFHS 0.6±0.07%, NS; SD 0.17±0.04). As expected, HOMA-IR was correlated to both MuTGC and HTGC. Cardiac index (CI) was reduced in HFHS mice (HFHS 0.47 ±0.03 vs SD 0.73±0.07 μL/min/g) and EMPA improved cardiac index (0.60 ±0.05, p=0.04) at M1 and M3. In the two HFHS group, Cardiac index was interestingly, positively correlated with ketone levels at M1 (r=0.54, p=0.007) but not with Glycaemia, HOMA-IR, HTGC and MuTGC.

Conclusion: Empagliflozin induced a drastic decrease of ectopic fat in liver and muscle, promoting insulin-sensitivity. Empaglifozin prevented the deleterious effect of HFHS diet on cardiac function that seems correlated to a shift in myocardial fuel source involving ketones.

Supported by: Boehringer-Ingelheim

Disclosure: **F. Maurice:** None.

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Dapagliflozin for treatment of prednisone induced hyperglycaemia: a double-blind randomised clinical trial

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Background and aims: Prednisone treatment for acute exacerbation of chronic obstructive pulmonary disease (COPD) is complicated by hyperglycemia in patients with and without pre-existent diabetes mellitus. Hyperglycemia is associated is with longer hospitalization and higher mortality. Treatment can be challenging because of severe insulin resistance. SGLT-2 inhibitors are an interesting treatment option because they are beta-cell independent and because of the quick onset of action. We therefore performed a double-blind randomized placebo controlled study to compare the effectiveness of dapagliflozin to placebo.

Materials and methods: We enrolled 46 subjects hospitalized for COPD exacerbation who were treated with prednisone, ≥ 30 mg daily for 5-14 days and had either a known history of type 2 diabetes or a random blood glucose > 10 mmol/L at admission. Subjects were treated with dapagliflozin 10 mg or placebo as add-on to their routine diabetes medication during their course of prednisone therapy. Subjects continued study medication as long as they were treated with prednisone. Glycemic control was evaluated using a blinded continuous glucose monitor.

Results: Glucose values were in the target range (3.9-10 mmol/L) 54.0 % of the time in the dapagliflozin group and 53.7 % in the placebo group.

There was no difference in mean glucose concentration (dapagliflozin 10.1 mmol/L, placebo 10.4 mmol/L). There were no severe hypoglycemic events. Five subjects in the dapagliflozin group had a non-severe hypoglycemic event versus 6 subjects who were treated with placebo. There was no difference in incidence of urogenital events between the groups.

Conclusion: Once daily dapagliflozin as add-on to routine diabetes medication did not result in better glycemic control in patients with prednisone-induced hyperglycemia during COPD exacerbation. There was no difference in incidence of hypoglycemic events. The glucose lowering efficacy of a short course of dapagliflozin is probably not strong enough to counter hyperglycemic effects of prednisone therapy.

Clinical Trial Registration Number: NCT02253121

Supported by: Research grant for an investigator initiated research project, AstraZeneca

Disclosure: **M.C. Gerards:** Grants; A research grant for an investigator initiated project (Astra Zeneca).

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Population pharmacokinetic-pharmacodynamic analysis to characterise the effect of empagliflozin on renal glucose threshold in patients with type 1 diabetes

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Background and aims: Empagliflozin is a sodium glucose cotransporter 2 (SGLT2) inhibitor that increases urinary glucose excretion (UGE) by lowering the renal threshold for glucose (RT_G). The objectives of this analysis were to quantify the effect of the SGLT2 inhibitor empagliflozin on renal glucose reabsorption in patients with type 1 diabetes (T1DM) using a mechanistic population pharmacokinetic-pharmacodynamic (PK-PD) model, and to compare results with analyses in patients with type 2 diabetes (T2DM).

Materials and methods: The PK-PD model was developed using data from a randomized Phase II study in which patients with T1DM received oral placebo or empagliflozin 2.5 mg, 10 mg, or 25 mg QD as adjunct to insulin. The PK-PD data set was comprised of 75 T1DM subjects contributing 1814 plasma empagliflozin concentrations and 895 urine glucose observations

Results: Empagliflozin pharmacokinetics were described using a two-compartment model with sequential zero and first-order absorption and a lag time. The pharmacodynamic model assumed that UGE was dependent on plasma glucose and renal function (accounting for UGE before saturation of reabsorption) and that empagliflozin lowered RT_G. Calculated RT_G with placebo was 181 mg/dL, and with empagliflozin (steady state) 1 mg and 2.5 mg was 53.4 mg/dL and 12.5 mg/dL, respectively. Empagliflozin 10 mg and 25 mg yielded minimal RT_G values. The final model was evaluated using visual predictive checks and found to be consistent with observed data.

Conclusion: Although estimated PK-PD parameters were generally comparable between patients with T1DM and patients with T2DM, population PK-PD simulations demonstrated that the lower RT_G in T1DM patients yielded a higher UGE compared with patients with T2DM.

Clinical Trial Registration Number: NCT01969747

Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance

Disclosure: **J. Mondick:** Employment/Consultancy; Employee of Metrum Research Group LLC who served as a paid consultant to Boehringer Ingelheim.

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Exposure to SGLT-2 inhibitors can reverse stearic acid-induced lipotoxicity in human endothelial progenitor cells

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Background and aims: Lipotoxicity is implicated in the association among insulin resistance, type 2 diabetes, endothelial dysfunction and increased cardiovascular (CV) risk. In a previous research of ours, lipotoxicity impaired the function of key players in the process of endothelial repair, i.e. the human circulating angiogenic cells (CACs, also known as "early" endothelial progenitor cells), the bioavailability of

which predicts CV mortality in humans. Specifically, we showed that in CACs stearic acid (SA) induced a JNK-mediated pro-inflammatory response and lipoapoptosis, through the activation of endoplasmic reticulum (ER) stress. The SGLT-2 inhibitor (SGLT-2i) empagliflozin (EMPA) reduces the risk of CV mortality in people with type 2 diabetes, apparently beyond glucose control, perhaps by impairing Na^+/H^+ exchanger (NHE) flux in target cells, in an cariporide-like fashion. Goal of our study was to test the hypothesis that SGLT2-i can restrain SA-induced lipotoxicity in human CACs.

Materials and methods: CACs were isolated after culturing lymphomonocytes obtained from healthy subjects on fibronectin-coated dishes in endothelial medium (EGM-2) for 7 days. Digital PCR technology was used to test the expression of SGLTs in CACs. CACs were incubated with physiological concentrations of stearic acid (SA) (100 μmol/L) with/without SGLT-2i [EMPA and dapagliflozin (DAPA)] from 1 to 100 μmol/L over time to assess the effects on a) apoptosis (activation of caspases 3/7) b) pro-inflammatory cytokine [interleukin(IL)-1β, IL-6, IL-8, monocyte chemoattractant protein (MCP)-1 and tumor necrosis factor (TNF)-α] and oxidative stress marker [superoxide dismutase 2 (SOD2), thioredoxin (TXN) and heme oxygenase-1 (HO-1)] gene expression by qPCR c) activation of JNK by western blotting. The potential NHE involvement was assessed by adding amiloride and cariporide (both NHE inhibitors) in SA-treated CACs. Statistical analysis: differences between groups were identified using repeated measure ANOVA (followed by Bonferroni post-hoc).

Results: SGLT-2 expression in human CACs was not detected. Both EMPA and DAPA (at 100 μmol/L) inhibited SA-stimulated expression of IL-1β, IL-8, TNF-α and MCP-1 ($p<0.005$ *vs* SA), but, unexpectedly, not via JNK de-activation. Expression of oxidative stress markers (SOD2, TXN, HO-1) was reduced by EMPA and DAPA as strongly as by the antioxidant N-acetyl-cysteine ($p<0.05$ *vs* SA). Amiloride -a non-specific NHE blocker- but not cariporide (a NHE1 specific inhibitor), mimicked the anti-inflammatory effects of EMPA ($p<0.05$).

Conclusion: EMPA and DAPA can curb SA-induced inflammation and oxidative stress in human CACs, probably through SGLT2-independent mechanism(s), of which NHE-inhibition is a viable candidate. This study may be instrumental in disclosing at least some of the mechanisms involved in EMPA- (and possibly DAPA)-mediated CV protection.

Disclosure: **V. Spigoni:** None.

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Effects of empagliflozin on day-to-day variability of home blood pressure and heart rate in patients with type 2 diabetes

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Background and aims: Recent evidence suggests that day-to-day variability of self-measured home blood pressure is strongly associated with cardiovascular and stroke mortality, and the EMPA-REG OUTCOM study documented impressive benefits with empagliflozin on cardiovascular and all-cause mortality and hospitalization for heart failure. However, the effects of empagliflozin in addition to standard care, on day-to-day variability of self-measured home blood pressure (BP) and heart rate (HR) at home in patients with type 2 diabetes (T2DM) at high cardiovascular risk are not known.

Materials and methods: We followed twenty-three consecutive T2DM patients (mean age: 69 years old, 12 men, mean BMI: 29.0 kg/m^2, mean HbA1c: 7.1%) with cardiovascular risk (CHD: 13 patients(pts), hypertension: 17 pts, atrial fibrillation: 5 pts, cardiac failure: 5 pts, stroke: 1 pt) to add 10 mg of empagliflozin once daily for three months. Home BP and HR were measured once every morning at home, using an oscillometric device. The variability of BP and HR were defined as the standard deviations (SD) of measurements which were performed on seven consecutive days.

Results: For home BP, empagliflozin significantly reduced systolic blood pressure (SBP) from 130±11 mmHg at baseline to 126±11 mmHg at the first week(1W) of the administration ($P<0.05$). SBP achieved the target home BP goal (125±11 mmHg) at the second week(2W) and was maintained during the study (4W: 124±10 mmHg, 8W: 125±8 mmHg, 12W: 124±10 mmHg, $P<0.01$). As regards day-to-day variability of SBP, SD mmHg and coefficient of variation (CV: SD/mean) % decreased from 7.3 ±3.5 mmHg and 5.6±2.7 % at baseline to 6.7±2.5 mmHg and 5.4±1.9 %, respectively, at 1W (4W: 5.8±2.3 mmHg and 4.6±1.9 %, 8W: 5.2 ±2.3 mmHg and 4.2±1.9 %, $P<0.05$). In diastolic blood pressure (DBP), there was a significant reduction of SD and CV compared with that at baseline (baseline: 4.9±1.6 mmHg and 6.9±2.3 %, 4W: 4.3 ±1.6 mmHg and 6.3±2.3 %, 8W: 3.9±1.8 mmHg and 5.5±2.5 %, 12W: 4.4±1.6 mmHg and 6.2±2.3 %, $P<0.05$); however, there was no change of DPB (baseline: 71±10 mmHg, 4W: 68±8 mmHg, 8W: 71±9 mmHg, 12W: 71±8 mmHg). Similarly, there was a decreasing trend in SD and CV of HR (baseline: 3.9±1.2 beats per minute (bpm) and 6.3±1.9 %, 1W: 4.3±2.6 bpm and 6.9±4.1%, 4W: 3.3±1.3 bpm and 5.4±2.1 %, 8W:3.5 ±1.8 bpm and 5.7±3.0 %, 12W: 3.1±1.3 bpm and 5.0±2.2 %, $P<0.1$), although there was no significant change in HR (baseline: 62±13 bpm, 1W: 63±11 bpm, 4W: 61±11 bpm, 8W: 61±11 bpm, 12W: 61±12 bpm).

Conclusion: *1,* Empagliflozin significantly reduced systolic home BP, without increasing HR in T2DM patients. *2,* Empagliflozin tended to reduce the day-to-day variability of self-measured morning home BP and HR in T2DM patients.

Table. Baseline characteristics			
Diabetic treatment	N	Cardiovascular medication	N
Diet only	4	ARB	17
Biguanide	13	ACE inhibitor	4
α-Glucosidase inhibitor	4	Ca channel blocker	17
Sulfonylurea	2	β-blocker	9
DPP-4 inhibitor	3	Diuretic	4
Insulin	3	Statin	15

Disclosure: **T. Misawa:** None.

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Empagliflozin (EMPA) reduces heart failure outcomes irrespective of blood pressure (BP), low density lipoprotein cholesterol (LDL-C) and HbA_{1c} control

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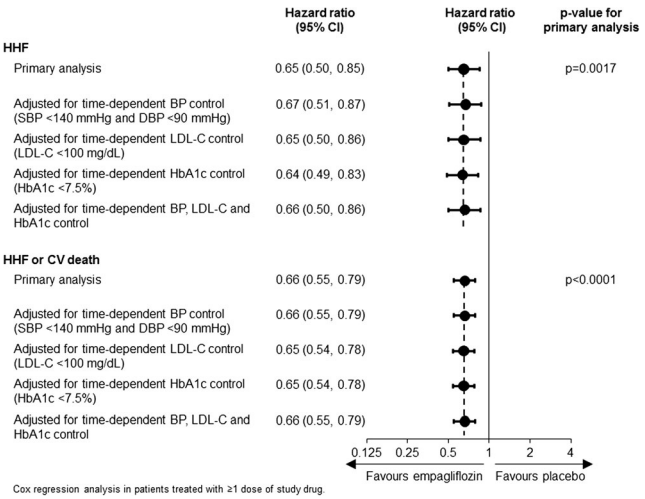
Background and aims: In EMPA-REG OUTCOME, EMPA given in addition to standard of care reduced the risk of hospitalisation for heart failure (HHF) (HR 0.65 [95% CI 0.50, 0.85]) and the composite of HHF or cardiovascular (CV) death (0.66 [0.55, 0.79]) vs placebo (PBO) in patients with type 2 diabetes and established CV disease. We investigated the effects of controlling BP, LDL-C and HbA1c on treatment differences in HF outcomes.

Materials and methods: Patients were randomised to EMPA 10 mg, EMPA 25 mg, or PBO. HHF and the composite of HHF or CV death were assessed in the pooled EMPA group vs PBO adjusting for control of BP, LDL-C and HbA1c at baseline and during the study as time-dependent covariates. Control was defined as systolic BP (SBP)

<140 mmHg and diastolic BP (DBP) <90 mmHg, LDL-C <100 mg/dL, and HbA1c <7.5%.

Results: Adjusting for control of BP, LDL-C or HbA1c at baseline and during the study, HRs for HHF with EMPA vs PBO ranged from 0.64 to 0.67, and HRs for HHF or CV death ranged from 0.65 to 0.66 (Figure). Adjusting for control of all 3 parameters, the HR (95% CI) for HHF was 0.66 (0.50, 0.86) and for HHF or CV death was 0.66 (0.55, 0.79) (Figure).

Conclusion: EMPA reduced HHF and HHF or CV death to the same extent when analyses were adjusted for control of BP, LDL-C and HbA1c over time, suggesting that these risk reductions were preserved irrespective of control of conventional risk factors during the study.



Clinical Trial Registration Number: NCT01131676

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Effects of the SGLT-2 inhibitor empagliflozin on vascular function and central haemodynamics in patients with type 2 diabetes

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Background and aims: Empagliflozin is a selective sodium-glucose cotransporter 2 (SGLT-2) inhibitor indicated for the treatment of type 2 diabetes. Its application leads to improved cardiovascular, renal and heart failure outcome in secondary prevention. To better understand these effects, we examined vascular function and central hemodynamics in patients with type 2 diabetes.

Materials and methods: In this prospective, double blind, randomized, placebo-controlled, crossover, interventional single center study 76 patients with untreated type 2 diabetes were randomized to empagliflozin 25 mg orally once daily or placebo. After 6 weeks of treatment with either empagliflozin or placebo and 1 week wash-out-phase, patients received the other compound for another 6 weeks (cross-over). We analyzed the effects of empagliflozin on central hemodynamics and vascular function as assessed by central systolic blood pressure (BP) (primary endpoint), central pulse pressure, forward and backward wave amplitude and augmentation index under office (Sphygmocor, AtCor, Australia) as well as ambulatory conditions (Mobilograph, IEM, Aachen).

Results: After 6 weeks of therapy fasting plasma glucose, HbA1c, body weight (all p<0.001) and office systolic (p<0.001) and diastolic (p<0.021) BP were significantly lower with empagliflozin than with placebo. Treatment with empagliflozin reduced central systolic BP (114 ± 12 vs 119 ± 14 mmHg, p<0.001), central diastolic BP (74.4 ± 6.9 vs 76.8 ± 8.2 mmHg, p=0.004) and central pulse pressure (39.5 ± 9.9 vs 42.2 ± 11 mmHg, p=0.012) compared to placebo. Forward (p=0.006) and backward (p=0.026) reflection amplitude, assessed under office conditions, were also significantly lower with empagliflozin than with placebo. Under ambulatory conditions over 24 hours we also observed lower central systolic (117 ± 9 vs 119 ± 9 mmHg, p=0.059) and diastolic (79 ± 7 vs 81 ± 7 mmHg, p=0.011) BP after 6 weeks treatment with empagliflozin compared to placebo. Pulse wave velocity under ambulatory conditions was also reduced after 6 weeks with empagliflozin (p=0.016).

Conclusion: Our study demonstrated consistent significant improvements of vascular function and central hemodynamics with empagliflozin under office and ambulatory conditions. Our data support the concept that empagliflozin exerts beneficial effects on cardiovascular and heart failure outcome via improved vascular function.

Clinical Trial Registration Number: NCT02471963

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Disclosure: **R.E. Schmieder:** Lecture/other fees; Boeringer Ingelheim International GmbH.

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Renal protective effect of sodium dependent glucose transporter 2 inhibitor in combination with the renin angiotensin system blockers in early stage of diabetic nephropathy

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Background and aims: The secondary analysis of the Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients showed that subjects treated with empagliflozin, an inhibitor of sodium-dependent glucose transporter 2 (SGLT2), had a significantly lower risk for the progression macroalbuminuria, even though empagliflozin did not prevent incident albuminuria. However, the effects of combined SGLT2 and the renin-angiotensin system (RAS) inhibition on microalbuminuria have not been well-established. Our aim of this retrospective study was to determine the renoprotective effects of combined SGLT2 and RAS inhibition, using urine albumin/creatine ratio (UACR) and estimated glomerular filtration rate (eGFR) as indicators, in subjects with type 2 diabetes (T2D) with early nephropathy (defined as UACR less than 300mg/g).

Materials and methods: Thirty-seven subjects with T2D of outpatients (the mean age: 54.0 years, BMI: 28.1, duration of diabetes: 12.2 years) who have continued to use SGLT2 inhibitor for six months or longer at our University Hospital were selected, and were divided into two groups. One group was already treated with RAS inhibitor (SGLT2i+RASi, n=12), the other group was treated without RAS inhibitor (SGLT2i, n=25). The changes of UACR, eGFR, and metabolic parameters after treatment with SGLT2 inhibitor were compared with the changes of those parameters before treatment using paired Student's *t*-test or Chi square test. Multiple linear regression analysis was performed to evaluate the effects of covariates on albuminuria reduction.

Results: The mean body weight and HbA1c were improved from baseline to six months after the treatment with SGLT2 inhibitor (75.6kg to 73.7kg, 8.1% to 7.8%, respectively, p<0.05). However, UACR or eGFR was not changed six months after treatment with SGLT2 inhibitor compared with baseline (51.0 ± 61.8 vs 54.3 ± 73.3 mg/g, 85.3 ± 21.4 vs 86.1 ± 21.8, respectively). In the subgroup analysis, the mean UACR was decreased 27.5 ± 18.4mg/g in the SGLT2i+RASi group, while it was

increased 13.0 ± 8.9mg/g in the SGLT2i group ($p < 0.05$). Similarly, the proportion of subjects who recognized UACR reduction was significantly higher in the SGLT2i +RASi group than that in the SGLT2i group (66.7% vs 32.0%, $p < 0.05$). However, there was no difference in body weight, HbA1c, eGFR or hematocrit between SGLT2i+RASi group and SGLT2i group. On multiple linear regression analysis, a reduction of eGFR ($p<0.001$), a baseline of UACR($p=0.004$) and the combination with RAS inhibitors ($p=0.002$) were independent predictors to explain the reduction of UACR according to SGLT2i treatment.

Conclusion: Administration of SGLT2 inhibitor reduces urine albumin excretion in T2D subjects who had been prescribed RAS inhibitors. This finding supports the potential use of SGLT2 inhibitor in combination with RAS blockers in patients with type 2 diabetes at high risk of progression of nephropathy.

Disclosure: **D. Kukidome:** None.

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SGLT-2 inhibitors affect on plasma renin activity and aldosterone concentration with the alteration of urinary sodium excretion and haematocrit in type 2 diabetes

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Background and aims: SGLT-2 inhibitors (SGLT-2i) have shown that an inhibition of sodium glucose cotansporter-2 in the renal tubule results in an excretion of urinary glucose and Na. The purpose of this study is to examine alterations of plasma renin activity (PRA) and aldosterone concentration (PAC) after treatment with SGLT-2 inhibitor in type 2 diabetes mellitus (T2D).

Materials and methods: We measured PRA and PAC after resting at supine position for 30 minutes in 18 T2D patients (age 68±18 years old, men: female = 12:6) after treatment with SGLT-2 inhibitors (tofogliflozin: 9 cases, empagliflozin: 4cases, canagliflozin: 4cases dapagliflozin: 1case) for more than 1 months, and also selected as control 18 non-diabetic patients adjusted for age and sex from 2014 to 2016. Moreover, we could measured each metabolic parameters such as blood glucose, HbA1c, serum lipids level, urinary Na/creatinine and blood pressure, and PRA and PAC before and after treatment with SGLT-2 inhibitors (SGLT-2i) for 1month in 7 T2D patients. We analyzed these results with paired and unpaired *t* test using JMP12.2.0.

Results: SGLT-2i significantly increased PRA and decreased PAC levels compared with control as follows; PRA in control group and SGLT-2i group 0.9±0.5 ng/ml/h and 5.8±8.9 ng/ml/h, $p<0.04$, PAC in control group and SGLT-2i group 99±44 pg/ml and 72±36 pg/ml, $p<0.05$. Moreover, when we examined each metabolic parameter before and after treatment with SGLT-2i for 1 month in 7 T2D patients, each body weight, blood pressure, and HbA1c was significantly decreased compared with before treatment in 6 T2D patients ($p<0.04$). PRA value after treatment with SGLT-2i was significantly increased compared with before (before 3.25±3.13, after 5.98±4.78, $p<0.05$), but not PAC. Moreover, correlation between the alteration of PRA and PAC and urinary Na/creatinine and hematocrit were measured. Each alteration of PRA and PAC significantly correlated with alteration of urinary Na/creatinine and hematocrit, respectively ($P<0.05$).

Conclusion: SGLT-2i increases urinary sodium excretion, which affects macula densa in juxta-glomerular apparatus, and finally stimulates secretion of renin, but the alteration of aldosterone and hematocrit was correlated. SGLT-2i-induced increases in urinary sodium excretion affect stimulation of renin secretion and SGLT-2i-induced increase in urinary glucose excretion may affect on osmotic diuresis and aldosterone secretion.

Disclosure: **T. Ishizuka:** None.

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The effects of the SGLT2 inhibitors on the Japanese type 2 diabetes patients with chronic kidney disease

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Background and aims: Some large-scale clinical trials with the sodium-glucose cotransporter-2 inhibitors (SGLT2i) have revealed significant improvements in cardiovascular events and even diabetic nephropathy in patients with type 2 diabetes mellitus(T2DM). The frequency of SGLT2i use by general practitioners is expected to increase; however, it is not clear whether similar results are observed in Japanese patients with T2DM. This study is aimed to clarify the renal effects of the SGLT2i in the Japanese T2DM patients with chronic kidney disease (CKD).

Materials and methods: Data from T2DM patients with CKD who are visiting members of the Kanagawa Physicians Association, and who were taking SGLT2i administration were extracted. Clinical findings at the time of the survey and the beginning of SGLT2i administration were examined.

Results: Among 863 registered cases, 761 cases were analysed (male: female = 491: 270, age 59.6±13.0 years). The administration period was 14.4 months on average. The distribution of eGFR (mL/min/1.73m^2) was as follows: ≥ 60 is 74.2%; 30-59, 25.8%; and <30, 0.3%. Six SGLT2i were used: ipragliflozin (n=236), empagliflozin (n=123), tofogliflozin (n=144), dapagliflozin (n=112), canagliflozin (n=88), and luseogliflozin (n=54). Seventy-seven patients (10.1%) were administered SGLT2i alone, while 434 (57.0%) were also treated with metformin. DPP4 inhibitors were administered to 423 patients (55.6%), sulfonylureas to 260 (34.2%), insulin to 195 (25.6%), and GLP1 analogues to 101 patients (13.3%). Clinical findings at the beginning of administration versus at the time of the survey are shown as mean ± SD. Body weight (kg), 76.8±16.4 vs. 74.6±16.0 ($p<0.01$); Hemoglobin A1c (HbA1c, %), 8.1±1.5 vs. 7.4±1.2 ($p<0.01$); blood pressure (BP) at the hospital (mmHg), 138.3 ± 19.7/79.2 ± 12.7 vs. 132.7 ± 17.0/77.5 ± 11.3 ($p<0.01$); BP in the morning at home (n=71; mmHg), 129.5 ± 13.9/76.3 ± 13.6 vs. 128.5 ± 13.5/74.8 ± 11.2 ($p=0.55$). Improvements in both weight loss and HbA1c were found in 62.8% of cases. eGFR decreased from 78.1 ± 24.1 to 75.4 ± 24.3 mL/min/1.73m^2 ($p<0.01$), and logarithmic value of albumin-creatinine ratio (ACR) was significantly decreased from 4.13 ± 1.56 to 3.94 ± 1.55 ($p<0.01$). The changes of logarithmic value of ACR based on the presence (+) or absence (-) of concomitant medications were; DPP4 inhibitors (+/-), -0.24 ± 1.03 vs. -0.12 ± 0.92 ($p=0.11$); GLP1 analogue (+/-), -0.14 ± 1.04 vs. -0.20 ± 0.97 ($p=0.57$); Renin-angiotensin system inhibitors(+/-), -0.26 ± 1.06 vs. -0.12 ± 0.89 ($p=0.06$). In this study, the degree of adherence with dietary treatment of each patients was divided into four groups ("good" (n=17), "fair" (n=117), "a little bad" (n=291), and "bad" (n=77)). The change of logarithmic value of ACR were -0.56 ± 0.85, -0.17 ± 0.89, -0.22 ± 0.96, and 0.02 ± 0.88, respectively, which was considered to be related to adherence with dietary treatment.

Conclusion: Although the use of concomitant drugs varies between Japan and other countries, this retrospective study confirmed that the results of large-scale clinical trials were also observed in the real world in Japan. The importance of dietary therapy was also partially observed, and further discussion on the relationship with background factors or differences between each SGLT2i should be considered in the future.

Disclosure: **K. Kobayashi:** None.

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Empagliflozin and incidence of events consistent with acute renal failure, including acute kidney injury: pooled safety analysis in >12,000 individuals

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Background and aims: SGLT2 inhibitors may alter renal hemodynamics, often reflected as a modest decrease in renal function shortly after drug initiation. Although GFR generally stabilizes during chronic treatment, reports of acute kidney injury (AKI) have emerged, representing an important safety consideration.
Materials and methods: To investigate events consistent with acute renal failure (ARF) including AKI with empagliflozin (EMPA), we analyzed pooled safety data from >12,000 patients with T2D who were randomized (1:1:1) to EMPA 10 mg, 25 mg, or PBO in 15 Phase I-III trials (including EMPA-REG OUTCOME) plus 4 extension studies. ARF incidence was assessed using investigator-reported adverse events (without adjudication) coded according to the narrow standardized MedDRA query. AKI was captured by the MedDRA preferred term 'AKI'.
Results: Total exposure (patient-years) was 7782, 7754 and 7369 for EMPA 10 mg, 25 mg and PBO, respectively. The incidence rates of either ARF (table) or AKI were similar for the two EMPA doses and PBO. As expected, ARF and AKI events increased with decreasing renal function, yet the overall risk with EMPA was similar to PBO across all subgroups of baseline eGFR.
Conclusion: This large pooled analysis of >12,000 patients with T2D does not suggest an increased risk for acute renal events with EMPA in the setting of controlled clinical trials. When initiating EMPA, careful monitoring of renal function according to local prescribing information is recommended.

	Placebo		Empagliflozin 10 mg		Empagliflozin 25 mg	
Acute Renal Failure	n/N (%)	Rate /100-patient yrs	n/N (%)	Rate /100-patient yrs	n/N (%)	Rate /100-patient yrs
All patients*	159/4203 (3.8)	2.19	137/4221 (3.2)	1.78	141/4196 (3.4)	1.84
eGFR ≥90	13/1172 (1.1)	0.71	9/1204 (0.7)	0.45	10/1233 (0.8)	0.49
eGFR 60 to <90	56/2298 (2.4)	1.42	56/2285 (2.5)	1.35	53/2216 (2.4)	1.30
eGFR 45 to <60	55/529 (10.4)	5.22	45/530 (8.5)	3.96	42/531 (7.9)	3.72
eGFR 30 to <45	32/197 (16.2)	7.87	24/192 (12.5)	6.23	34/197 (17.3)	8.86
eGFR <30	3/7 (42.9)	37.65	3/9 (33.3)	21.35	2/16 (12.5)	7.59

*Baseline eGFR subgroups (mL/min/1.73m^2) according to MDRD (Modification of Diet in Renal Disease) formula. Baseline eGFR measurements available for 12,616 participants (PBO, N=4203; EMPA 10 mg, N=4220; EMPA 25 mg, N=4193). Renal safety profile was assessed using investigator-reported AEs. ARF was assessed based on the narrow standardized MedDRA query for the condition, which included the MedDRA preferred term AKI.

Clinical Trial Registration Number: NCT00885118, NCT00789035, NCT00558571, NCT00749190, NCT01011868, NCT01193218, NCT01210001, NCT01177813, NCT01159600, NCT01289990, NCT01131676, NCT01164501, NCT01370005, NCT01306214, NCT01947855
Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance
Disclosure: **R. Agarwal:** Non-financial support; Boehringer Ingelheim.

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Safety update on dapagliflozin across the phase 2b/3 clinical trial programme

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Background and aims: Safety and tolerability of sodium-glucose cotransporter-2 inhibitors (SGLT2i) are of interest. Dapagliflozin is a highly selective SGLT2i for the treatment of type 2 diabetes.
Materials and methods: Data were pooled from 13 placebo-controlled trials of up to 24 weeks' duration (Table). To detect rare adverse events (AEs), larger placebo/comparator-controlled pools of 21 trials (≤208 weeks; dapagliflozin, n=5936; control, n=3403) and 30 trials (≥12 weeks; dapagliflozin, n=9195; control, n=4629) assessed diabetic ketoacidosis and lower-limb amputations, respectively.
Results: Over 24 weeks, AE and serious AE rates were similar for dapagliflozin vs placebo; 60 vs 56% and 5 vs 5%, respectively. Rates of hypoglycaemia, volume depletion AEs and urinary tract infection were balanced between groups (Table). Genital infections were more frequent with dapagliflozin vs placebo (6 vs 1%), fractures were balanced between groups (0.3 vs 0.7%) and renal function AEs occurred in 3 vs 2% of patients (most common was decreased creatinine clearance; 1.1 vs 0.7%). In the 21-study pool, 1 serious event of diabetic ketoacidosis and 3 events of ketonuria/metabolic acidosis occurred with dapagliflozin; estimated incidence for any of these events was 0.03 (95% CI: 0.010, 0.089). In the 30-study pool, lower-limb amputations occurred in 8 (0.1%) dapagliflozin vs 7 (0.2%) control patients.
Conclusion: In summary, dapagliflozin was well tolerated across the clinical trial programme. AE rates were generally balanced for dapagliflozin vs placebo/control, including special interest AEs such as hypoglycaemia, fractures, amputations and diabetic ketoacidosis.

PBO/comparator-controlled 30-study pool: Amputation	DAPA total (N=9195)	Control (N=4629)	PBO/comparator-controlled 21-study pool: DKA	DAPA total (N=5936)	Control (N=3403)
Patients with lower-limb amputations, n (%)	8 (0.1)	7 (0.2)	SAE of DKA, n	1	0
			AE ketonuria / metabolic acidosis, n	3	0
			Incidence DKA*, % (95% CI)	0.02 (0.004, 0.059)	0
			Incidence DKA/metabolic acidosis*, % (95% CI)	0.03 (0.010, 0.089)	0
ST PBO-controlled 13-study pool: Patients with events, n (%)			**DAPA 10 mg (N=2360): 998 p-y**	**PBO (N=2295): 958 p-y**	
Fractures			8 (0.3)	17 (0.7)	
Hypoglycaemia; Major event; AE → discon.			324 (14); 3 (0.1); 1 (<0.1)	284 (12); 2 (0.1); 0	
Renal function†			76 (3)	42 (2)	
Volume depletion§			27 (1)	17 (1)	
UTI, Total; Females			110 (5); 85 (4)	81 (4); 64 (3)	
Genital infection, Total; Females			130 (6); 84 (4)	14 (1); 11 (<1)	

Adverse events are based on a predefined list of preferred terms; Includes data after rescue; *Estimated incidence; †Includes renal failure or impairment, creatinine renal clearance decreased/increased/abnormal, blood creatinine increased/decreased, glomerular filtration rate decreased/increased/abnormal, cystatin C increased, renal function test abnormal, urine flow or output decreased/increased/abnormal, anuria. §Hypotension/hypovolemia/dehydration. DAPA total includes DAPA 2.5, 5, 10, 20 and 50 mg groups combined. Control includes placebo with/without background medications or active control including benchmark treatments. AE=adverse event; DAPA=dapagliflozin; discon.=discontinuation; LT=long term; PBO=placebo; p-y=patient-years; SAE=serious adverse event; ST=short term; UTI=urinary tract infection; wks=weeks

Table. Adverse events of special interest in the dapagliflozin Phase 2b/3 clinical trial programme

Clinical Trial Registration Number: NCT00263276, NCT00357370, NCT00528372, NCT00528879, NCT00643851, NCT00660907, NCT00663260, NCT00831779; NCT00673231, NCT00680745, NCT00683878, NCT00736879, NCT00855166, NCT00859898, NCT00972244, NCT00976495, NCT00984867, NCT01031680 + 12 more studies
Supported by: AZ
Disclosure: **A.J.L. Scheen:** None.

PS 073 Metformin

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Factors associated with the discontinuation of metformin therapy: results from 11,539 patients in the global DISCOVER study

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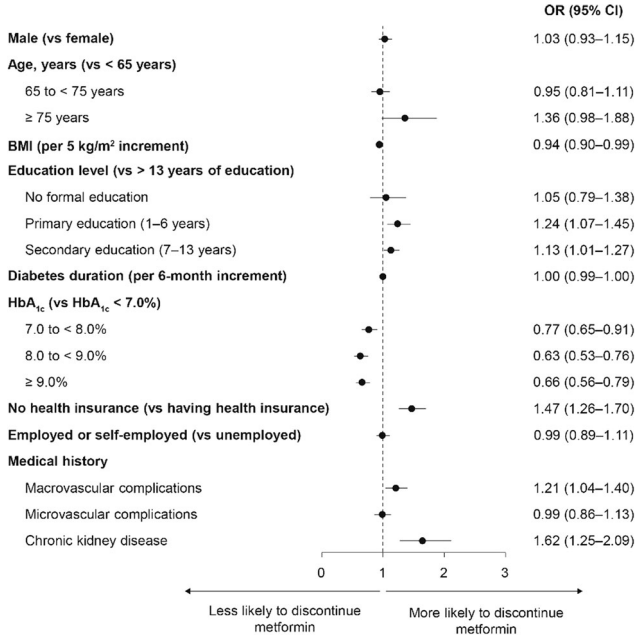
Background and aims: Clinical guidelines recommend metformin as first-line glucose-lowering therapy in patients with type 2 diabetes mellitus (T2DM), with the addition of a second agent if HbA_{1c} levels remain uncontrolled. Some patients may discontinue metformin, most often because of intolerance. However, the frequency of, and factors associated with, metformin discontinuation have not been well described on a global scale.
Materials and methods: DISCOVER is a 3-year observational study of patients with T2DM initiating a second-line therapy. Treatment patterns, reasons for treatment changes, and clinical variables were recorded at baseline using a standardized electronic case report form. A multivariate hierarchical logistic regression model, with country as a random effect, was used to assess factors associated with metformin discontinuation.
Results: Data were assessed from a total of 13 245 patents across 32 countries. Of these, 11 539 (87.1%) used metformin as first-line therapy, either as monotherapy (7977 patients, 69.1%) or as part of a combination (3562 patients, 30.9%). Among these, 4083 (35.4%) discontinued metformin when initiating second-line therapy (37.2% of those who used initial metformin monotherapy; 31.3% of those who used metformin as part of a combination). The rate of metformin discontinuation varied greatly across countries (9.7-98.0%). Factors associated with the likelihood of discontinuing metformin are shown in the Figure. Patients with chronic kidney disease (relative to without) and those without health insurance (relative to with) were more likely to discontinue metformin. Patients with $HbA_{1c} \geq 7.0\%$ were less likely to discontinue metformin than those with $HbA_{1c} < 7.0\%$. When compared with those aged < 75 years, patients aged ≥ 75 years had an OR for discontinuation of 1.38 (95% CI 1.00-1.90).
Conclusion: Metformin was discontinued in more than one-third of patients initiating a second-line therapy. Although discontinuation may be appropriate in some patients, this rate is considerably higher than would be expected. Adverse effects may be the reason for discontinuation in patients with $HbA_{1c} < 7.0\%$.

Figure. Likelihood of discontinuing metformin when initiating a second-line glucose-lowering therapy. ORs were calculated using a hierarchical logistic regression model, with country as a random effect and adjusted for all variables in the figure.



Clinical Trial Registration Number: NCT02322762
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Small intestine as the main site of blood glucose-lowering effect during an early response to single dose of metformin

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Background and aims: Metformin is the most prescribed drug for the treatment of type 2 diabetes. During long-term treatment, metformin inhibits hepatic gluconeogenesis while enhancing glucose uptake in skeletal muscle. Although it is known that the gut has an important role in the effects of metformin, its precise involvement is still not fully understood, especially in the context of acute metformin administration. Thus, we studied acute effects of metformin on glucose uptake in tissues as well as glucose transport across intestinal epithelium in dietary obese mice.
Materials and methods: Adult C57BL/6J mice were fed a corn oil-based high-fat diet (HF; lipids ~35% wt/wt) for 8 weeks. Thereafter overnight fasted mice were given a single dose of metformin (Met; 400 mg/kg body

weight) or saline (Sal) by oral gavage. After 30 minutes mice were subjected to either oral glucose tolerance test (OGTT) or the analysis of glucose uptake using ^{18}F-fluorodeoxyglucose and PET/μCT or radiolabeled assays using [^{3}H]2-deoxyglucose. The same experimental setup was used to measure *ex vivo* glucose transport across intestinal epithelium, when mice were sacrificed 30 min after metformin gavage and two segments of jejunum and ileum were used to prepare everted gut sacs. Data are presented as means ± SE. Data were analyzed with *t*-test at the significance level $p = 0.05$.

Results: Incremental area under the glycaemic curve during OGTT (as a marker of glucose intolerance) was decreased in Met when compared to Sal group (Met, 587±79 vs. Sal, 1713±109 mmol/180 min), while plasma insulin levels did not differ between the Met and Sal group 30 min after glucose administration. The analysis using PET/μCT did not reveal any differences in radioisotope accumulation between Met and Sal group one hour after ^{18}F-FDG gavage. Glucose uptake using [^{3}H]2-deoxyglucose was unchanged in skeletal muscle, adipose tissue or kidney of Met vs. Sal group. On the contrary, decreased glucose uptake was observed in the livers of mice from Met group (Met, 61±6 vs. Sal, 98±13 dpm/mg tissue; $p<0.05$). Furthermore, glucose transport across intestinal epithelium was reduced in proximal jejunum (Met, 0.4±0.2 vs. Sal, 1.7±0.2 mmol), and in both proximal (Met, 1.3±0.3 vs. Sal, 4.1±0.4 mmol) and distal (Met, 2.9 ±0.3 vs. Sal, 4.6±0.6 mmol) ileum in the Met group.

Conclusion: Together our results support the idea that the inhibition of transepithelial glucose transport in the intestine is responsible for blood glucose-lowering during an early response to single dose of metformin.

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Changes in metabolites in response to metformin during the 18-month Copenhagen Insulin and Metformin Therapy (CIMT) Trial

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Background and aims: Metformin is the first-line treatment in type 2 diabetes. However, not all patients benefit from treatment. Our aim was (1) to investigate the effects of metformin on the plasma metabolome, of which e.g. increased levels of branched chain and aromatic amino acids are known to be associated with type 2 diabetes, giving molecular insight into its known HbA_{1c}-lowering effect, and (2) to find out, whether metabolomic profile can predict the HbA_{1c}-outcome of the treatment.

Materials and methods: The CIMT trial was a multicenter trial from May 2008 to December 2012. Participants were randomized to 18 months of treatment with metformin/placebo and to three different insulin regimens. In total, 87 metabolites were analyzed in all participants (n=371) with LC/MS at baseline and follow-up. Metabolite levels were standardized and analyzed with a linear mixed effect model in R, adjusting for age, sex, BMI, and previous insulin treatment. Associations between metabolites and HbA_{1c} were analyzed with a linear regression model with the same adjustments. Variables with a multiple testing-corrected $p<0.1$ are reported.

Results: At baseline, subjects who were treated with metformin before the trial (n=313) had higher levels of leucine/isoleucine (one peak) and five lysophosphatidylethanolamines (LPEs C16:0, C18:1, C20:4, 20:5 and C22:6), and lower levels of carnitine and valine, when compared to metformin-naïve subjects (n=58). As an effect of metformin treatment in the trial at follow-up, again, leucine/isoleucine was elevated and carnitine and valine were reduced compared to baseline. In addition to these confirmatory findings, we found lower levels of hydroxycinnamic acid, cinnamic acid, phenylacetamide and tyrosine. We did not find significant associations between baseline metabolites and follow-up HbA_{1c} in the randomized population ($p>0.16$). However, we found an association to HbA_{1c} at baseline between those that had been on metformin before the trial and the metformin-naïve patients: The levels of carnitine C10:1 ($p_{interaction}$=0.025), carnitine C12:1 ($p_{interaction}$=0.067) and leucine/isoleucine ($p_{interaction}$=0.027) were negatively associated with HbA_{1c} at baseline in the metformin treated group compared to the metformin-naïve group.

Conclusion: Contrary to what might have been expected, in this exploratory analysis of changes in the plasma metabolome associated with metformin use, our results indicate that metformin is associated with increased levels of LPEs and leucine/isoleucine which are known to be associated with diabetes and insulin resistance. Meanwhile, there were improvements in the levels of valine, tyrosine and carnitine which are also associated with insulin resistance and mitochondrial dysfunction. This differential response deserves further study. Because of the small number of metformin-naïve patients in this study, we were not able to identify metabolites that can predict a favourable HbA_{1c}-response to metformin.

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Metformin exposure in diabetic subjects is similar to patients without diabetes

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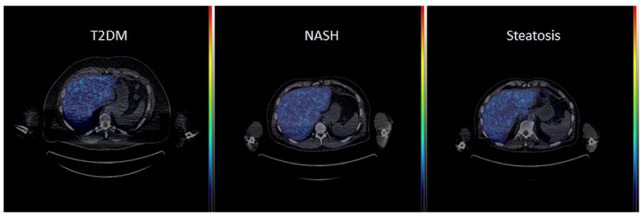
Background and aims: The effectiveness, low cost and favorable side effect profile of metformin, has made it first-line anti-diabetic treatment in type 2 Diabetes Mellitus (T2DM) worldwide. A study has demonstrated a 80-fold difference in steady state plasma metformin concentrations in patients with T2DM with no correlation to decrease in HbA1c after adjusting for baseline HbA1c. Plasma metformin concentration is therefore considered a poor surrogate marker of the glucose lowering effect of metformin. It is well accepted that the anti-hyperglycaemic effect of metformin involves reduction of hepatic glucose production and thus requires hepatic uptake of the drug. Under physiological pH metformin is an organic cation and relies on the membrane transporters, OCT1 and MATE1, for hepatic uptake. To determine hepatic exposure to metformin in diabetic subjects *in vivo*, we have used ^{11}C-metformin Positron Emission Tomography (PET) to investigate diabetic and non-diabetic patients with non-alcoholic steatohepatitis (NASH) or simple steatosis. As secondary endpoint, mRNA expression of OCT1 and MATE1 was investigated under the hypothesis that the level of metformin exposure was associated to their level of expression.

Materials and methods: Diabetic subjects with NASH (n=4), non-diabetic subjects with NASH (n=7) or simple steatosis (n=7) where liver biopsies had been obtained for diagnostic purposes, were studied using PET after intravenous injection of ~167 MBq ^{11}C-metformin. Volume of distribution (Vd) was evaluated using Pmod (PMOD Technologies LLC). Liver biopsies were analyzed using qPCR and normalized to 40S ribosomal protein S18.

Results: Hepatic exposure to metformin in diabetic patients with NASH (Vd 1.91 ± 0.09) is similar to non-diabetic patients with NASH (Vd 2.27 ± 0.15) and simple steatosis (Vd 2.39 ± 0.25) p=0.32, One-Way ANOVA. Preliminary data from qPCR analysis on liver biopsies show no significant difference in mRNA expression of OCT1 and MATE1 among

diabetic and non-diabetic patients (p=0.17 and p=0.18 respectively), nor was there an apparent correlation between transporter expression and Vd.
Conclusion: This study demonstrates that metformin exposure *in vivo* is stable in diabetic and non-diabetic human subjects with non-alcoholic fatty liver disease (NAFLD). Approximately 70% of patients with T2DM have NAFLD as part of the metabolic syndrome, and their hepatic disease does not influence metformin distribution.



Supported by: NNF Excellence Project Grant
Disclosure: **E.I.O. Sundelin:** None.

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Differences in the correlation of HbA_{1c} versus fasting plasma glucose in large cohorts of drug-naive versus metformin treated patients

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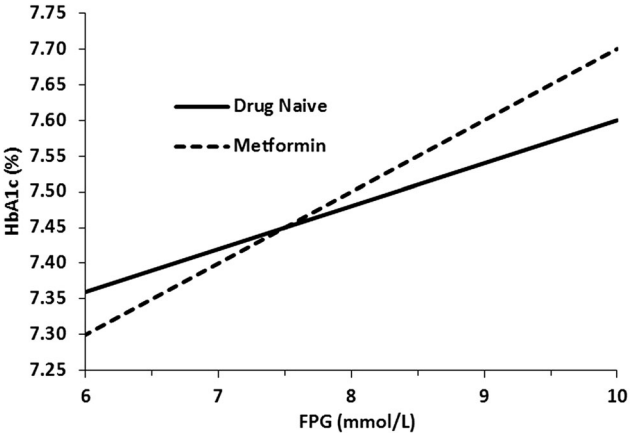
Background and aims: We previously tested the relative contribution of fasting plasma glucose (FPG) and postprandial glucose (PPG) to HbA1c using an algorithm developed by the A1c-Derived Average Glucose (ADAG) study group. The algorithm estimates average glucose exposure to calculate apparent PPG by subtracting FPG. The ADAG algorithm predicted 24 hour PPG exposure in drug-naive patients but was not robust enough to assess this PPG exposure in metformin treated patients. The current analysis was carried out in an attempt to further investigate the difference between drug-naive and metformin treated patients.
Materials and methods: The baseline data (before treatment) of drug-naive (n=2445) and of metformin treated patients (n=2727) from the pooled vildagliptin database were utilised. The slope of HbA1c versus FPG for the overall population and for the groups with HbA1c <8% (drug-naive, n=933 and metformin, n=1634) and ≥8% (drug-naive, n=1512 and metformin, n=1093) were calculated.
Results: The overall population slopes of HbA1c versus FPG were 0.21 ±0.007, R^2=0.44 in drug-naive and 0.21±0.005, R^2=0.58 in metformin from the same intercept of 6.5%. In patients with HbA1c <8%, the slopes were 0.06±0.007, R^2=0.56, intercept=7.0% in drug-naive and 0.10 ±0.006, R^2=0.29, intercept=6.7% in metformin treated patients. The HbA1c ≥8% slopes were 0.13±0.007, R^2=0.20, intercept=7.7% in drug-naive and 0.13±0.008, R^2=0.30, intercept=7.6% in metformin. Thus, there was no difference in overall population or in patients with HbA1c ≥8% between drug-naive and metformin, although the slope was lower and the intercept was higher in patients with HbA1c ≥8%. However, in patients with HbA1c <8% the slopes were lower and from a lower intercept than in patients with HbA1c ≥8%. Furthermore, in patients with HbA1c <8% from a 0.3% higher intercept the drug-naive slope was lower than the metformin slope (figure). We assume that a lower slope (adjusted for intercept) of HbA1c versus FPG is due to a greater contribution of PPG to the total glucose exposure.
Conclusion: Thus, the data are consistent with a greater PPG contribution to HbA1c in patients with HbA1c <8% in both drug-naive and metformin treated patients. The apparent higher PPG contribution in drug-naive than in metformin treated patients with HbA1c <8% may explain why the ADAG algorithm predicted 24 hour PPG exposure in drug-naive patients but was not robust enough to assess this PPG exposure in metformin treated patients. Furthermore, the apparent higher PPG contribution in metformin treated patients with HbA1c <8% is greater than that in metformin treated patients with HbA1c >8%, suggesting that metformin may reduce PPG independent of its baseline effect to inhibit hepatic glucose production and that this contributes to its reduction in HbA1c.

Figure: Baseline HbA1c versus baseline FPG in patients with type 2 diabetes mellitus with baseline HbA1c levels <8%



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Disclosure: **B. Ahrén:** None.

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Comparative assessment of glycaemic achievements with second line anti diabetes therapy intensification: real world evidence based choices for patients and providers

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Background and aims: There are multiple options for second-line anti-diabetic drugs (ADDs), but little is known about their effects on glycaemia in clinical practice. The aim of this real-world study was to inform clinicians and patients about the likelihood of achieving desired glycaemic control with each of the major second line ADDs when added to metformin.
Materials and methods: Centricity Electronic Medical records contains extensive primary and ambulatory care clinical data from the USA. A cohort of patients with T2DM on metformin, who added and continued a second line ADD for at least 6 months, and without history of cardiovascular, renal or malignant diseases, was identified with: DPP-4 inhibitor (DPP-4i, n=22,085), GLP-1 receptor agonist (GLP-1RA, n=5,491), sulfonylurea (SU, n=67,291), thiazolidinedione (TZD, n=11,696), and insulin (INS, n=15,957). Adjusted change in HbA1c, the probability of reducing HbA1c below 7% or by at least 1.5% were evaluated over 6, 12 and 24 months of continued therapy - stratified by HbA1c categories of 7-8%, 8-9%, 9-12% and > 12% at therapy intensification (baseline).
Results: The 5 treatment groups had similar mean follow-up of 4 years and variable baseline characteristics: age (mean: 53-59 years), gender (male: 35-53%), weight (mean: 98-110 kg), and HbA1c (mean: 7.8-9.3%). Mean unadjusted HbA1c changes at 6 months were 0.8/ 0.8/ 0.7/ 1.0/ 0.8 % in SU/ DPP-4i/ GLP-1RA/ INS/ TZD groups respectively. In the 8-9% baseline HbA1c group, adjusted HbA1c reductions were consistent over 2 years and were similar in the GLP-1RA, TZD and DPP-4i groups, while SU and INS users experienced significantly smaller reductions (6- and 12-month HbA1c changes in Table 1). Patients in the

DPP-4i, GLP-1RA and TZD groups had a significantly higher probability of reducing HbA1c by at least 1.5% or below 7% at 6 months and over 2 years (probability range: 35 - 50%) compared to SU or INS groups. The SU group had a higher probability of reducing HbA1c by at least 1.5% (probability range: 27 - 32%) compared to the INS group (probability range: 20 - 30%) over 2 years. In the 9-12% baseline HbA1c group, HbA1c reductions were similar in GLP-1RA, TZD, DPP-4i and SU groups at 6 months (mean: 1.9 - 2.2%). While the probability of reducing HbA1c by at least 1.5% was similar in all ADD groups at 6 months (probability range: 57-70%), the likelihood of better glycaemic achievements was significantly higher at 12 and 24 months in the incretins and TZD groups (Table 1).

Conclusion: In metformin treated patients with T2DM, intensification with SU or INS offers relatively lower probability of short- and long-term glycaemic achievements, in comparison to incretins or TZDs.

Table 1: Adjusted reduction in HbA1c (95% CI), probability to reduce HbA1c for at least 1.5% (95% CI), and probability to reduce HbA1c below 7% (95% CI) at 6 and 12 months post second-line initiation by baseline HbA1c category and by second-line drug class in type 2 diabetes patients with no history of cardiovascular, chronic kidney disease or cancer.

Baseline HbA1c category		8-9%			9-12%		
Comparator Group	Time	|ΔHbA1c|	P(|ΔHbA1c|≥1.5%)	P(HbA1c<7%)	|ΔHbA1c|	P(|ΔHbA1c|≥1.5%)	P(HbA1c<7%)
MET + SU	6 months	0.8 (0.82, 0.86)	0.3 (0.31, 0.32)	0.3 (0.31, 0.32)	1.9 (1.89, 1.95)	0.6 (0.62, 0.63)	0.2 (0.23, 0.25)
	12 months	0.7 (0.67, 0.71)	0.3 (0.28, 0.30)	0.3 (0.28, 0.29)	1.9 (1.90, 1.96)	0.6 (0.61, 0.63)	0.3 (0.24, 0.25)
MET + DPP-4i	6 months	1.0 (0.96, 1.03)	0.4 (0.35, 0.38)	0.4 (0.35, 0.38)	2.2 (2.15, 2.25)	0.7 (0.67, 0.70)	0.3 (0.30, 0.33)
	12 months	0.9 (0.90, 0.98)	0.4 (0.35, 0.38)	0.4 (0.35, 0.38)	2.4 (2.31, 2.43)	0.7 (0.69, 0.72)	0.4 (0.33, 0.36)
MET + GLP-1RA	6 months	1.1 (0.99, 1.17)	0.4 (0.39, 0.47)	0.4 (0.39, 0.47)	2.0 (1.89, 2.17)	0.6 (0.59, 0.67)	0.3 (0.27, 0.34)
	12 months	1.1 (0.93, 1.17)	0.4 (0.39, 0.48)	0.4 (0.40, 0.49)	2.3 (2.08, 2.42)	0.7 (0.62, 0.70)	0.4 (0.30, 0.39)
MET + INS	6 months	0.6 (0.58, 0.71)	0.3 (0.25, 0.30)	0.3 (0.27, 0.31)	1.8 (1.71, 1.82)	0.6 (0.57, 0.60)	0.2 (0.23, 0.26)
	12 months	0.4 (0.36, 0.51)	0.2 (0.22, 0.27)	0.3 (0.22, 0.27)	1.8 (1.70, 1.82)	0.6 (0.55, 0.58)	0.2 (0.22, 0.25)
MET + TZD	6 months	1.1 (1.06, 1.17)	0.4 (0.37, 0.42)	0.4 (0.38, 0.43)	2.1 (2.02, 2.19)	0.7 (0.65, 0.70)	0.3 (0.29, 0.33)
	12 months	1.1 (1.04, 1.17)	0.4 (0.39, 0.45)	0.5 (0.42, 0.48)	2.4 (2.24, 2.45)	0.7 (0.68, 0.73)	0.4 (0.35, 0.40)

Disclosure: **O. Montvida:** None.

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Clinical and economic outcomes in type 2 diabetes patients treated with fixed-dose versus loose-dose combination anti-hyperglycaemic therapies

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Background and aims: Medication adherence is required for successful type 2 diabetes (T2D) treatment and adequate A1C reduction. Prior research has demonstrated that fixed-dose combination (FDC) regimens lead to greater patient adherence than loose-dose combination (LDC) regimens. Given the new entrants into the FDC market, this study aimed to compare adherence and clinical and economic outcomes between patients initiating FDC and LDC anti-hyperglycemic regimens in a contemporary, real-world setting.

Materials and methods: his retrospective, observational study used claims data from *Truven Health* MarketScan Commercial and Medicare Supplemental Databases (2013-2015) to identify and compare T2D patients initiating metformin-containing FDC or LDC oral anti-hyperglycemic regimens. Outcomes were measured in the 12 months following initiation. Adherence was defined as the proportion of days covered (PDC) by the initiated regimen derived from the days' supply of pharmacy claims for regimen medication(s); patients with PDC ≥80% were deemed adherent. A logistic model was fit to determine the odds of being adherent for the FDC versus LDC cohort, adjusted for key baseline characteristics, including index-regimen non-metformin class. The highest recorded A1C values in the year before and after initiation were used for analysis among the subset of patients with laboratory data available. Annual T2D-related costs were summed using all claims with a primary diagnosis of T2D or claims for anti-hyperglycemic medications.

Results: The identified cohorts of 22,456 FDC patients and 21,172 LDC patients were well balanced on key baseline characteristics, including mean age (both cohorts: 55.2 years) and sex (women: FDC = 40.8%, LDC = 41.8%). Additionally, among the subset of patients with laboratory data (FDC N=842; LDC N=873), the proportion of patients with baseline A1C values <7.0 were not significantly different between the cohorts (FDC: 17.3% vs. LDC: 16.7%; p=0.735). A majority of the FDC cohort initiated on a metformin+dipeptidyl peptidase-4 (DPP-4) inhibitor regimen (77%), while metformin+sodium glucose cotransporter-2 (SGLT2) inhibitor regimens were rare (1.3%). Among the LDC cohort, the most frequently initiated regimen was metformin+sulfonylurea (73%). Nearly 80% of FDC medications were brand-named, versus only 23% of LDC regimens. In the year following initiation, FDC patients were significantly more likely to be adherent compared with LDC patients (41% vs. 29%; p<0.001). In the adjusted logistic model, the odds of being adherent were 1.69 times greater for the FDC cohort compared with the LDC cohort (p<0.001). The proportion of patients achieving an A1C value <7.0 during the year following initiation was 7% higher among the FDC cohort (37% vs. 30%, p=0.001). Compared with the LDC cohort, the mean T2D-related pharmacy costs were higher among FDC patients (FDC: $2,142 vs. LDC: $917; p<0.001); however, T2D-related medical costs were significantly lower (FDC: $1,483 vs. LDC: $1,717; p<0.001).

Conclusion: This study provides real-world evidence that FDC anti-hyperglycemic regimens are associated with higher adherence, lower T2D-related medical costs, and improved A1C outcomes compared with LDC regimens. These findings strengthen evidence for prescribing FDC medications to achieve significant clinical improvements in patients with T2D requiring combination therapy.

Disclosure: **A. Vlahiotis:** Employment/Consultancy; Anna is an employee of Truven Health Analytics, an IBM Company. Truven Health was paid by AstraZeneca to conduct this study.

PS 074 SGLT2 inhibitors and the heart

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Effects of canagliflozin on biomarkers of cardiovascular stress in older patients with type 2 diabetes

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Background and aims: Sodium glucose co-transporter 2 (SGLT2) inhibitors may reduce cardiovascular (CV) risk in patients with type 2 diabetes mellitus (T2DM). We examined the effects of canagliflozin (CANA) on the CV stress biomarkers N-terminal pro-B type natriuretic peptide (NT-proBNP) and high sensitivity troponin I (hsTnI) in older patients with T2DM.
Materials and methods: In this exploratory analysis of a subset of T2DM patients (age 55-80 y, mean = 64 y; 56% male, T2DM duration = 12 y) randomized to placebo (PBO; N = 216), CANA 100 mg/d (N = 229), or CANA 300 mg/d (N = 221) for 104 weeks, serum levels of NT-proBNP (Roche) and hsTnI (Abbott) were measured. Median percent change from baseline was compared at weeks 26, 52, and 104. For these analyses, CANA groups were pooled.
Results: Baseline characteristics were similar between groups. From a baseline median of ≈50 pg/mL, NT-proBNP increased with PBO, but changed minimally with CANA over 104 weeks (Table). From a baseline median of ≈3.3 pg/mL, hsTnI also increased with PBO, but was reduced or unchanged with CANA. Hodges-Lehmann estimates (95% confidence interval) of the median percent differences between CANA and PBO at weeks 26, 52, and 104, respectively, were -13.2% (-25.4, -1.7), -16.3% (-28.9, -4.0), and -27.6% (-43.2, -11.5) for NT-proBNP; and -8.3% (-14.0, -2.5), -11.9% (-18.0, -5.6), and -10.0% (-17.3, -2.6) for hsTnI ($p<0.05$ for each between-group difference).
Conclusion: CANA delayed rise in NT-proBNP and hsTnI over 2 years compared with PBO in older T2DM patients. These results suggest attenuation in CV stress with CANA, consistent with the anticipated CV protective effect of SGLT2 inhibitors.

Table. Median percent change in NT-proBNP and hsTnI from baseline to 26, 52, and 104 weeks.

Parameter*,†	PBO	Pooled CANA	Difference vs PBO (95% CI)
NT-proBNP			
Week 26	17.5 (11.8)	−3.6 (8.6)	−13.2 (−25.4, −1.7)‡
Week 52	13.2 (8.1)	−1.2 (8.0)	−16.3 (−28.9, −4.0)§
Week 104	39.1 (10.9)	8.7 (8.7)	−27.6 (−43.2, −11.5)§
hsTnI			
Week 26	4.5 (3.6)	−5.8 (4.0)	−8.3 (−14.0, −2.5)§
Week 52	3.2 (3.8)	−6.9 (4.0)	−11.9 (−18.0, −5.6)§
Week 104	11.5 (4.4)	0.0 (4.6)	−10.0 (−17.3, −2.6)§

CI, confidence interval; SE, standard error. *Data are median percent change (SE) unless otherwise indicated. †Results are data as observed for patients with values at baseline and at each specific time point (NT-proBNP: n = 180, 156, and 148 for PBO and n = 379, 368, and 318 for CANA at weeks 26, 52, and 104, respectively. hsTnI: n = 172, 145, and 140 for PBO and n = 344, 329, and 294 for CANA at weeks 26, 52, and 104, respectively). ‡Nominal p<0.05. §Nominal p<0.01. Nominal p values for the difference in median percent change between the CANA and PBO groups are based on the Wilcoxon rank sum test. SEs for the median percent change were estimated using the bootstrap technique by simulated repeated samples for each biomarker and treatment group.

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The sodium-dependent glucose transporters (SGLT) as a new promising pharmacological target in human ischaemic hearts

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Background and aims: Recently, EMPAREG OUTCOME trial has shown that empagliflozin reduces cardiovascular mortality and the rate of hospitalization for heart failure, in patients with type 2 diabetes with previous cardiovascular events. The mechanisms underlying the cardioprotective effects of this sodium/glucose transporter 2 (SGT2) inhibitor are still unknown, though a direct action of the drug on the cardiomyocytes could be hypothesized.
Materials and methods: We evaluated the expression of SGLT1 and SGLT2 by quantitative-real time-RT-PCR and western blot analyses, in tissue biopsies of healthy, ischemic and hypertrophic human hearts.
Results: In this study, we evaluated the relative expression of SGLT2 and SGLT1, the two most relevant members of the SGLT family being potentially responsive to empagliflozin, in tissue biopsies of healthy (n=9), ischemic (n=9) and hypertrophic (n=6) human hearts. We found no expression of SGLT2 in either normal or pathological conditions, whereas SGLT1 was expressed in normal myocardial tissue and was significantly upregulated in ischemia and hypertrophy, in association with increased phosphorylation in activating domains of the intracellular second messengers AMP-activated protein kinase (AMPK), extracellular-signal regulated kinase 1 and 2 (ERK-1/2) and mammalian target of rapamycin (mTOR).
Conclusion: Our findings suggest that the hyper-expression of SGLT1 in cardiomyocytes may represent a potential pharmacological target for cardioprotection.
Disclosure: **A. Di Franco:** None.

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An SGLT-2 inhibitor reverses impaired ventricular repolarisation in patients with type 2 diabetes

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Background and aims: EMPA-REG OUTCOME trial demonstrated that empagliflozin, an SGLT-2 inhibitor, reduced cardiovascular events, including sudden cardiac death and heart failure, in patients with type 2 diabetes mellitus (T2DM), but its mechanisms remain unclear. It has been shown that increased ventricular repolarization heterogeneity is associated with the risk of ventricular arrhythmias and sudden cardiac death and that ventricular repolarization heterogeneity is often observed in patients with T2DM. In the present study, we hypothesized that treatment with an SGLT-2 inhibitor reverses ventricular repolarization heterogeneity in patients with T2DM.
Materials and methods: We retrospectively analyzed data for consecutive patients with T2DM in whom a 12-lead electrocardiogram was recorded before and after treatment with an SGLT2 inhibitor at outpatient clinics from April 2014 to October 2016. The changes in corrected QT

dispersion (QTcd: the difference between the maximum and minimum of the QTc intervals) and Tpeak-Tend interval at V5 lead (Tpeak-Tend), indices of ventricular repolarization heterogeneity, after treatment with an SGLT-2 inhibitor were calculated.

Results: Forty-six patients enrolled in this study; thirty-three (72%) were men, age was 60.4±10.7 (SD) years, and median duration of diabetes was 10.5 years. Six SGLT-2 inhibitors were used for treatment in the study subjects (ipragliflozin, n=17; dapagliflozin, n=16; empagliflozin, n=7; canagliflozin, n =3; luseogliflozin, n=2; tofogliflozin, n=1). Duration of treatment with an SGLT-2 inhibitor was 241.5±170.5 days. Treatment with SGLT-2 inhibitors reduced HbA1c (7.7±1.2 vs. 7.5±1.4%), body weight (77.8±13.9 vs. 74.7±12.5 kg) and systolic blood pressure (132.8 ±17.9 vs. 125.8±11.9 mmHg). QTcd was significantly reduced after treatment with an SGLT-2 inhibitor (51.9±19.2 vs. 45.5±11.6 msec, p<0.01), though heart rate and mean QTc interval were not altered. Interestingly, improvement in QTcd was clearly evident in patients with prolonged QTcd before the treatment; when the patients were divided into two subgroups by the median of QTcd (48.8 msec), improvement of QTcd was seen in the high-QTcd subgroup but not in the low-QTcd subgroup. Similarly, improvement of Tpeak-Tend was observed (96.0±6.6 vs 90.1 ±8.3 msec, p<0.01) only in a subgroup with Tpeak-Tend being higher than the median value (84.0 msec). There was a weak but significant positive correlation between changes in QTcd and changes in systolic blood pressure (Spearman's ρ=0.32 p<0.05), while no correlation was found between changes in QTcd or Tpeak-Tend and changes in HbA1c.

Conclusion: These findings suggest that an SGLT-2 inhibitor reverses impaired ventricular repolarization heterogeneity in patients with T2DM, independent of its effect on glycemic control. The favorable effect on ventricular repolarization heterogeneity may be a potential mechanism of recently reported reduction of cardiovascular events by empaglifozin.

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Disclosure: **T. Sato:** None.

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Does baseline HbA_{1c} or change in HbA_{1c} predict the reduction in cardiovascular (CV) death with empagliflozin? Results from EMPA-REG OUTCOME

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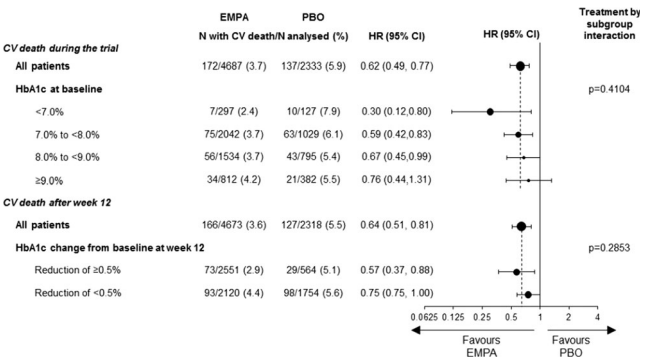
Background and aims: In the EMPA-REG OUTCOME trial, EMPA given in addition to standard of care significantly reduced CV death by 38% vs placebo (PBO) (HR 0.62; 95% CI 0.49, 0.77) in patients with type 2 diabetes (T2DM) and established CV disease. We investigated whether baseline HbA1c or change in HbA1c influenced the effect of EMPA on CV death.

Materials and methods: Patients were randomised to EMPA 10 mg, EMPA 25 mg, or PBO. Background glucose-lowering therapy was to remain unchanged for 12 weeks and then be adjusted to achieve glycaemic control according to local guidelines. CV death was analysed in the pooled EMPA group vs PBO in subgroups by (1) baseline HbA1c (<7.0%; 7.0 to <8.0%; 8.0 to <9.0%; ≥9.0%) and (2) reduction from baseline in HbA1c at week 12 (≥0.5%; <0.5%). Differences in risk between treatment groups were assessed using a Cox proportional hazards model.

Results: A total of 7020 patients were treated. Median observation time was 3.1 years. The benefit of EMPA vs PBO on CV death was consistent irrespective of baseline HbA1c or change in HbA1c from baseline at week 12 (Figure).

Conclusion: In patients with T2DM and established CV disease, the reduction in CV death with empagliflozin appeared to occur irrespective of either baseline HbA1c or the early glycaemic response to the medication.



Clinical Trial Registration Number: NCT01131676
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Disclosure: **S.E. Inzucchi:** Honorarium; Merck & Co, Intarcia Therapeutics, Inc., Lexicon Pharmaceuticals, Janssen, Sanofi, AstraZeneca, Boehringer Ingelheim, Novo Nordisk, vTv Therapeutics.

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EMPA-REG OUTCOME: consistent reduction in risk of cardiovascular (CV) outcomes and mortality with empagliflozin (EMPA) irrespective of sulphonylurea (SU) use at baseline

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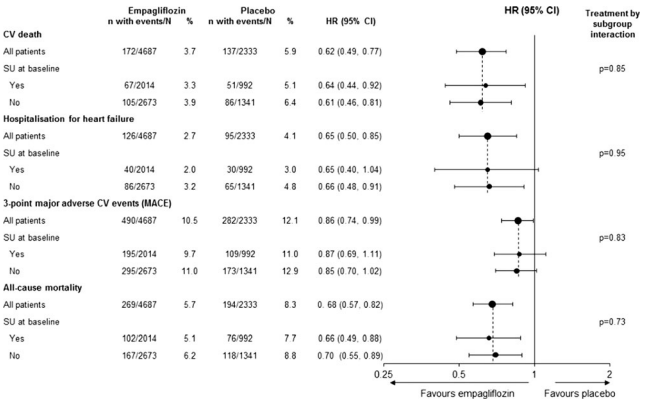
Background and aims: In EMPA-REG OUTCOME, EMPA given in addition to standard of care significantly reduced CV death by 38% and all-cause mortality by 32% vs placebo (PBO) in patients with T2DM and established CV disease. We investigated CV outcomes and adverse events (AEs) by SU use at baseline.

Materials and methods: Patients were randomised to receive PBO, EMPA 10 mg, or EMPA 25 mg. Background glucose-lowering therapy was to remain unchanged for 12 weeks then be adjusted to achieve glycaemic control according to local guidelines. Differences in risk of CV outcomes between EMPA and PBO across subgroups by use of SU at baseline were assessed using a Cox proportional hazards model.

Results: In total, 2333, 2345 and 2342 patients received PBO, EMPA 10 mg and EMPA 25 mg, of whom 992 (42.5%), 985 (42.0%) and 1029 (43.9%), respectively, were taking SU (alone or with other glucose-lowering medications) at baseline. At week 164, the adjusted mean (SE) change from baseline in HbA1c was -0.33% (0.03) with EMPA pooled and 0.10% (0.05) with PBO in patients who were taking SU at baseline (adjusted mean [95% CI] difference: -0.43% [-0.54, -0.31]) and -0.36% (0.03) with EMPA and -0.03% (0.04) with PBO in patients who

were not taking SU at baseline (adjusted mean [95% CI] difference: -0.33% [-0.43, -0.22]). AEs were consistent with the known safety profile of EMPA. Confirmed hypoglycaemic AEs (plasma glucose ≤70 mg/dL and/or requiring assistance) were reported in 23.4%, 24.5% and 25.0% of patients in the PBO, EMPA 10 mg and EMPA 25 mg groups who were taking SU at baseline, and 31.2%, 30.5% and 29.7% of patients in these groups not taking SU at baseline, respectively. Hypoglycaemic AEs requiring assistance were reported in 1.1%, 1.2% and 1.0% of patients in the PBO, EMPA 10 mg and EMPA 25 mg groups who were taking SU at baseline and 1.9%, 1.5% and 1.5%, respectively, of patients in these groups not taking SU at baseline, respectively. Risk reductions in CV outcomes and all-cause mortality with EMPA pooled vs PBO were consistent irrespective of SU use at baseline (Figure).

Conclusion: In patients with T2DM and established CV disease, the proportion of patients with hypoglycaemic AEs was similar between the PBO and EMPA groups in patients who were and were not taking SU at baseline. EMPA reduced the risk of CV and all-cause mortality, 3-point major adverse CV events and heart failure irrespective of SU use at baseline.



Clinical Trial Registration Number: NCT01131676
Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance
Disclosure: **J.T. George:** Employment/Consultancy; Employee of Boehringer Ingelheim.

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Reduction in hospitalisation for heart failure with empagliflozin is consistent across categories of baseline HbA_{1c} and change in HbA_{1c}: results from EMPA-REG OUTCOME

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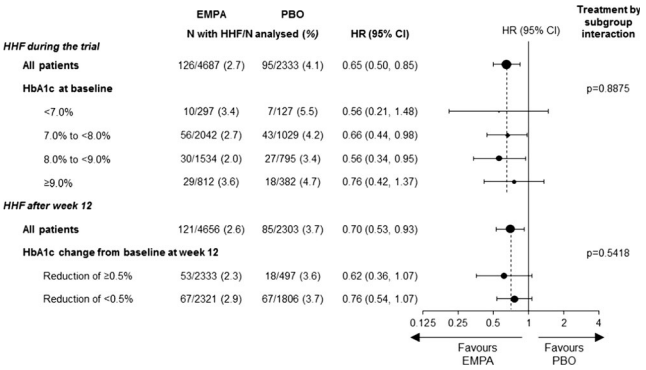
Background and aims: In the EMPA-REG OUTCOME trial, EMPA given in addition to standard of care significantly reduced the risk of hospitalisation for heart failure (HHF) vs placebo (PBO) (HR 0.65; 95% CI 0.50, 0.85) in patients with type 2 diabetes (T2DM) and established CV disease. We investigated whether baseline HbA1c or change in HbA1c influenced the effect of EMPA on HHF.

Materials and methods: Patients were randomised to receive EMPA 10 mg, EMPA 25 mg, or PBO. Background glucose-lowering therapy was to remain unchanged for 12 weeks and then be adjusted to achieve glycemic control according to local guidelines. HHF was analysed in the pooled EMPA group vs PBO by categories of (1) baseline HbA1c (<7.0%; 7.0 to <8.0%; 8.0 to <9.0%; ≥9.0%) and (2) reduction from baseline in HbA1c at week 12 (≥0.5%; <0.5%). Differences in risk between treatment groups were assessed using a Cox proportional hazards model.

Results: A total of 7020 patients were treated. Median observation time was 3.1 years. The benefit of EMPA vs PBO on HHF was consistent irrespective of baseline HbA1c or change in HbA1c from baseline at week 12 (Figure).

Conclusion: In patients with T2DM and established CV disease, the reduction in HHF with empagliflozin appeared to occur irrespective of either baseline HbA1c or the early glycaemic response to the medication.



Clinical Trial Registration Number: NCT01131676
Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance
Disclosure: **H.J. Woerle:** Employment/Consultancy; Employee of Boehringer Ingelheim.

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EMPA-REG OUTCOME: empagliflozin (EMPA) reduced the risk of cardiovascular (CV) outcomes and mortality irrespective of metformin (MET) use at baseline

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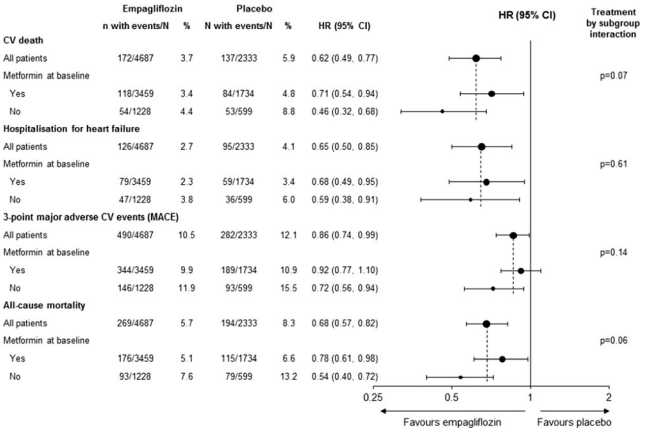
Background and aims: In the EMPA-REG OUTCOME trial, EMPA given in addition to standard of care significantly reduced CV outcomes and all-cause mortality vs placebo (PBO) in patients with T2DM and established CV disease. We investigated CV outcomes and adverse events (AEs) by use of MET at baseline.

Materials and methods: Patients were randomised to receive PBO, EMPA 10 mg, or EMPA 25 mg. Background glucose-lowering therapy was to remain unchanged for 12 weeks then be adjusted at the investigator's discretion to achieve glycemic control according to local guidelines. Differences in risk of CV outcomes between EMPA and PBO across subgroups by use of MET at baseline were assessed using a Cox proportional hazards model.

Results: In total, 2333, 2345 and 2342 patients received PBO, EMPA 10 mg and EMPA 25 mg, of whom 1734 (74.3%), 1729 (73.7%) and 1730 (73.9%), respectively, were taking MET (alone or with other glucose-lowering medications) at baseline. In patients who were and were not taking MET at baseline, risk reductions in CV outcomes and all-cause mortality with EMPA vs PBO were consistent (Figure). At week 164, the adjusted mean (SE) change from baseline in HbA1c was -0.32% (0.03) with EMPA pooled and 0.08% (0.04) with PBO in patients who were taking MET at baseline (adjusted mean [95% CI] difference: -0.40% [-0.49, -0.31]) and -0.41% (0.04) with EMPA and -0.14% (0.07) with PBO in patients who were not taking MET at baseline (adjusted mean [95% CI] difference: -0.27% [-0.42, -0.11]). AEs were consistent with the known safety profile of EMPA. Confirmed hypoglycaemic AEs (plasma glucose ≤70 mg/dL and/or requiring assistance) were reported in 26.1%, 26.5% and 27.3% of patients in the PBO, EMPA 10 mg and EMPA 25 mg groups who were taking MET at baseline, and 32.9%, 32.1% and 28.4% of patients not taking MET at baseline, respectively. Hypoglycaemic AEs requiring assistance were reported in 1.2%, 1.4% and 1.2% of patients in the PBO, EMPA 10 mg and EMPA 25 mg groups who were taking MET at baseline and 2.5%, 1.5% and 1.5% of patients not taking MET at baseline, respectively.

Conclusion: In patients with T2DM and established CV disease, EMPA reduced the risk of CV outcomes and all-cause mortality irrespective of MET use at baseline, The proportion of patients with hypoglycaemic AEs was similar between the PBO and EMPA groups in patients who were and were not using MET at baseline.



Clinical Trial Registration Number: NCT01131676
Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance
Disclosure: **S. Hüttner:** Employment/Consultancy; Employee of Boehringer Ingelheim.

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Empagliflozin decreases risk of kidney function decline in type 2 diabetes: slope analyses in patients with / without heart failure at baseline from the EMPA-REG OUTCOME trial

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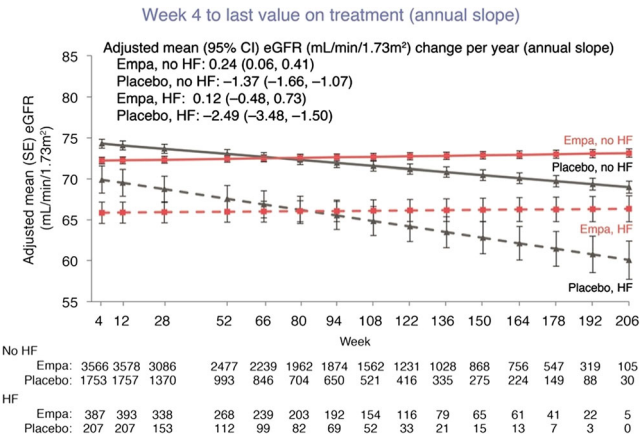
Background and aims: Progressive decline of kidney function is common in patients with diabetes, whether or not they have heart failure (HF). In the EMPA-REG OUTCOME trial, empagliflozin decreased the risk for the composite of CV death and hospitalization for HF (HR 0.66; 95% CI 0.55, 0.79; p<0.0001) in patients with type 2 diabetes and established cardiovascular risks. Empagliflozin also slowed the progression of kidney disease and decreased the risk of events indicative of kidney disease progression. Here, we report the rate of change in eGFR in patients with and without HF in the EMPA-REG OUTCOME trial.

Materials and methods: 7020 patients receiving standard care were randomized (1:1:1) to empagliflozin 10 mg, 25 mg or placebo. Treatment differences in the average rate of change in eGFR for pre-specified trial phases were assessed using a linear-regression, random-intercept and time-coefficient model and results are depicted for mean slopes. Patients with and without HF at baseline were assessed separately.

Results: Empagliflozin caused an acute drop in eGFR during the first 4 weeks, followed by a long-term stable eGFR during a median treatment time of 2.6 years (Figure), and a rapid return towards baseline after cessation of empagliflozin. In contrast, there was a steady decline in eGFR in the placebo group during the long-term treatment phase. The pattern of eGFR changes were similar in patients with (n=706) or without baseline (n=6314) HF.

Conclusion: Empagliflozin slowed eGFR decline in patients with type 2 diabetes, whether or not they were reported to have had HF at the start of treatment. Slope analyses using multiple eGFR readings from individual patients represent an emerging useful method to document changes in kidney function over time. The kidney effects of empagliflozin on patients with HF (with or without diabetes) is being further investigated in ongoing trials in patients with HFrEF (EMPEROR-reduced) and HFpEF (EMPEROR-preserved).



Clinical Trial Registration Number: NCT01131676
Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance
Disclosure: **A.K. Cheung:** Lecture/other fees; Boehringer Ingelheim.

PS 075 Understanding gestational diabetes

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LRG1 as a predictive marker for pre-eclampsia in women with type 1 diabetes

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Background and aims: Pregnant women with type 1 diabetes (T1DM) have a 4-fold increased risk for pre-eclampsia (PE) vs. their non-diabetic counterparts. PE is characterised by new-onset hypertension (>140/90mmHg), with proteinuria (>300mg/24h) and/or organ damage developing after 20 wks gestation: it can be fatal to both mother and child. Currently, PE is hard to predict, and the only cure is delivery. Improved biomarkers and specific treatments are urgently needed. We aim to explore the utility of Leucine-Rich alpha-2-Glycoprotein-1 (LRG1), a marker of inflammation and angiogenesis, as a predictor of PE in pregnant type-1 diabetic (T1DM) women.
Materials and methods: This is a prospective study of 62 pregnant women: 23 with T1DM who developed PE, 21 with T1DM who remained normotensive, and 18 healthy non-diabetic women as reference controls. The two T1DM groups were matched for age, duration of diabetes, HbA1c and parity. Fasting plasma samples were collected during the second trimester (21.7±1.4 weeks of gestation) and analysed for LRG1 (ELISA, IBL). Samples were diluted at 1:2000 as determined by previous validation studies. sFlt, the current 'gold standard' anti-angiogenic biomarker for PE prediction, was previously measured in this cohort (ELISA, R&D Systems).
Results: LRG1 protein levels were significantly increased in women with T1DM who subsequently developed PE vs. those who did not (50.7 ± 2.4 vs 40.8 ± 2.5 μg/mL, $p<0.01$, mean ± SEM). This significant increase preceded the clinical signs and symptoms of PE. In comparison, sFlt did not predict PE at this gestational age.
Conclusion: Women with T1DM who later developed PE had significantly higher plasma LRG1 in the second trimester of pregnancy vs. those who remained normotensive. LRG1 may have utility as an early predictor of PE, and could provide novel insights into disease mechanisms for PE in diabetic women.
Supported by: DFENI
Disclosure: **A.H.Y. Cheung:** None.

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NMR-based metabolic phenotyping of serum in patients with gestational diabetes

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Background and aims: Gestational diabetes mellitus (GDM) is associated with adverse maternal and fetal outcomes and confers to the affected women a high lifetime risk for the development of type 2 diabetes. Because of the importance of early intervention in preventing these complications, there is still scope for devising improved first-trimester biomarker determination for the diagnosis and risk stratification of gestational diabetes. Metabolomic profiling, the systematic study of small-molecule products of biochemical pathways, has been used to predict, diagnose and monitor several metabolic disorders, including GDM. Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy is one of the preferred tools for the metabolomic analysis of biological matrixes. It is a rapid and reproducible technique, requires minimum sample pretreatment, and provides an overall qualitative and quantitative metabolic assay, including detailed information on molecular structure. We employed NMR spectroscopy combined with Multivariate Data Analysis to characterize the metabolic signature of blood serum in a Caucasian cohort of 19 pregnant healthy women who developed GDM compared to 19 non-diabetic pregnant healthy women.
Materials and methods: The two groups studied were matched for age, BMI and serum lipid parameters. Blood samples were collected between the 26th and 30th week of pregnancy just before the OGTT test and before any therapeutic intervention was applied. The serum ^{1}H NMR spectra were recorded at 298 K on a 500 MHz Bruker Avance DRX NMR spectrometer operating at a field strength of 11.74 Tesla and running on TopSpin 2.1 suite. Orthogonal projection to Partial Least Squares Discriminant Analysis (OPLS-DA) was applied to construct statistical models of serum metabolic data.
Results: The two dimensional OPLS-DA scores plot revealed a distinct separation between pregnant women with and without GDM with minimal overlapping. The significance of the OPLS-DA metabolic models via cross-validated ANOVA test was calculated as a p value of 0.002 (significant <0.5). According to the OPLS-DA coefficient plot, the serum metabolic phenotype of pregnant women with GDM was characterized by lower concentrations of lactate, alanine, glutamine and glutamate and higher concentrations of 2-hydroxybutyrate, 3-hydroxybutyrate, and to a lesser extent of branched chain amino acids (leucine, valine and isoleucine). Of note, elevated branched chain amino acids that have been considered as a risk factor for type 2 diabetes were only marginally elevated in our study, a finding that is in consistent with previous studies in GDM.
Conclusion: Apart from hyperglycemia, GDM is associated with a characteristic metabolic profile that possibly reflects the disturbances in various metabolic pathways. The role of these alterations in the pathogenesis of the complications of GDM as well as their impact on the increased risk for the subsequent development of type 2 diabetes remain to be established.
Disclosure: **L. Spanou:** None.

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Changes in lipid and amino acid levels during pregnancy and early postpartum period in gestational diabetes

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Background and aims: The increasing prevalence of gestational diabetes mellitus (GDM) poses a risk for permanent diabetes development in future life for both mother and child. We hypothesize that changes in non-esterified fatty acids (NEFAs) and amino acids (AAs) in plasma previously associated with type 2 diabetes development may reflect more serious abnormalities of insulin secretion and sensitivity reflected by more severe disturbances of glucose tolerance during pregnancy associated with higher *postpartum* metabolic risk. The aims of this study were to determine NEFAs (C12:0 to C22:6) and all proteinogenic and several nonessential AAs during pregnancy and *postpartum* period in women with GDM and those with physiological pregnancy and to study the relationships between selected metabolites and anthropometrical and biochemical data.
Materials and methods: A total of 40 women (20 GDM cases diagnosed according to WHO criteria and 20 healthy counterparts) were included in the study. All subjects underwent OGTT with 75g of glucose load between 24-28th week of pregnancy, moreover, women with GDM history underwent repeated *postpartum* OGTT up to 12 months after delivery. Plasma levels of NEFAs were determined by gas chromatography with flame ionization

detector. Plasma essential and several nonessential AAs were determined by capillary electrophoresis with subsequent MS detection.
Results: Decreased levels of dodecanoic and tetradecanoic acid were found in GDM group at 2nd trimester (P=0.02 a P=0.05, Mann-Whitney). There was a significant decrease in docosahexaenoic acid levels *postpartum* within GDM group (P=0.03, Wilxocon). Five NEFAs (C16:0, C17:0, C18:0, C18:1, C18:2) were positively correlated with age, two NEFAs (C16:1, C18:3) were negatively correlated with offspring birth weight, plasma levels of docosahexaenioc acid were positively correlated with systolic blood pressure in 2nd trimestr of pregnancy and C14:0, C16:1 and C18:1 levels during pregnancy were correlated with glycaemia levels (all P<0,05, Spearman). Decreased levels of several AAs (Ala, Asn, Gln, Glu, His, Lys, Ser, Trp and hydroxyproline) were found in GDM pregnancy (all P<0.05, Mann-Whitney). Levels of almost all analyzed AAs were decreased *postpartum* in GDM group compared to their levels during pregnancy (all P<0.04, Wilcoxon). Negative correlations between glutamic acid, histidine and hydroxyprolin levels and respective values of OGTT glucose levels and AUC during pregnancy were found (all P<0.05, Spearman).
Conclusion: Our data support a possible link between GDM and selected proteomic and lipidomic parameters. Pathophysiological relevance and predictive potential of altered metabolomic pattern in GDM for future maternal metabolism and/or offspring health remain to be studied.
Supported by: AZV 16-28040A
Disclosure: **A. Pleskacova:** Grants; Ministry of Health of Czech Republic, grant number 16-28040A.

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Impact of maternal triglyceride and novel metabolic parameters on neonatal anthropometrics in Asians

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Background and aims: In Caucasian populations, maternal obesity and triglyceride (Tg)(in addition to glucose) have been found to modulate neonatal anthropometrics via intrauterine fetal programming. Less is known of these associations during pregnancy in Asians, in whom strength of/ thresholds for correlation may differ given that visceral adiposity/insulin resistance in Asians is elevated at a lower BMI. Hence we aimed to determine the relationship between maternal BMI/metabolic variables (including Tg) and neonatal anthropometrics in Malaysian mother-offspring pairs.
Materials and methods: In this prospective observational study, we recruited both women with gestational diabetes mellitus(GDM) and normal glucose tolerance(NGT)(excluding pregestational diabetes) from a tertiary combined multidisciplinary antenatal clinic. Demographic, anthropometric and clinical data were obtained during an interview/examination using a structured questionnaire. Blood was drawn for insulin, C-peptide and Tg during 75g OGTT at 14-32 weeks gestation. At birth, neonatal anthropometrics were assessed and data such as maternal weight gain during pregnancy, mode of delivery etc. extracted from records.
Results: We recruited 470 women: 125 GDM, 225 lean NGT , 90 nonlean NGT(BMI>27.5 kg/m2). In the group as a whole (n=470), pregravid BMI, fasting Tg, fasting glucose and HOMA2-%S correlated significantly and independently with BW Ratio (r=0.193, 0.148, 0.222,-0.142 respectively). Total gestational weight gain in pregnancy was the strongest significant independent predictor of BW (r=0.219). Pregravid BMI, fasting Tg, fasting glucose, HOMA2-%S and total gestational wt gain in pregnancy were also independently predictive of BWR in the NGT mothers (p<0.05). Mean BW in this population of well-treated GDM mothers in a tertiary centre BW was nonsignificantly lower in GDM compared with Lean NGT offspring(2959g vs 3060g). 7 (4.7%) offspring fulfilled criteria for macrosomia, BW ≥ 4 kg. There were 10 LGA babies(2.1%) as defined by Fenton antenatal growth charts. Calculated 90th centile threshold in this Asian population(GDM+NGT) for BW was 3.6 kg. In the whole group,fasting Tg >3.6 mmol/l(95th centile)(OR 31.9) and gestational weight gain>10kg (OR 13.6)was independently predictive of LGA status. Tg >3.6 mmol/l (OR 16.2) and gestational wt gain > 20 kg (OR 4.7) were independently predictive of a sum of skinfold thickness >90th centile.
Conclusion: These findings indicate that maternal hypertriglyceridemia is an important risk factor for LGA status and increased neonatal adiposity in Asian offspring independent of glucose, in treated GDM and NGT mothers. Gestational weight gain and insulin resistance also adversely impact neonatal anthropometrics. Our findings indicate that in addition to maternal glycemia, hypertriglyceridemia and insulin resistance may prove to be important metabolic targets in pregnancy.
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Inflammation is differently modulated in offspring of mothers with gestational diabetes as compared to offspring of NGT obese women

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Background and aims: Both pre-pregnancy obesity and gestational diabetes mellitus (GDM) confer, each independently, the offspring an increased metabolic risk. Aim of the present cohort study was to compare the inflammatory profile of normal weight controls (NWCs), GDM and normal glucose tolerant obese (NGT_Ob) mothers and its impact on the offspring's cytokine release and body weight at birth.
Materials and methods: Serum levels of leptin, adiponectin, their ratio (L/A) as estimate of insulin resistance, interleukin (IL) 6, IL8, IL10, tumor necrosis factor (TNF) alpha, TNFRII, Intercellular Adhesion Molecule 1 (ICAM1), sCD14 were assayed at birth in maternal and cord blood of 350 normal weight, 51 NGT obese and 42 GDM women from the "Feeding" cohort study. Cytokine values are reported after log transformation.
Results: Leptin (4.1 ± 0.7; 3.5 ± 1.0; 2.9 ± 0.9 ng/ml) was significantly increased in NGT_Ob and GDM vs. NWCs (p<0.001 for all). The L/A (21.2 ± 21.9; 10.8 ± 18.5) and ICAM1 (6.5 ± 1.0; 5.7 ± 0.9 ng/ml) were significantly increased only in NGT_Ob vs. NWC women (p<0.001), while TNFRII was significantly increased in GDM women vs. NWCs (6.8 ± 0.4; 6.6 ± 0.4 pg/ml; p<0.03). GDM offspring showed the highest leptin (2.8 ± 1.2; 2.3 ± 1.1; 2.1 ± 1.0 ng/ml), L/A (3.3 ± 7.7; 1.3 ± 2.0) and proinsulin levels (2.9 ± .9; 2.6 ± 0.7; 2.5 ± 0.7 pmol/L) (p<0.001 for all). The best predictors of child body weight z-score at birth were the child's proinsulin and leptin concentrations (β=0.334, p<0.0001), while maternal age, leptin, pre-pregnancy body mass index, and gestational weight gain were excluded variables. Comparison of offspring born with large for gestational age (LGA) demonstrated no difference in proinsulin levels, while confirmed significantly higher leptin (3.2 ± 1.2; 2.7 ± 1.2; 2.3 ± 0.9 ng/ml), L/A (7.0 ± 12.8; 2.0 ± 2.8; 1.1± 1.1) and sCD14 (1.8 ± 1.6; 0.7 v 0.4; 1.0 ± 0.8 μg/ml) levels in GDM (N=14) than in NGT_Ob (N=8) and NWCs offspring (N=46).
Conclusion: Newborns of GDM mothers exhibit increased proinsulin, leptin, L/A and sCD14 levels than offspring of obese NGT mothers. While proinsulin seems to explain the higher birth weight of the children, leptin and sCD14 might partially explain the risk of developing low-grade inflammation and metabolic disturbances in adult life.

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Chemerin and omentin-1 circulating and gene expression levels in adipose tissue and placenta in women with gestational diabetes

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Background and aims: Gestational diabetes mellitus (GDM) is defined as a state of glucose intolerance characterized by β-cell dysfunction and insulin resistance. Maternal adipose tissue and the placenta secrete various adipokines such as chemerin and omentin-1 that could play a role in the development of insulin resistance in human pregnancy. We examined whether chemerin and omentin-1 mRNA and protein production is altered in GDM women and whether this might relate to indices of insulin resistance.
Materials and methods: Maternal peripheral blood, visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT) and placenta tissues were obtained from GDM women -with and without obesity- and normal glucose tolerant (NGT) pregnant women, at the time of the Cesarean section. We developed a real-time quantitative RT-PCR using fluorecently labelled hybridization probes to detect relative chemerin and omentin-1 mRNA levels in VAT, SAT and the placenta. Circulating chemerin and omentin-1 protein levels were measured in all women by use of standard commercial ELISAs. Fasting plasma glucose and insulin (RIA) levels were measured from maternal peripheral blood.
Results: Circulating chemerin was significantly higher in GDM-obese compared to NGT-non-obese women (Table) and correlated positively with BMI and HOMA-IR at delivery. Circulating omentin-1 was significantly lower in GDM (obese and non-obese) compared to NGT-non-obese women (Table) and was negatively correlated with BMI. Chemerin mRNA is expressed significantly higher in both fat depots (SAT and VAT) compared to placenta in all women (6-to 24-times, $p<0.05$) and its expression in VAT in GDM-obese was higher compared to NGT-non-obese women (Table). Omentin-1 is expressed significantly higher in VAT compared to SAT (50- to 100-times, $p<0.01$) and its expression in placenta was negligible in all women (Table)
Conclusion: Maternal adipose tissue and especially VAT is the main site of chemerin and omentin-1 mRNA expression in at term human pregnancy. Chemerin is elevated and omentin -1 protein production is decreased in GDM-obese women. This finding together with the positive association of chemerin with markers of insulin resistance, suggest a potential role of these adipokines and more especially of chemerin in the development of insulin resistance in these women.

	NGT (n=23)		GDM (n=15)	
	Non-Obese	Obese	Non-Obese	Obese
Fasting Glucose (mg/dl)	68.0±5.5	76.9±4.4	78.4±6.4	79.9±4.3
Fasting Insulin (μIU/ml)	7.9±0.8	8.7±1.6	7.2±1.0	14.6±2.6*¶
HOMA-IR	1.4±0.2	1.7±0.4	1.4±0.3	2.9±0.7*
Chemerin levels (ng/ml)	167.3±10.0	212.8±36.2	209.4±37.6	215.2±17.2*
Omentin-1 levels (ng/ml)	31.9±6.7	20.3±3.6	17.7±2.5*	19.2±1.6*
VAT chemerin mRNA levels (AU)	5.5±1.4§	10.4±3.1§	5.8±2.6§	16.1±9.2*§
SAT chemerin mRNA levels (AU)	4.3±1.6§	7.1±1.8§	3.9±1.3§	5.5±1.9§
Placenta chemerin mRNA levels (AU)	0.7±0.3	0.5±0.1	0.6±0.4	1.5±0.9
VAT omentin-1 mRNA levels (AU)	6.9±2.1¥	5.5±2.0¥	12.7±7.0¥	7.1±2.3¥
SAT omentin-1 mRNA levels (AU)	0.1±0.1	0.1±0.03	0.5±0.5	0.1±0.03
Placenta omentin-1 mRNA levels (AU)	ND	ND	ND	ND

Values represent means ± SEM, *p<0.05 vs NGT-Non-Obese, ¶ p<0.05 vs GDM-Non Obese , § p<0.05 vs. placenta chemerin mRNA levels , ¥, p<0.01 vs. SAT-omentin-1 mRNA levels

Disclosure: **P.C. Tsiotra:** Employment/Consultancy; YES.

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Gestational diabetes modulates cholesterol homeostasis in human fetoplacental endothelium

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Background and aims: Gestational diabetes mellitus (GDM) is associated with hyperinsulinemia, hyperlipidemia and disordered cholesterol metabolism in the maternal circulation. Cholesterol can be efficiently delivered into the fetal circulation across human term fetoplacental endothelial cells (HPEC) via ABCA1 and ABCG1. The aim of this study was to investigate how GDM affects cholesterol metabolism in HPEC and to explore the underlying mechanism(s) of alteration.
Materials and methods: HPEC were isolated from fetal arterial vessels of term placentas from GDM or healthy subjects. Cholesterol biosynthesis and esterification were determined by TLC using cholesterol precursor [^{14}C]-acetate. HPEC were labeled with [^{3}H]-cholesterol or [^{3}H]-24S-hydroxycholesterol to perform sterol efflux assays, using fetal serum, HDL_3, or apoA-I as acceptors. Cellular ROS production was measured using H_2DCFDA dye. Oxysterol levels in HPEC and umbilical cord plasma were quantified by GC-MS.
Results: GDM promoted cholesterol biosynthesis in HPEC by up-regulating SREBP2 mRNA expression and HMGCR mRNA and protein levels. Total cellular cholesterol levels were maintained through that GDM significantly enhanced cholesterol release from HPEC as a result of up-regulation of ABCA1 and ABCG1. Similar results were obtained when control HPEC were incubated with LXR against TO901319, 27-hydroxycholesterol, 24(S)-hydroxycholesterol, 7-ketocholesterol, or 7β-hydroxycholesterol. GDM conditions further upregulated Cyp27A1 transcription and increased the production of reactive oxygen species (ROS). ROS generated oxysterols were significantly increased in GDM HPEC. 24(S)-hydroxycholesterol release to fetal serum was more efficient in GDM than in control HPEC.
Conclusion: The GDM environment modulates cholesterol homeostasis by enhancing cholesterol biosynthesis which is compensated by increasing cholesterol efflux in response to LXR activation due to increasing oxysterol presence in the fetoplacental endothelium.
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Disclosure: **Y. Sun:** None.

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Elucidating the role of novel angiogenesis-related proteins, FKBPL and SIR-1, in trophoblast cells exposed to diabetic stimuli: potential implications for preeclampsia

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Background and aims: Diabetic vascular complications are closely related to irregular angiogenesis, oxidative stress and lipid peroxidation. Preeclampsia (PE) is a pregnancy condition characterised by high blood pressure, proteinuria and multi-organ dysfunction. PE occurs in 4-6% of pregnancies; however in women with diabetes the incidence of PE is increased four-fold. Furthermore, PE does not only have short-term risks, but long-term is associated with cardiovascular disease and/or Type 2 diabetes in both mothers and offspring. Despite research efforts in this area, currently there are no reliable early biomarkers, preventative or treatment strategies for PE, other than delivery. Moreover, paucity of mechanistic data is impeding the development of successful preventative

and curative therapies of PE, particularly in the context of diabetes. The novel angiogenesis-related protein, FKBPL, is a critical regulator of developmental and pathological angiogenesis and closely associated with nutrient-sensing protein, SIRT-1. Therefore, FKBPL may play a key role in the pathophysiology of PE associated with diabetes.

Materials and methods: Three different trophoblast cells lines (HTR8.SV.neo, BeWo and Jar) were exposed to diabetic stimuli: normal vs. high glucose (5.5 vs. 10, 20, 40 mM), native vs. highly oxidised, glycated low density lipoprotein (N- vs. HOG-LDL; 25 μg protein/ml), or hypoxia (1%); then FKBPL and SIRT-1 protein expression and mRNA levels were measured in cell lysates. FKBPL and SIRT-1 were also measured in placental extracts using western blotting.

Results: FKBPL and SIRT-1 protein expression differed across the three trophoblast cell lines, with Jar cells expressing the highest levels of FKBPL and SIRT-1, BeWo cells expressing the lowest level of SIRT-1 and HTR8.SV.neo cells expressing the lowest level of FKBPL. In BeWo cells treated with high vs. normal glucose (48 h), FKBPL mRNA levels were increased 1.3-fold (10mM; p=0.001; n=3), 1.21-fold (20mM; p=0.03; n=3) and 1.46-fold (40mM; p=0.007; n=3). In HTR8.SV.neo cells treated with HOG-LDL (24 h) a 2-fold increase of FKBPL protein was demonstrated compared to native LDL (p=0.048; n=4). No significant changes in FKBPL or SIRT-1 protein expression were observed in Jar cells treated with HOG-LDL, perhaps because of higher FKBPL protein expression in Jar vs. HTR8.SV.neo (p=0.03; n=3). Exposure of HTR8.SV.neo cells to hypoxia (1%) led to 2.4- and 3.8-fold reductions in FKBPL (p=0.003; n=3) and SIRT-1 (p=0.02; n=3) protein expressions, respectively. In concert, we have previously published that endothelial cells (HMEC-1) exposed to hypoxia (0.1%) exhibited reduced FKBPL secretion. FKBPL is also strongly expressed in placental extracts.

Conclusion: Our data suggest that FKBPL and SIRT-1 may have important regulatory and biomarker roles in the development of PE as a vascular complication of diabetes. Pregnancies complicated by both diabetes and PE might exhibit elevated levels of FKBPL/SIRT-1 in early gestation (hyperglycaemia, oxidative stress) and reduced levels in late gestation (placental hypoxia). We plan to investigate this in longitudinal plasma samples from patients with and without diabetes, and with and without PE.

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Disclosure: **L. McClements:** None.

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Amino acid profile in metformin vs insulin treated women with gestational diabetes

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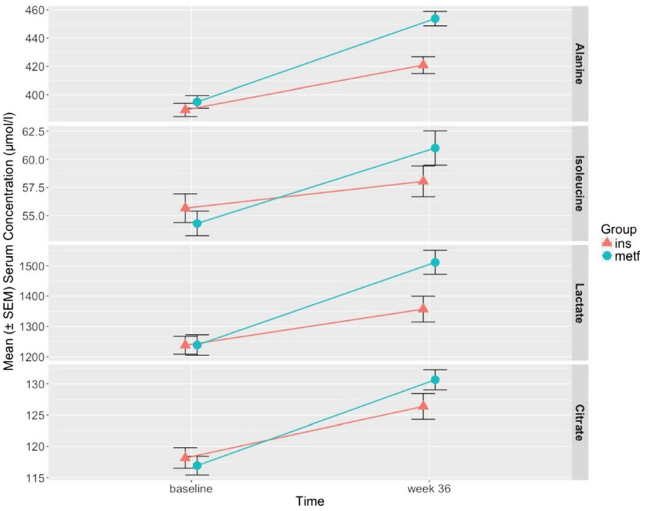
Background and aims: Gestational diabetes mellitus (GDM) is a growing health concern worldwide. While recent evidence is supporting the safety of metformin use in the treatment of GDM, the detailed data considering long term consequences and the effect on maternal and fetal metabolism is mostly lacking. Amino acids influence intermediary metabolism. Changes in aromatic and branched chain amino acids have been associated with insulin resistance and type 2 diabetes. This study was conducted to investigate whether metformin treatment of GDM affects the amino acid profile of the mother or the fetus and to find out if it causes adverse or favourable changes in the metabolome compared to conventional insulin treatment.

Materials and methods: 217 pregnant women diagnosed with GDM were randomized to receive either metformin (110) or insulin (107). Serum samples were available from 99 metformin and 91 insulin treated women at the time of GDM diagnosis (mean 30th week of pregnancy). Another serum sample was collected at 36th week of pregnancy. Cord blood samples were obtained from new-borns exposed to metformin (n=98) or insulin (n=98) during gestation. NMR spectroscopy was used to determine serum concentrations of 9 amino acids (alanine, glutamine, glycine, isoleucine, leucine, valine, histidine, phenylalanine and tyrosine), 3 ketone bodies (acetate, acetoacetate and 3-hydroxybutyrate), and 5 glycolysis related metabolites (glucose, lactate, pyruvate, citrate and glycerol). Student's t-test and Pearson correlations were used for statistical analysis.

Results: The rise in alanine (58.8 μmol/l vs. 31.5 μmol/l, p<0.0001) and isoleucine (6.69 μmol/l vs. 2.37 μmol/l, p=0.023) during the treatment was larger in the metformin group compared to insulin. Other branched chain, aromatic or other amino acids were not affected by metformin. Serum lactate (273 μmol/l vs. 119 μmol/l, p=0.015) and citrate (13.7 μmol/l vs. 8.22 μmol/l, p=0.017) increased slightly more in the metformin group. Alanine or isoleucine concentrations at 36th week of pregnancy or their changes were not associated with glycemic control (HbA1c) of the mothers, birth weight of the baby or maternal weight gain. In cord serum samples, the only difference was higher alanine in the metformin group (487 μmol/l vs. 446 μmol/l, p<0.001).

Conclusion: Metformin treatment of GDM causes a similar clearcut increase in serum alanine concentration as previously reported in non-pregnant, non-diabetic subjects. Alanine is a major source for liver gluconeogenesis. Its increase in serum in the metformin group may be a consequence of the suppressing effect of metformin on gluconeogenesis. Overall, the observed changes in the metabolome were small and the data are unlikely to raise health concerns in the mother or the fetus. However, the mechanism behind the altered amino acid profile as well as its clinical significance need yet further studies.



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Exposome of placenta: the effect of maternal adipose tissue metabolism on the placenta

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Background and aims: The placenta is exposed to different environmental signals during pregnancy. The so-called exposome of the placenta can

alter the proper placental development and therefore affect the correct fetal development. Recently, preliminary studies have pointed to a possible communication between the placenta and the expandability of the adipose tissue during gestation. The aim of this work is to investigate the different effects of metabolic situations with alterations in the adipose tissue (increased or altered distribution) in placental metabolism in rodents.

Materials and methods: For the obese pregnant model, 8-weeks-old wistar rats were fed standard diet or moderated cafeteria diet (MFD). Body mass index (BMI), biochemical parameters and different cytokines were measured at day 30. After 35 days with the diet, rats were crossed and gestation was followed until day 20 (G20). For the model with alteration of adipose tissue, 3-month-old peroxisome proliferator-activated receptor gamma 2 knockout (PPARγ2KO) mice were crossed and sacrificed at day G18. We performed GTT (glucose 1g/kg BW) and ITT (insulin 0.75U/kg BW). Lipid metabolism gene expression, enzymatic activities and metabolomic analysis was measured in placenta form both models.

Results: Our data showed that a MFD significantly increased body weight (BW) of rats compared with rats fed control diet in the first week (227. 3± 2.3 g *vs.* 215.1 ± 3.2 g (P<0. 01), with significant differences during the 35 days with the diet. However, at G20, neither differences were detected in the BW nor the placental weight of rats fed MFD in comparison with rats fed control diet. Although fetuses number per litter was similar, the fetuses weight from obese mothers was significantly lower ($p<0.01$) compared with fetuses from control diet mothers. Only cholesterol content was significantly decreased ($p<0.05$) in placenta from obese rats; however, Glut 1 and lipin gene and protein expression was significantly increased in obese compared with control diet rats. Cystathionine was significantly increased in the metabolomic analysis in obese pregnant rats. Although pregnant PPARγ2KO mice showed increased BW at G18, no differences were found in placenta or fetuses weight. Deletion of PPARγ2 during gestation revealed different fat distribution, significant increased triglyceride content in liver, but not differences in lipid content in placenta, despite decreased lipropotein lipase gene expression. Of note, the placentas from PPARγ2KO mice presented some degree of inflammation, with significant increase of the ratio iNOS/Arginase.

Conclusion: Impairment of adipose tissue expandability during pregnancy has an effect on metabolic adaptations in the pregnant that can alter the placenta exposome. These adaptations are promoted in placental lipid and carbohydrate metabolism, with the purpose of normal gestation to term.

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Pregnancy outcomes in women diagnosed with type 1 diabetes in childhood: a national electronic record-linked cohort study

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Background and aims: The Brecon register is a near complete (98% ascertainment) community-based register of type 1 diabetes (T1D) diagnosed prior to age 15 in Wales since 1995 (n=3289.) This national approach means the Brecon Cohort is representative of all individuals with childhood onset T1D in Wales, and avoids the biases observed in previously published clinic-based studies. This study aims to utilise the Brecon register to compare pregnancy outcomes in young women (up to age 35) with childhood onset T1D and women without childhood onset T1D (general population.) We will also describe the relationship between pregnancy outcomes, maternal age and duration of T1D, which is not currently understood. This study forms part of a programme of work aimed at identifying individuals that develop early complications of T1D, who are likely to benefit from new interventions, such as immunotherapy to preserve beta cells. Since pregnancy outcomes are strongly influenced by glycaemic control at conception, they are especially likely to be influenced by beta cell function in the years after diagnosis.

Materials and methods: The Brecon register was linked to multiple national datasets in the Secure Anonymised Information Linkage (SAIL) databank, to conduct an electronic record-linked cohort study of pregnancy in women under 35 in Wales from 1995-2013. The outcome measures were pre-eclampsia, preterm birth (<37 weeks), low birth weight (≤2.5kg), small for gestational age (SGA) (≤10th centile), macrosomia (≥4kg), large for gestational age (LGA) (≥90th centile), congenital malformations, stillbirths and hospital admissions in the first year of life. Confounders including maternal age and socioeconomic status, parity, mode of delivery, gestation, birth weight, gender, smoking and breastfeeding were adjusted for as appropriate. Analysis involved logistic regression, with estimates of effects between groups reported as odds ratios (OR) with 95% confidence intervals (95%CI.)

Results: 197,796 births were eligible for inclusion. 330 births were to mothers with childhood onset T1D. Baseline characteristics were comparable, with the exception of mode of delivery (66% Caesarean section in mothers with childhood onset T1D vs 18.5% in the general population) and mean gestation at delivery (35.7 vs 39.7 weeks.). All adverse outcomes except SGA were more common in mothers with childhood onset T1D; pre-eclampsia crude OR 3.31 (95% CI 2.46, 4.46), preterm birth 11.30 (9.09, 14.06), macrosomia 1.80 (1.35, 2.40), LGA 2.28 (1.70, 3.05), low birth weight 2.45 (1.83, 3.28), stillbirths 10.50 (6.12, 18.01), congenital malformations 2.88 (1.99, 4.18), admissions during first year of life 3.35 (2.68, 4.19), SGA 0.18 (0.08, 0.37.) All except stillbirths and congenital malformations remained significant after adjusting for confounders.

Conclusion: Our study demonstrates that a record-linkage approach can be used to describe pregnancy outcomes in a community-based cohort. Pregnancy outcomes remain poor in mothers with childhood onset T1D, despite significant advances in obstetric and diabetes care. Measures to preserve beta cell function may improve outcomes, and further studies are required to explore this.

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Disclosure: **L. Allen:** Grants; Diabetes Research and Wellness Foundation provided funding for this study.

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Risk factors for neonatal acidosis in women with type 1 diabetes

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Background and aims: Type 1 diabetes is associated with increased risk of adverse neonatal outcomes, including neonatal asphyxia. Low pH values in the umbilical artery have strong association with neonatal outcomes and have been widely adopted in clinical practice, especially in high-risk populations. The aim of this study was to identify factors associated with low arterial pH values (pH< 7.10) in infants of type 1 diabetic mothers.
Materials and methods: Clinical and laboratory data of 1069 women with type 1 diabetes and their infants from a period between 1993 and 2015 were extracted from clinical records of a tertiary care center of our university. Between 1993 and 2005 the recommended HbA1c in pregnancy was < 7.0 %, and from 2006 < 6.1%. Data of 280 women were excluded from the analysis due to: miscarriage (pregnancy loss < 22 weeks), multiple pregnancy and incomplete data. Finally, data of 789 women was included in the analysis. Based on pH values in the umbilical artery of their infants, women were divided into 2 groups: NORMAL pH- pH ≥ 7.10 and LOW pH- pH<7.10. Determinants of the LOW pH in umbilical artery were identified by logistic regression with data presented as odds ratios and 95% confidence intervals.
Results: 72 (9.1%) infants had LOW pH in the umbilical artery. LOW pH values were associated with decreased Apgar score at 1 minute (0.76 [0.70-0.82]) and 5 minute (0.77 [0.69-0.85]) after birth. Maternal age, age at diagnosis of diabetes and diabetes duration had no association with LOW umbilical artery pH. Maternal pre-pregnancy BMI, the presence of diabetic vascular complications, chronic hypertension, and gestational hypertension/preeclampsia had no impact on LOW umbilical artery pH. LOW pH values were not associated with gestational age at delivery and the degree of prematurity (late to moderate preterm 32-37 weeks, very preterm- 32-28 weeks and extremely preterm- < 28 weeks). There was association between maternal HbA1c [%] analyzed before delivery and LOW pH in the umbilical artery- 1.40 [1.11-1.78], P=0.005. Similar association was found for HbA1c analyzed between 20-24 weeks- 1.29 [1.03-1.63], P=0.026. There was no association between the first trimester HbA1c level as well as lack of preconception care and LOW pH in the umbilical artery. There was association between urgent cesarean section (N=53) and LOW pH in the umbilical artery 1.64 [1.11-2.44], P=0.01 and this association was independent of HbA1c analyzed before delivery. There was no association between both neonatal LGA (birthweight> 90 percentile) and SGA (birthweight < 10 percentile) and LOW pH in the umbilical artery.
Conclusion: Lack of efficient glycemic control after first trimester of pregnancy is the strongest predictor of neonatal acidosis in women with type 1 diabetes. However in the group of urgent cesarean sections for fetal distress, low umbilical artery pH values cannot be explained by maternal hyperglycemia.
Disclosure: **P. Gutaj:** None.

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Maternal and perinatal outcomes in all diabetes pregnancies and controls in Kronoberg 2009-2012

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Background and aims: To compare maternal and perinatal outcomes in women with pregestational (PDM) type 1 (T1D) or type 2 diabetes (T2D) and gestational diabetes mellitus (GDM) and compare these to those of pregnancies not complicated with diabetes. In Kronoberg nurses take greater responsibility than is usual in prenatal diabetes care.
Materials and methods: Retrospective records review of 281 pregnancies during 2009-2012, including all 145 complicated by diabetes in the region. Rates of maternal (abortion, preeclampsia, pre-delivery, vaginal/cesarean sectio delivery, CS) and fetal outcomes (large for gestational age (LGA), macrosomia (> 4500g), congenital malformations) were assessed, and examined for potential predisposing or contributing factors such as maternal age, ethnicity, obesity, weight gain, parity, HbA1c levels and insulin doses.
Results: There were 48 (33%) PDM and 97 (67%) GDM pregnancies, that were compared to 136 pregnancies not complicated by diabetes. Among the PDM 37 women (77.1%) had T1D and 11 (22.9%) T2D. The PDM mothers were more physically active (p<0.01), and had normal pregestational BMI (p=0.05) but tended to gain more weight during pregnancy compared to the GDM mothers (p=0.06). Compared to GDM the PDM group rates of CS (24.7% and 41.7%, p=0.03), preterm deliveries (12.3% and 37.5%, p=0.0001) and LGA (13.4% and 53.2%, p=0.0001) were increased, and pregestational BMI was associated with macrosomia (p=0.028). In the GDM group, obesity was associated with LGA (p=0.038) while excessive weight gain tended to be associated (p=0.078). In comparison to T1D the T2D women had higher pregestational BMI (34.0 kg/m^2 vs 26.7 kg/m^2, p=0.01), tended to be older (33.5 vs 29.8, p=0.075) and a greater proportion were unemployed (81.8% vs 27.8%, p=0.02). HbA1c in the second (p=0.04) and third trimesters (p=0.08) of pregnancy was lower in T2D compared to in T1D. In T1D significantly more infants were LGA, 59.5% vs 27.3% in T2D (p=0.05). Among pregnant women with any diabetes type more were of non-Caucasian origin (p=0.0001), delivered by CS (p=0,042) and were smokers (p=0.001), compared to the non-diabetic women, while weight gain during pregnancy was higher (11.1 kg vs 13.1 kg, p=0.005) among the women without any diabetes. There was a tendency towards more LGA neonates in the diabetic pregnancies (p=0.059).
Conclusion: Obesity and excessive weight gain in pregnancies complicated by diabetes were found to be associated with increased risk of macrosomic and/or LGA neonates. That the weight gain during pregnancy was lower in the diabetic pregnancies, and that the frequency of LGA in GDM was not elevated indicates that this model of antenatal diabetes care is successful in those respects. The increased prevalence of LGA in Type 1 diabetes despite maternal BMI and mostly good glycemic control warrants both increased clinical attention and further investigation.
Clinical Trial Registration Number: 2013/375-31
Disclosure: **A. Stogianni:** None.

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Is fasting plasma glucose in early pregnancy a better predictor of adverse obstetric outcomes than HbA_{1c}?

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Background and aims: Emerging data sustains that an early pregnancy HbA1c ≥ 5.9% identifies a group of women at high risk for poorer pregnancy outcomes regardless of gestational diabetes mellitus (GDM) diagnosis. The usefulness of different HbA1c cut-off points has not been fully evaluated yet. Fasting plasma glucose (FPG) is an inexpensive parameter and previous studies have also reported significant graded associations between first trimester FPG levels below those diagnostic of diabetes and adverse pregnancy outcomes albeit without an obvious threshold at which

risk increased. Our aim is to determine, in a multi-ethnic cohort, the suitability of first-trimester FPG and HbA1C levels to identify women without diabetes at increased pregnancy risk.

Materials and methods: A prospective study was conducted between April 2013-September 2015. Universal testing for FPG and HbA1c levels at the first antenatal bloods was performed. All women were screened for GDM at 24-28 weeks' gestation using a two-step approach. Primary outcomes were macrosomia and preeclampsia. We assessed different FPG and HbA1c cut-off points for associations with primary outcomes. Odds ratios were adjusted by nulliparity, pre-pregnancy body mass index, previous macrosomia, ethnicity, pregnancy weight gain, GDM diagnosis and anemia.

Results: 1228 pregnancies were included for outcome analysis. Adjusted odds ratios for associations between different first trimester FPG and HbA1c cut-off values and primary outcomes are shown in the table. There was no association between FPG levels and the risk of developing macrosomia or preeclampsia. Women with HbA1c≥5.8% showed an increased risk of macrosomia. A HbA1c≥5.9% threshold was associated with a higher risk of preeclampsia.

Conclusion: In a multiethnic population, first-trimester FPG levels are not a better predictor of adverse pregnancy outcomes than HbA1c.

Adjusted odds ratios for associations between first trimester FPG and HbA1c cut-off values and primary outcomes

	Macrosomia (n) in each group (below vs above threshold)	OR (95% CI)	Preeclampsia (n) in each group (below vs above threshold)	OR (95% CI)
FPG 84 mg/dl	22/447 vs. 55/782	1.14 (0.63-2.08)	12/447 vs. 34/782	1.91 (0.87-4.2)
FPG 88 mg/dl	36/705 vs. 41/524	1.40 (0.80-2.47)	21/705 vs. 25/524	1.97 (0.99-3.9)
FPG 92 mg/dl	51/901 vs. 26/328	1.38 (0.76-2.53)	31/901 vs. 15/328	1.74 (0.87-3.5)
FPG 96 mg/dl	60/1049 vs. 17/180	1.54 (0.75-3.14)	39/1049 vs. 7/180	1.28 (0.53-3.1)
FPG 100 mg/dl	70/1134 vs. 7/95	0.95 (0.31-2.86)	44/1134 vs. 2/95	0.69 (0.15-3.1)
HbA1c 4.8%	9/120 vs. 68/1109	0.55 (0.23-1.30)	3/120 vs. 43/1109	1.63 (0.38-7.0)
HbA1c 5.0%	21/319 vs. 56/910	0.77 (0.41-1.42)	15/319 vs. 31/910	0.63 (0.3-1.28)
HbA1c 5.4%	57/872 vs. 20/357	0.80 (0.42-1.51)	35/872 vs. 11/357	0.95 (0.45-1.9)
HbA1c 5.8%	67/1145 vs. 10/84	**2.69 (1.16-6.24)**	42/1145 vs. 4/84	1.65 (0.54-4.9)
HbA1c 5.9%	69/1181 vs. 8/48	**3.14 (1.18-8.34)**	42/1181 vs. 4/48	**3.19 (1.03-9.9)**
HbA1c 6.0%	69/1199 vs. 8/30	**7.69 (2.6-22.69)**	44/1109 vs. 2/30	2.78 (0.6-12.9)

Disclosure: **L. Mañé:** None.

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Study on pregnancy outcomes in patients with prepubertal onset of type 1 diabetes

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Background and aims: The work was initiated to study peculiarities of pregnancy in patients with prepubertal onset of type I diabetes mellitus (DMI).

Materials and methods: We examined 27 patients with prepubertal onset of DMI. Carbohydrate metabolism was assessed according to the level of glycemia (laboratory and self-monitoring with the Akki-check Nano swab) and glycated hemoglobin (HbaAc). DCA Vantage Siemens (USA) was used to measure glycated hemoglobin (HbA1c) by means of latex agglutination inhibition. Certified by the National Glycohemoglobin Standardization Program this method became the reference one. All patients were basally-bolus insulin therapy Detemir + Novorapid. All patients were examined in compliance with protocol of management for patients with pre-gestational diabetes.

Results: Mean age of the examinees, primagravidas, was 24.3 ± 4.9, the disease onset age and duration being 9.9 ± 3.9 and 11.2 ± 3.4, respectively. Pre-gestational, gestational and post-gestational HbA1c was 8.1 ± 1.1 %, 6.81 ± 1.5 % and 8.8 ±1.6 %, respectively, that is, as it can be seen, reduced through pregnancy. 27 patients received intensive basal-bolus therapy with recombinant human insulin, daily dose requirement changing by gestational age. Thus, daily doses in the first and second trimesters were 46.7 ± 11.0 and 51.6 ± 15.6 U/d, respectively, the one after delivery being 48.6 ± 9.7 U. Hypoglycemic episodes were registered almost in all examinees 3-4 times a week in the first half of pregnancy, severe hypoglycemia being observed in three patients, the severest one through coma being registered in one patient. As to vascular complications, diabetic retinopathy of various degrees was found in 11 patients (50 %), III-IV degree diabetic nephropathy being found in four. Diabetic nephropathy was found progressing, but transient and resolving in six months after delivery. As to pregnancy outcomes, therapeutic abortion was performed in three patients, due to morning sickness in one woman and to deterioration of the kidneys in two. Cesarean section was performed at various gestational ages in 17 (70.8%), at 34-35 week in five patients, at 36-37 week in six women, at 38-39 week in another six. Natural delivery was performed in seven patients at 38-39 week.

Conclusion: Prepubertal DM onset poses high risk of complications in pregnancy. Risk of unfavorable pregnancy outcome is the highest one upon prolongation of pregnancy with contraindications.

Supported by: Clinical observation of patients and learning

Disclosure: **L. Danyarova:** Employment/Consultancy; Introduction of patients.

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Contribution of telemedicine in the management of gestational diabetes: comparison of two remote follow-ups, using mydiabby platform or a h24 hotline

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Background and aims: Telemedical management of gestational diabetes (GD) can be more effective than the conventional or classical management by improving and simplifying the course, increasing patients and healthcare users satisfaction and enabling significant reductions in costs. We compared data from a center in the "Ile de France" using the myDiabby telemedical platform (TLM group), to those of a center in the "Hauts de France", carrying out a conventional monitoring, enriched with a hotline (HOT group).

Materials and methods: The available TLM / HOT data were collected for all 300 and 203 patients followed in the two centers respectively, during the year 2015. The main maternal characteristics, essential pregnancy data and major events of childbirth were compared. All data were entered into a computerized program. In addition, a satisfaction questionnaire was sent to the patients and the medical team of the TLM group. After an initial training workshop, patients in the TLM group were only followed by e-mails exchanged on a secure messaging system, which could also be used to send an electronic chart of self-monitoring of blood glucose. Those in the HOT group had episodic visits and could call the nurses by H24 hotline, communicate their blood glucose results, which were then reported manually. Specialised nurses assisted mothers in insulin dose adjustment, under the supervision of medical doctors.

Results: (TLM / HOT) For maternal characteristics, the mean age was 33 / 30.5 years, patients were less obese in the first group (BMI>25: 20/80%), and we found history of GD in 15/19% of the mothers. 42/21% of mothers were more than 35 years old; 5/8% have a history of foetal macrosomia and 46/40% had a family history of type 2 diabetes. GD was early diagnosed in 25/44%, insulin was needed in 32/48% of the cases. Mean term delivery were the same at 39wks, with 28/26% caesarean section. The average weight of the babies was the same, 3333/3314 grams, of which respectively 3 and 10% were > 4000 grams. In the TLM group, women were less obese but older, with more history of diabetes, less early diagnosis and less insulin, same term, caesarean sections, and the same mean weight of babies but less macrosomes. Differences between the two populations were moderate except for hospitalizations. While only 2.5% of women in the TLM group were hospitalized, this figure rose to 27% in the HOT group, an identical figure was

found at the national level. Time saving in decision making, improved overall visibility, catch-up of loss sight were advised as benefits by the TLM medical team and patients in this group all wished to recommend this follow-up for identical situations.

Conclusion: The HOT group has already benefited from improved support thanks to a dedicated team and hotline. "Mother-child" outcomes are of high quality in an area where medical facilities are lagging behind the national average. An orientation in this group towards telemedicine is an option, the results are equal or better with a major benefit at the level of hospitalizations whose drop close to disappearance is both an organizational benefit, an improvement in the quality of life of the Future mother and a very important minimization of the overall cost of care.

Disclosure: **S.N. Ngambou:** None.

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Reported rates of pregnancy outcomes with Gla-100 and Gla-300: results from a post-marketing survey of pharmacovigilance data

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Background and aims: Approximately 2-5% of pregnancies are complicated with diabetes, and hyperglycaemia has been associated with an increased risk of birth defects compared with the general population. Maintenance of normal glycaemic levels, including with use of exogenous insulin, is recommended to minimise the risks of foetal and maternal complications during pregnancy. Insulin glargine 100 U/ml (Gla-100) and 300 U/ml (Gla-300) are long-acting insulin analogues that have been shown to provide good glycaemic control with low risk of hypoglycaemia in people with diabetes; as such, they may offer beneficial treatment options in pregnancy. Currently, there are no randomised controlled trials specifically designed to investigate the rate of birth defects in pregnancies where insulin glargine is administered.

Materials and methods: Using Medical Dictionary for Regulatory Activities (MedDRA) terms, a cumulative search of Sanofi's global pharmacovigilance database was performed to identify pregnancy outcomes for women using insulin glargine.

Results: Post-marketing searches identified 2695 (574 solicited, 2121 unsolicited) cases of exposure to Gla-100 during pregnancy, and 43 (28 solicited, 15 unsolicited) for Gla-300. Cases were reviewed to remove non-relevant reports, leaving 2681 cases of Gla-100 use in pregnancy and 42 cases with Gla-300 (**Table**). Cumulative patient exposure was 90,860,252 and 572,677 patient-years for Gla-100 and Gla-300, respectively. Reporting rates for adverse events of specific interest were 29.5/1,000,000 patient-years with Gla-100 and 73.3/1,000,000 patient-years with Gla-300. Congenital, familial and genetic anomalies were rare with Gla-100 (82, 3.1%) and were mostly cardiac (42, 1.6%), limb (9, 0.3%) or gastrointestinal (6, 0.2%) malformations; no congenital, familial or genetic anomalies were observed with Gla-300 for the reporting period. The rates observed for Gla-100 were consistent with the rate of birth defects reported for the general population, being ~3% of all live births. Spontaneous abortions with insulin glargine were also rare (4.3% with Gla-100, 0.0% with Gla-300) and consistent with miscarriage rates observed in the general population (10%).

Conclusion: Rates of spontaneous abortions and congenital anomalies were low for Gla-100 and Gla-300 and consistent with rates in the general population. These results indicate the use of insulin glargine during pregnancy is not associated with any specific adverse effects on pregnancy and no specific foetal malformations or neonatal toxicity.

Table. Pregnancy events and reporting rates for Gla-100 and Gla-300

MedDRA search term	Gla-100		Gla-300	
	Number of events (%)[ac]	Reporting Rate[bd] (per 1,000,000 patient-years)	Number of events (%)[ac]	Reporting Rate[be] (per 1,000,000 patient-years)
Total number of pregnancy exposures	**2681 (N/A)**	**29.51**	**42 (N/A)**	**73.34**
Abortion induced	12 (0.4)	0.13	0 (0.0)	N/A
Abortion spontaneous	114 (4.3)	1.25	0 (0.0)	N/A
Congenital, familial, and genetic disorders	82 (3.1)	0.9	0 (0.0)	N/A
Ectopic pregnancy	5 (0.2)	0.06	0 (0.0)	N/A
Exposures via breast milk	78 (2.9)	0.86	2 (5.0)	3.49
Premature birth	82 (3.1)	0.9	1 (2.0)	1.75
Exposure via father	5 (0.2)	0.06	2 (5.0)	3.49
Stillbirth	19 (0.7)	0.21	0 (0.0)	N/A
Live birth/Unknown outcome	2284 (85.2)	25.14	37 (88.0)	64.61

[a] Some cases may be counted more than once owing to multiple events reported in one case
[b] The reporting rate is based on cumulative exposure as there are no data by gender
[c] Percentage calculation is based on total number of pregnancy/lactation related cases and events
[d] Gla-100 sales data cumulative to 30 June 2016 (8,290,997,962 counting units sold = 90,860,252 patient-years, data collected from IMS Health)
[e] Gla-300 sales data cumulative to 30 June 2016 (17,418,926 counting units sold = 572,677 patient-years, data collected from IMS Health)

Supported by: Sanofi
Disclosure: **D. Ozkaya:** Employment/Consultancy; Sanofi.

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The CONCEPTT-Diet study: an analysis of diet and glycaemia in UK women with type 1 diabetes before and during pregnancy

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Background and aims: CONCEPTT is a multicentre randomised controlled trial evaluating the impact of real-time continuous glucose monitoring (RT-CGM) on maternal glycaemic control before and during pregnancy. CONCEPTT-Diet is a prospective observational study of UK participants aiming to investigate the relationship between maternal diet and glycaemia.
Materials and methods: Women with Type 1 diabetes, aged 18-40 years, using insulin pump therapy or multiple daily injections with baseline HbA1c 48-86 mmol/mol (6.5-10%) were eligible for the study. Participants were randomised to either RT-CGM or Home Glucose Monitoring (HGM) added to standard insulin delivery. UK participants who consented to be involved in the dietary study completed a 3-day food diary at run-in and at 24 weeks (pre-pregnant cohort) or at 34 weeks gestation (pregnant cohort). Dietary analysis was performed using validated software Dietplan 6.0.
Results: There were 54 participants in the pre-pregnant cohort. Baseline mean energy intake was 1577 kcal/day, of which 42% was derived from carbohydrates (mean 175 g/day) and 41% from fat (mean 71 g/day). 49% of mean daily carbohydrate intake was from recommended sources. In the HGM group (n=30), mean daily protein consumption increased between baseline and follow-up, due to increased consumption of meat, bread and cheese. Carbohydrate intake from confectionary increased in both HGM and CGM groups, with greater change observed in the CGM group (40% v. 25%). In the pregnant cohort (n=44), mean daily energy intake was 1764 kcal, of which 43% was from carbohydrates (mean 204 g/day) and 40% from fat (mean 78 g/day). 49% of mean carbohydrate intake was from recommended sources. At baseline, macronutrient intake was more evenly spread throughout the day with larger snacks and smaller meals compared to the pre-pregnant cohort. No significant change in macronutrient consumption was observed in the HGM group (n=25) between baseline and follow-up. In the CGM group (n=19), mean fat consumption decreased by 10 g/day, but baseline fat intake was high (mean 85 g/day). Carbohydrate intake from confectionary also decreased in both groups with greater change observed in the CGM group (40% v. 30%).
Conclusion: Participants in this study demonstrate increased energy consumption from fats and corresponding lower consumption from carbohydrates compared to the background UK population. More than half the carbohydrates consumed are from non-recommended sources. Mean daily protein consumption increased between baseline and follow-up in the women randomised to HGM before pregnancy but not during pregnancy. Mean carbohydrate intake from confectionary decreased in all groups with a greater change observed in the women receiving RT-CGM.
Clinical Trial Registration Number: NCT01788527
Supported by: JDRF CCTN, NIHR
Disclosure: **S. Neoh:** None.

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Are pregnancy outcomes different in women with IADPSG-diagnosed gestational diabetes treated with insulin compared to those receiving medical nutritional therapy only

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Background and aims: Use of IADPSG diagnostic criteria for GDM diagnosis is now common in Europe. In Ireland, the prevalence of GDM using these criteria is 12.4%. The objective was to assess if women with GDM diagnosed using IADPSG criteria treated with insulin have comparable pregnancy outcomes to those treated with medical nutritional therapy (MNT) only.
Materials and methods: This retrospective cohort study included 752 GDM women on insulin (GDM-I) and 567 GDM women on MNT only (GDM-M). All women were managed within the Atlantic Diabetes in Pregnancy clinical network. Maternal outcomes examined were pregnancy-induced hypertension (PIH), preeclampsia (PET), antepartum (APH) postpartum hemorrhage (PPH), polyhydramnios, and cesarean delivery. Fetal outcomes examined were shoulder dystocia (SD), malformations, hypoglycemia, prematurity, mortality, neonatal intensive care unit admission (NICU), macrosomia (>4kg) and large and small for gestational age (LGA> 90th centile, SGA < 10th centile).
Results: GDM-I women were more obese (BMI>30kg/m^2) compared to GDM-M women (66.5% vs 49.2%; p<0.01) and more likely to have a family history of type 2 diabetes (68.1% vs 61.3%p=0.01). There were no differences in ethnicity, parity or smoking between groups. Systolic BP (121 vs 119 mm Hg; p<0.01) was greater in the GDM-I group at baseline. Results of the OGTT showed a higher fasting blood glucose (5.3 vs 4.9 mmol/l; p<0.01) and a higher 2-hour blood glucose (7.8 vs 7.4 mmol/l; p<0.01) in women with GDM-I compared to GDM-M. Women with GDM-I had a greater risk of polyhydramnios (aOR 2.33, 95%CI 1.31-4.14) and were more likely to deliver by cesarean section (aOR 1.67, 95%CI 1.25-2.23). There was no difference between the groups for rates of PET, PIH, APH and PPH. Perinatal mortality, congenital malformations and rates of SGA were also similar between groups. Infants of women with GDM-I compared to GDM-M were delivered earlier (39 vs 40 weeks; p<0.01) but rates of prematurity were similar, and had a greater median birth weight (3595 vs 3440 grams; p<0.01). Rates of macrosomia (22.2% vs 12.7%; p<0.01) and LGA (19.7% vs 12.5%; p<0.01) were greater in GDM-I vs GDM-M but rates of SD and hypoglycemia were similar. GDM-I vs GDM-M mothers had a higher HbA1C (5.6% vs 5.4%; p<0.01) despite treatment. Infants of GDM-I mothers were more likely to require NICU admission (aOR 4.88. 95%CI 3.54- 6.73). On subgroup analysis, a BMI ≥30 kg/m^2 had a greater impact on rates of macrosomia (26.5% vs 16.2%; p<0.01) and LGA (22.5% vs 15.1% p<0.01) in GDM-I compared to GDM-M women. In addition, a BMI ≥ 25 kg/m^2 had a greater impact on rates of elective cesarean section (48.9%, 31.6%, p<0.01) in GDM-I compared to GDM-M women. As the maternal BMI increased, the rates of admission to NICU increased in both groups but was greater in the GDM-I group (p<0.01).
Conclusion: GDM-I and GDM-M mothers have similar rates of maternal medical morbidities despite having a greater baseline risk factor profile. Despite this, the rate of delivery by CS remains greater primarily driven by elective intervention in the GDM-I group. Infant morbidities are similar between groups but macrosomia and LGA continue to be greater. This may be secondary to a higher starting maternal BMI or inadequate glycemic control. Perhaps in women with a higher baseline risk profile, blood glucose targets need to be even tighter. This requires further investigation.
Disclosure: **D. Bogdanet:** None.

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Type 1 diabetes treated with insulin pump or conventional insulin therapy in pregnancy: tertiary hospital outcomes

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Background and aims: Good metabolic control at conception and during pregnancy is important for diabetic women, in order to reduce the risk of fetal and maternal adverse outcomes. It is still uncertain whether continuous subcutaneous insulin infusion (CSII) provides better maternal and child outcomes over multiple daily injections (MDI). The aim of this study was to compare metabolic control and maternal-fetal outcomes in women with type 1 diabetes managed on CSII compared with MDI.
Materials and methods: Retrospective study of type 1 diabetes pregnant women data, who attended specialized appointments on a tertiary hospital, from 2012-2016. Macrosomia defined as weight >4000 g, large for gestational age (LGA) as percentile >90 and preterm delivery as < 37 weeks.
Results: From a total of 54 type 1 diabetic pregnant women, 48 patients were included: 22 CSII and 26 MDI, with similar ages (33 vs 30 years), diabetes duration (16 vs 15 years) and complications prevalence (microvascular 45 vs 40%; none macrovascular cases registered). Preconception hemoglobin A_{1C} (HbA_{1C}) was lower in CSII group (7,8 vs 10,1%, p<0,05), as well as global pregnancy HbA_{1C} (6,7 vs 7,5%, p<0,05). This reflects the lower first and second trimesters' HbA_{1C} in CSII compared to MDI (7,2 vs 8,0%, p<0,05 and 6,6 vs 7,3%, p<0,05); however there was no statistically significant difference for third trimester (6,6 vs 7,1%, p=0,106). For maternal outcomes, the difference wasn't statistically significant for weight gain (13 vs 11 kg), preconception or final body mass index, severe hypoglycemia (5 vs 0%), ketoacidosis (0 vs 9%), cesarean delivery (68 vs 74%) and gestational age at delivery (38 vs 37 weeks). Incidences of pregnancy losses (14 vs 15%) and preterm delivery (32 vs 46%) were similar among both groups. For fetal outcomes, a higher birthweight was calculated for CSII (3549 vs 2984 g, p<0,05). Albeit there were more macrosomic (21 vs 6%) and LGA (47 vs 39%) babies in CSII, the difference was not statistically significant. Secondary neonatal outcomes did not statistically differ between CSII and MDI: congenital anomalies (0 vs 3% - cardiac anomaly), neonatal hypoglycemia (12 vs 0%), jaundice (12 vs 7%), perinatal injuries among vaginal deliveries (9 vs 11%) and neonatal intensive care admission (17 vs 33%).
Conclusion: As reported by other authors, there was a lower HbA_{1C} in CSII group without increased risk of severe hypoglycemia or diabetic ketoacidosis. Despite the better metabolic control, the proportion of LGA and macrosomia was higher than expected in this group. The lower mean birthweight observed in the MDI group was probably influenced by a pronounced low birthweight case on a preterm delivery. The lower HbA_{1C} achieved before conception with CSII proves that this method is a useful tool to accomplish metabolic goals.
Disclosure: **C. Silvestre:** None.

941

Treatment with insulin detemir vs NPH during pregnancy in women with gestational diabetes: comparison of glycaemic control and pregnancy outcome

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Background and aims: In the literature there is only one study comparing insulin detemir (Det) versus neutral protamine Hagedorn (NPH) in women with gestational diabetes mellitus (GDM) regarding safety and efficacy. The objective of this retrospective study was to compare glycemic control, pregnancy outcome as well as fetal/neonatal outcome between treatments with insulin detemir and insulin NPH in women with GDM.
Materials and methods: A total of 192 women with GDM were included and two groups were formed. The first group comprised 98 women who received detemir and the second group comprised 94 women who received NPH. GDM was diagnosed based on IADSPG/WHO criteria. At the beginning all women were recommended to follow a specific diet and to perform self-monitored glucose measurements. Treatment with insulin was initiated based on: fasting blood glucose >95mg/dl or 1-hour postprandial glucose >130mg/dl and/or evidence of macrosomia or polyhydramnios on fetal ultrasound. All patients who needed rapid-acting insulin were excluded. Data regarding medical history, parameters of glycemic control, time and mode of delivery and neonatal outcomes were recorded.
Results: Demographics and baseline characteristics in both groups were similar: ethnicity (Greek: Det 87% vs NPH 82%), age (Det 36±4 vs NPH 37±5yrs), prepregnancy body mass index (Det 29±6 vs NPH 28±7kg/m^2), education, family history of diabetes mellitus type 2 (Det 43% vs NPH 43%), hypertension (0% in both groups), glycemic control estimated by Hemoglobin A1c (HbA1c) at diagnosis (median: Det 5.3 vs NPH 5.4%). There were no differences with respect to the week of insulin initiation (Det 27.7±7 vs NPH 27±7.5 weeks), the total insulin dose (median: Det 540 vs NPH 527 IU), the duration of insulin therapy (median: Det 53 vs NPH 56 days), the daily insulin dose/weight at the start and end of insulin treatment (median: Det 0.1 vs NPH 0.1 IU/kg and median Det 0.14 vs NPH 0.13 IU/kg respectively), as well as the number of insulin injections per day**.** Maternal overall weight gain during pregnancy (Det 10 ±7 vs NPH 12±9 kg), and weight gain per week since the start of insulin through to delivery (median: Det 40 vs NPH 110g/wk) did not differ between the groups. The detemir group had slightly lower, although significant, HbA1c level at the end of gestation (median: Det 5.2 vs NPH 5.4%, p=0.035). There were no hypoglycemias or allergic reactions in both groups. Further, there were no differences regarding perinatal/neonatal outcomes: time (median: Det 38 vs NPH 38 weeks) and mode of delivery (Cesarean section: Det 62 vs NPH 48%), pre-term delivery (Det 14 vs NPH 21%), Apgar score (median: Det 9 vs NPH 9), birth weight (Det 3298±571 vs NPH 3031±589g), birth weight adjusted for gender and gestational age (Det 56^{th}±19 vs NPH 50^{th}±11.5 percentile), percentage of macrosomia (weight >90^{th} percentile) (Det 5.6% vs NPH 0%) and small for gestational age (<10^{th} percentile) (0% in both groups).
Conclusion: Glycemic control and pregnancy outcome were equally effective in women using insulin either detemir or NPH during pregnancy.
Disclosure: **E. Anastasiou:** None.

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Three years experience with FGM/CGM in pregnant women with type 1 diabetes

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Background and aims: In pregnancies in women with Type 1 diabetes (T1D), the risk is increased for malformations, accelerated growth of the fetus, preeclampsia, premature delivery, shoulder dystophia, sectio and neonatal intensive care. A tight blood glucose control is vital to reduce complications during pregnancy and delivery. The use of flash glucose measurements (FGM) or continuous glucose measurements (CGM) enables a strict glucose control throughout the whole pregnancy. The aim of this study was to explore the outcome of pregnancies after use of FGM/CGM.
Materials and methods: All pregnant women with T1D at our hospital are offered a FGC/CGM system during pregnancy. To date we have had 46 women who have completed the pregnancy wearing a FGM/CGM. FreeStyle Libre® (Abbott) is most used. In case of non-awareness of hypoglycaemia, systems with alarm, as Dexcom® or MiniLink® REAL Time Transmittor (Medtronic) are preferred. Glucose curves were weekly transferred via Diasend system to the diabetologist. Insulin doses were adjusted by the diabetologist every week after phone calls to the patients. Target for glucose control was between 4-8 mmol/L.
Results: During pregnancy the women had a median increase of insulin dose from 43 to 84.5 E/day. Weight went from 71.9 to 86.6 kg. HbA1c decreased from 56.8 to 44.1 mmol/mol. Mean glucose was 7.5±0.9

mmol/L during the whole pregnancy. The main time, 52 %, was spent within glucose target between 4-8 mmol/L. Hypoglycaemia defined as glucose below 4 mmol/L was present during 12% of time and 37% of the time the women had glucose above 8 mmol/L. Gestational length was 38 week, the frequency of preeclampsia was 8.7% and sectio frequency 38%. Birth weight was 3804 g. The frequency of neonatal hypoglycaemia was 13%.

Conclusion: It is possible to achieve almost normoglycemia in pregnant women with T1D with new FGM/CGM technology. The FGM/CGM system is necessary to ensure a safe pregnancy without severe hypoglycaemia. The outcome of these pregnancies was close to women without T1D.

Supported by: Regional Funds Region Skane

Disclosure: **M. Landin-Olsson:** None.

943

The EValuation Of Ketones INtensive monitoring in women with Gestational diabetes (EVOKING) study: preliminary results

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Background and aims: Women with gestational diabetes mellitus (GDM) are frequently asked to check their ketones levels by measuring ketonuria before breakfast. The presence of ketosis is associated with several adverse outcomes both during pregnancy and in the life of the newborn. Ketosis can be present before main meals (i.e. before lunch and dinner), and a fasting urine ketone measurement is not sufficient to catch all the daily ketosis episodes. Blood ketone monitoring which directly measures β-hydroxybutyrate in blood could be a more accurate test for detecting ketosis compared with a urine test which mainly measures acetoacetate. The aim of this study was to evaluate the effect of blood ketone intensive monitoring for the detection of ketosis in women with GDM with a negative urinary ketone test.

Materials and methods: This was a single center, observational, prospective study enrolling consecutive women with GDM cared for at the Diabetes and Pregnancy Unit of a University Hospital**.** Self-monitoring blood glucose tests (GlucoMen LX, Menarini, Italy), dietary advice and physical activity prescription was given according to standard care to all participating women. Only women with negative fasting urinary ketone tests were included. During the same gestational week (30 weeks) all participants were asked to perform a capillary blood ketone test before breakfast, before lunch and before dinner. Ketosis was defined as the presence for at least 25% of the time of fasting blood ketone levels >0.1 mmol/L and >0.2 mmol/L before lunch and dinner. Information on clinical and antrophometric characteristics, risk factors for GDM and laboratory tests was collected.

Results: Overall, a total of 101 women (mean age 34.7±4.8 years, pre-pregnancy BMI 28.2±5.2 kg/m^2, 59.4% insulin treated) were studied. Blood ketones were present in 37.6% of the cases before breakfast, 13.9% before lunch and 11.9% before dinner. The percentage of women with at least one daily presence of blood ketones was 40.6%.The presence of fasting blood ketones correlated closely with ketone presence before lunch (r=0.63, p<0.0001) and before dinner (r=0.55, p<0.0001) and with one hour after breakfast mean glucose levels (r=0.23, p=0.02). When comparing women with positive blood ketone tests with those with a negative test no differences was detected in clinical and antrophometric mothers' characteristics, risk factors for GDM, nor for insulin treated rate.

Conclusion: Blood ketone testing in women with GDM can detect a greater number of ketosis episodes than urinary ketone testing indicating the benefit of testing specifically for β-hydroxybutyrate. The presence of fasting blood ketone correlated closely with ketone positivity during the day and with glucose levels after breakfast. A detailed investigation of the relationship between blood ketones and pregnancy and neonatal outcomes will be available at the end of the EVOKING study. These preliminary results indicate that blood ketone measurement should be recommended to women with GDM.

Disclosure: **A. Di Benedetto:** None.

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Gestational diabetes treated with lifestyle modification, metformin and insulin

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Background and aims: Gestational diabetes (GDM) describes glucose intolerance first recognized during pregnancy. GDM rates are increasing globally and are associated with short- and long-term complications for the mother and her infant. Treatment options for GDM include lifestyle modification (LM) and medical therapies with insulin (INS) or metformin (MF). We reviewed outcome of all our patients with GDM attending our hospital in 2015 and 2016.

Materials and methods: Following risk factor based screening, a two-step approach with Glucose Challenge Test (GCT) and Oral Glucose Tolerance Test (OGTT) were performed to diagnose new patients with GDM in the antenatal clinics. All patients underwent lifestyle modification (LM) with diet and exercise. Medical therapies with metformin (MF) or insulin (INS) were initiated based on patient's capillary blood glucose (CBG) and the latest antepartum scan in order to maintain glycemic targets. Patients were followed until delivery and maternal fetal outcomes characteristics were recorded.

Results: We identified 683 patients with GDM attending our unit, with a mean age of 35.6 ± 4.5 years old. Majority of mothers were overweight and obese at booking (30% BMI 25 to 30kg/m^2, and 35% BMI above 30kg/m^2). There were 479 (70.1%) patients with new diagnosis of GDM while 204 (29.9%) patients with previous diagnosis of GDM. Most were European in origin (n=517, 75.9%) followed by Asians (n=116, 17%), Africans (n=23, 3.4%), Middle-Eastern (n=10, 1.5%), and patients from the American continents (n=12, 1.8%). A total of 423(61.9%) patients continued on LM alone while 116(17%), 111(16.3%) and 19(2.8%) patients required MF, INS and both treatment respectively. Most patients had normal spontaneous vaginal delivery (SVD, n=346, 50.7%) followed by Cesarean section (n=213, 31.2%) and assisted delivery (n=71, 10.4%) while 25(3.7%) patients had miscarriage. A total of 116 babies (17%) were macrosomic (>4kg). There were no differences in macrosomia (p=0.224) and delivery methods (p=0.269) between patients treated with LM, MF or INS. Patients from Asia (32/114 patients, 28.1%) and Africa (6/23 patients, 26.1%) required more INS treatment compared to Europeans (72/507 patients, 14.2%, p=0.006). There was no statistically significant difference in age between the three treatment methods. Mothers treated with INS had higher GCT values (11.24 ± 2.33 mmol/L) compared to MF-treated (9.35 ± 1.64 mmol/L) and LM-treated (9.41 ± 1.9 mmol/L, p <0.005). Plasma glucose at OGTT (fasting, 1 hour post OGTT) were also higher in INS-treated (5.8 ± 0.9, 12.41 ± 1.72 mmol/L) compared to MF-treated (5.19 ± 0.53, 11.18 ± 1.21 mmol/L) and LM-treated (4.7 ± 0.55, 10.7 ± 1.38 mmol/L, p <0.005) group respectively.

Conclusion: Our patients with GDM were older and overweight at baseline. Patients treated with INS had higher GCT values, higher fasting and 1-hour OGTT values compared to MF and LM. Equal numbers of patients were treated with metformin and insulin without differences in the outcome for mothers and babies. Based on these data, considering neonatal and maternal outcomes, metformin appears to be as safe as insulin and lifestyle modification in the treatment of GDM.

Disclosure: **W. Wan Mahmood:** None.

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Universal versus risk-factor-base screening for gestational diabetes: an analysis from a 5 year Portuguese cohort

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Background and aims: The criteria to screen for Gestational Diabetes Mellitus (GDM) are not internationally consensual. In opposition to the universal screening performed in Portugal, certain countries favour a risk-factor-based screening. Universal screening allows higher GDM detection rates but more women are submitted to an inconvenient test, additional medicalization of pregnancy and further maternal anxiety. Besides, the additional cases detected have in average a milder form of glucose intolerance and their pregnancy/neonatal outcomes may be similar to the background population. We aim to compare obstetric and neonatal outcomes in pregnant women with and without risk factors treated for GDM.
Materials and methods: Retrospective and multicentre study of 12006 pregnant women diagnosed with GDM between 2011 and 2015, in Portugal, by the International Association of the Diabetes and Pregnancy Study Groups (IADPSG) criteria. Data from the Portuguese National Register. Statistic analysis SPSSv20. Risk factors adapted from National Institute for Health and Care Excellence guidelines: Body Mass Index (BMI) superior to 30 kg/m^2, history of macrossomic newborns, history of GDM and first-degree relatives with type 2 diabetes mellitus (T2DM). We excluded women that had no data regarding risk factors (n=1563).
Results: At least one risk factor (RF) was found in 68,2% (n=7123) pregnant women with GDM. The most common RF was first-degree relatives with T2DM (71,8%, n=4966), followed by BMI superior to 30 kg/m^2 (45,9%, n=3267). In the pregnant women without RF versus with RF, insulin therapy was initiated in 38% (n=1256) vs 49% (n=3441) (p<0,001), the starting week of therapy was deferred one week (30 IQR 23-33 vs 29 IQR 21-32, p<0,001) and total daily doses of insulin were inferior (14 IQR 8-26 vs 18 IQR 10-31, p<0,001). Moreover, treatment with metformin was less frequent (6 vs 10%, p<0,001). Concerning obstetric complications, pre-eclampsia was more frequent in women with RF (3,4 vs 2,4%, p=0,006), but there were no differences in hydramnios or fetal death. Caesarean section was more commonly performed in women with RF (38,4 vs 30,6%, p<0,001). The newborns of pregnant women with RF were more frequently large-for-gestational-age (LGA) (5,1 vs 2,3%, p<0,001). The neonatal morbidity was higher in the newborns of pregnant women with RF (19,3 vs 17,5%, p=0,040), namely hypoglycaemia (4 vs 3,2%, p=0,044), neonatal hyperbilirrubinemia (11,6 vs 9,9%, p=0,014), respiratory distress syndrome (2,8 vs 2%, p=0,018) and congenital anomalies (3,4 vs 2,2%, p=0,002). The Diabetes Mellitus reclassification showed increased frequency of impaired fasting glycaemia, impaired glucose tolerance or diabetes in women with RF (8,6 vs 5,2%, p<0,001).
Conclusion: Almost a third of pregnant women would have remained undiagnosed if risk-based-factor screening were implemented in Portugal (31,8%, n=3320). Our results show that women without RF were less insulinized, had fewer caesareans, fewer LGA newborns and less neonatal complications than the women with RF, even after adjustment for age and maternal weight. However, we emphasize that of these women, 38% (n=1256) had inadequate metabolic control and required therapy with insulin. In the future, we intend to compare pregnant women with GDM and no RF with pregnant women without GDM to further ascertain about the impact of GDM in pregnancy outcomes.
Supported by: Portuguese Study Group of Diabetes and Pregnancy
Disclosure: **C. Matta-Coelho:** None.

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Gestational diabetes: the impact of new diagnostic criteria on prevalence and pregnancy outcomes

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Background and aims: Gestational diabetes mellitus (GDM) is associated with an increased risk of pregnancy complications. Early diagnosis and treatment of GDM may reduce these complications. In 2013, the WHO adopted the stricter cut-off glucose values of the IADPSG 2010 (WHO2013, fasting plasma glucose (FG) ≥5.1 and/or 2-hour glucose (2HG) ≥8.5 mmol/l). In the Netherlands, the WHO 1999 criteria (WHO1999, FG ≥7.0 and/or 2HG ≥7.8 mmol/l) are still used. In this study, we evaluated the impact on prevalence and pregnancy outcomes when applying the old WHO1999 vs. new WHO2013 criteria.
Materials and methods: 9628 women with singleton pregnancies were screened for GDM with a 75-g OGTT if they had risk factors or signs of GDM between January 2011 and January 2016. Women with GDM diagnosed according to WHO1999 criteria were treated for GDM. In 4431, pregnancy outcomes were retrospectively collected and compared between a non-GDM control group (WHO1999-/WHO2013-: FG <5.1 and 2HG <7.8 mmol/l) and two groups: GDM according to WHO2013 but not WHO1999 (WHO2013+/WHO1999-: FG ≥5.1-6.9 and 2HG <7.8 mmol/l); GDM according to WHO1999 but not WHO2013 (WHO2013-/WHO1999+: FG <5.1 and 2HG ≥7.8-8.4 mmol/l).
Results: Prevalence of GDM in the total cohort was 30% using WHO2013 and 20% using WHO1999. GDM was diagnosed solely on FG in 62% by WHO2013 contrasting to 1% by WHO1999. Compared with the non-GDM group (n=2851), women testing WHO2013+/WHO1999- (n=667, by definition not receiving diet treatment) were older, had a higher pre-gestational BMI (29.1 [IQR 24.8-33.5] vs. 25.2 [IQR 22.0-30.4] kg/m^2, P<0.001), had more often chronic hypertension (3.3% vs. 1.2%, P<0.001) and pregnancy induced hypertension (7.8% vs. 4.9%, P=0.003), showed higher rates of planned caesarean section (10.3% vs. 6.5%, P=0.001) and induction of labour (34.8% vs. 28.0%, P=0.001) and showed more often Apgar score <7 at 5 min (4.4% vs. 2.6%, P=0.015) and admissions to the neonatology dept. (15.0% vs. 11.1%, P=0.004). Frequency of foetal macrosomia and large for gestational age (LGA) neonates (>P90) was not significantly different to the non-GDM group (21.0% vs. 18.0%). Women testing WHO2013-/WHO1999+ (n=234, active treatment) had comparable rates of LGA neonates (15.4% vs. 18.0%) and emergency caesarean section (12.0% vs. 11.5%) compared with the non-GDM group. However, in this group 20.5% of the women needed treatment with insulin.
Conclusion: The lower FG cut-off in WHO2013 will have a major impact on the prevalence of GDM. Yet, it identified a group of women (WHO2013+/WHO1999-) with increased risk of adverse outcome. However, adopting the WHO2013 with a higher 2HG cut-off excluded women who now benefit from treatment. We conclude that change in diagnostic criteria for GDM will have considerable impact on prevalence and pregnancy outcomes.
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Disclosure: **S.H. Koning:** Grants; Novo Nordisk Netherlands provided an unrestricted research grant..

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Is first-trimester HbA_{1c} useful in the diagnosis of gestational diabetes?

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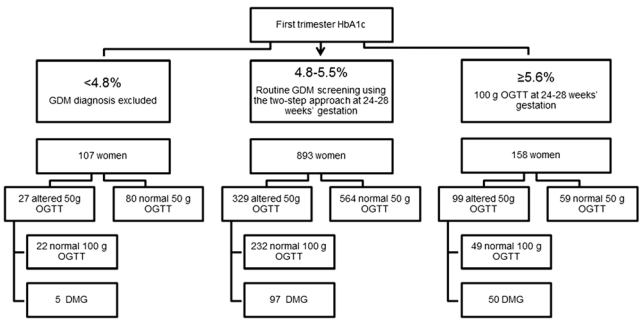
Background and aims: Some clinical guidelines recommend first-trimester universal screening with A1c (1T-A1c) for the diagnosis of unknown type 2 diabetes mellitus. 1T-A1c could have other potential uses such as early identification of women at risk of developing gestational diabetes mellitus (GDM) later in pregnancy. Our aim is to evaluate the utility of 1T-A1c in the diagnosis of GDM and to assess associated costs.
Materials and methods: Observational study of a prospective cohort of consecutive pregnant women attending obstetric follow-up between April 2013 and September 2015. All women had a 1T-A1c determination and GDM screening at 24-28 weeks of pregnancy using a two-step approach with a 50 g screen followed by a 100 g OGTT. Women with pregestational diabetes or undiagnosed type 2 diabetes in pregnancy were excluded. Sensitivity, specificity, positive (PPV) and negative predictive (NPV) values of different first trimester HbA1c cut-off points for the detection of GDM were calculated. A ROC curve was drawn to determine sensitivity and specificity of 1T-A1c in detecting GDM and a rule-in- rule-out diagnostic algorithm was proposed. Costs associated with blood extractions (equipment, salaries), accommodation, reagents and glucose load solution were included.
Results: 1,195 women were included and one hundred and fifty-six (11.85%) were diagnosed of GDM. The area under ROC curve for 1T-A1c to detect GDM was 0.679 (95% CI 0.631 - 0.727). The proposed rule-in-rule-out algorithm is shown in figure. A rule-out threshold for A1c of 4.8% had a sensitivity of 96.7% (95% CI 93.9-99.5), specificity of 10.1% (95% CI 8.3-12.0) and a NPV of 95.3% (95% CI 91.3-99.3). A rule-in value of 5.6% had a PPV of 31.6% (95% CI 24.4-38.9), a specificity of 89.3%(95% CI 87.4-91.2) and a sensibility of 32.9 (95% CI 25.4-40.4). The low PPV of this higher 1T-A1c threshold precludes its use for GDM diagnosis but it identifies a group of women with 31.6% risk of developing GDM, similar to the PPV of a 50 g OGTT. Therefore, an A1c cut-off value of 5.6% could be used to avoid a two-step diagnostic approach and patients could be referred straight to a diagnostic OGTT. Patients with an A1c value between 4.8% and 5.6% would be indeterminate and would require the two-step approach. The number of screening tests performed in the cohort according to 1T-A1c levels in the rule-in-rule-out algorithm is shown in figure. The global saving of the proposed algorithm would be of 8.8% of the total cost with the standard strategy.
Conclusion: 1T-A1c has not enough sensitivity nor specificity to diagnose GDM, although the use of a higher (5.6%) and lower (4.8%) threshold could simplify the GDM diagnostic process reducing the number of OGTTs and associated costs.



Disclosure: **L. Gortazar:** None.

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Is time to rethink early screening for gestational diabetes?

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Background and aims: The IADPSG Fasting Plasma Glucose (FPG) threshold of ≥92 mg/dl for the diagnosis of Gestational Diabetes (GDM) in early pregnancy are not recommended anymore and data regarding risk associated with hyperglycemia in early pregnancy are limited. In 2011 Italian guidelines suggested an early screening of GDM to be performed with a 75g OGTT at 16th-18th gestational weeks in women with obesity, previous GDM, or FPG 100-125 mg/dl at initial prenatal visit. With this study we have evaluated the effectiveness of such an early GDM screening.
Materials and methods: 1338 consecutive pregnant women underwent a 75g OGTT between January 2013 and December 2015 according to national guidelines and diagnosis of GDM was based on IADPSG/WHO 2013 criteria.
Results: 14.4% of screened women had high risk defined as the presence of least one of the following: BMI ≥30 kg/m^2, prior GDM, FPG 100-125 mg/dl at initial visit. Of these women, 84.3% had only one major risk factor (34.8% pGDM, 41.7% obesity and 7.8% high FPG at the first trimester); 7.0% had both pGDM and obesity, 6.1% had both pGDM and high FPG, while 2.6% had both high FPG and obesity. No women had all three major risk factors. Screening between 16th-18th was performed in 50% of cases and 28% of them repeated the OGTT later in pregnancy due to normal glucose tolerance at the first evaluation. Among high risk women, 40% of those with FPG 100 - 125 mg/dl in the first trimester, 53% of the obese ones, and 65% of those with pGDM underwent an early OGTT. The prevalence of GDM in high risk women was 67%. Among those performing early screening, GDM was detected in 40.7% (37/91 women) at the time of first screening and 37% (19/51) at 24th-28th gestational week. Among women performing only late screening, GDM was diagnosed in 74% (66/89) of the cases. GDM was diagnosed at the time of early screening in 56% of the women with pGDM, 67% of those with obesity and 80% of those with high FPG at the first trimester. The prevalence of GDM was higher in women with two risk factors, being 100% in those with obesity and high FPG in the first trimester.
Conclusion: On the basis of these data, we suggest that an early (16th-18th gestational weeks) screening for GDM should be implemented in high risk women, especially in those with FPG in the range 100-125 mg/dl at first prenatal visit and obesity. It remains to be determined whether early diagnosis and treatment may have a positive impact on perinatal outcomes.
Disclosure: **C. Bianchi:** None.

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Development of gestational diabetes is characterised by impaired fasting insulin sensivity and beta cell dysfunction at early gestation

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Background and aims: The pathophysiological components of impaired glucose metabolism (mainly impaired insulin action and β-cell dysfunction) could be quantified by advanced clinical examinations including clamp examinations as well as oral and intravenous glucose tolerance tests. However, also simple approximations from fasting measurements are available, which might be useful to provide an early and cheap identification of women with high risk for the later development of GDM.
Materials and methods: 112 pregnant women were included in this study and received a broad metabolic characterisation at early gestation (before 15+6 weeks of gestation) including fasting measurements of glucose,

insulin and C-peptide to provide information on insulin sensitivity by using the homeostatic model assessment of insulin resistance (HOMA-IR) and insulin secretion (HOMA-β). The disposition index (DI) was calculated as the product of HOMA-IR and HOMA-β to provide an estimation of β-cell function (i.e. the ability of the β-cells to adapt for insulin resistance). The amount of insulin action was additionally estimated by the quantitative insulin sensitivity check index from C-peptide (QUICKIc) to provide an estimate of insulin sensitivity from prehepatic measurements.

Results: GDM was diagnosed in 29 women, whereas 82 remained normal glucose tolerant (NGT) until end of gestation. Already at early pregnancy fasting surrogate measures of insulin action were considerably impaired in women who developed GDM as compared to those who remained NGT (HOMA-IR: 2.30 [IQR:1.45-3.93] vs. 1.42 [IQR:0.98-1.96], p<0.001). Moreover, a lower DI indicated β-cell dysfunction in subjects with later development of GDM (76 [IQR:58-97] vs. 115 [IQR:88-158], p<0.001). In particular, QUICKIc (ROC-AUC: 0.79, 95%CI: 0.68-0.90) as well as DI (ROC-AUC: 0.77, 95%CI:0.65-0.87) were closely related to the later development of GDM.

Conclusion: Later development of GDM is characterized by fasting surrogate measures of impaired glucose disposal already at early gestation. These approximations if insulin resistance and β-cell dysfunction might be useful to identify those women with particularly high risk for developing GDM.

Disclosure: **V. Falcone:** None.

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Gestational diabetes (GDM) diagnosed at 24-28 weeks' gestation in elderly and obese women already affects foetal growth and abdominal obesity

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Background and aims: Elderly and/or obese women might be associated with increased incidence of GDM and metabolic disturbances occurring early in pregnancy affecting fetal growth even before the diagnosis of GDM. We, thus, investigated whether altered glucose metabolism already exists and affects fetal growth at the time of GDM diagnosis.

Materials and methods: Medical records of 7,597 singleton pregnancies which have done 50-g glucose screening test (GST) were reviewed retrospectively. 1,186(15.6%) did 100-g OGTT with measurement of fasting plasma insulin and HbA1C. NGT (normal glucose tolerance) and GDM were diagnosed by ADA criteria. Insulin resistance (HOMA-IR) and secretion (HOMA-beta %) were calculated by homeostasis model assessment. We investigated gestational age estimated at the same day of 50-g GST by ultrasonographic measurement of abdominal circumference (GA-AC) and femur length (GA-FL). We also calculated ratio of GA-AC/GA-GST (GA at 50-g GST) and ratio of GA-AC/GA-FL as indices for fetal abdominal obesity. Total subjects were divided into 4 groups: group 1 (age <35 & prepregnancy BMI <25), 2 (<35 & ≥25), 3 (≥35 & <25), 4 (≥35 & ≥25), and GDM patients in each group were compared in subgroup analysis. Statistical analysis was performed using SAS 9.4.

Results: 1) Overall incidence of GDM was 5.1% (384/7,597). The incidence was the lowest in group 1 (3.1%, 147/4,721), higher in group 2 (8.8%, 23/261) and group 3 (6.7%, 163/2,432), and the highest in group 4 (22.5%, 51/227). **2)** HbA1C(%) in GDM was significantly higher than in NGT (5.3±0.5, 5.0±0.2, $p<.0001$). In subgroup with GDM, HbA1C in group 4 was significantly higher than in group 1 (5.6±0.55, 5.25±0.42, $p<.05$). **3)** HOMA-IR in GDM was significantly higher than in NGT (2.21±1.25, 1.95±0.90, $p<.001$). In subgroup with GDM, HOMA-IR in group 2 (3.84±1.45) and 4 (2.89±1.16) were significantly higher than in group 1 (2.08±1.31) and 3 (1.91±0.94), $p<.0001$. **4)** HOMA-beta in GDM and NGT were not significantly different from one other, but in subgroup with GDM, it was significantly higher in group 2 (253±207) than in group 1 (174±137) and 3 (140±75), $p<.0005$. **5)** Both GA-AC/GA-GST and GA-AC/GA-FL ratio were significantly higher in GDM (1.04±0.05, 1.02±0.05) than NGT (1.03±0.04, 1.01±0.04), $p<.003$. But only in group 3 and 4, GA-AC/GA-GST of GDM were higher than NGT. In subgroup with GDM, GA-AC/GA-FL in group 4 was significantly higher than group 1 (1.05 ±0.04, 1.03±0.04), $p<.05$. **6)** In correlation analysis, GA-AC/GA-GST and GA-AC/GA-FL ratio showed positive correlation with age (r=0.1140, r=0.0751, $p<.0001$), prepregnancy BMI (r=0.0945, r=0.0681, $p<.0001$) and HbA1C (r=0.1619, r=0.1452, $p<.0001$) and negative correlation with HOMA-beta (r=-0.1148, r=-0.1154, $p<.05$). **7)** In multiple linear regression analysis, age and HbA1C were significantly associated with GA-AC/GA-GST (β=0.0020, $p<.005$ and β=0.0179, $p<.05$) and GA-AC/GA-FL (β=0.0018, $p<.005$ and β=0.0171, $p<.05$).

Conclusion: GDM diagnosed at 24-28 weeks of gestation in elderly and obese women already affected fetal growth and abdominal obesity. Thus, earlier detection of metabolic dysfunction and appropriate intervention might be necessary.

Disclosure: **Y. Kim:** None.

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Experience from individualised monitoring of pregnancies with normal 2-hour OGTT

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Background and aims: NICE guideline has recommended diagnosis of gestational diabetes (GDM) on the basis of 75gm 2-hour OGTT, with fasting glucose >5.6mmol/L or 2-hour glucose >7.8mmol/L. For those with normal OGTT, it was unclear what are the perinatal and glycaemic outcomes especially those with borderline OGTT or progressive foetal risk factors. We sought to explore the value of individualised approach in subjecting this subset of pregnancies to glucose monitoring in a multidisciplinary setting.

Materials and methods: Excluding those with pre-existing diabetes prenatally, pregnant ladies at risk for GDM attending our centre who had 2-hour OGTT consecutively between 2013-2016 were reviewed. Pregnancies diagnosed with GDM on the basis of OGTT were subjected to self-monitored blood glucose (SMBG) and dietician review. Subsequent needs for treatment escalation with metformin or insulin as dictated by SMBG profile were then recorded in a database. Follow-up were made until fasting glucose was performed 6-week postnatally. We identified a second group of pregnancies who had normal OGTT but similar monitoring and management was prescribed by the attending multidisciplinary team on the basis of progressive foetal risk factors (polyhydramnios and increasing foetal abdominal circumference) or borderline but normal OGTT. The value of monitoring this subgroup based on individualised multidisciplinary approach was evaluated by comparing the perinatal and maternal glycaemic outcomes as depicted in Table 1. Data analysis was performed on IBM SPSS v23. Independent T-test and Fisher-exact test were used to examined relationship for continuous and categorical proportions respectively.

Results: During 2013-2016, 415 pregnancies were diagnosed to have gestational diabetes during OGTT. We have identified another 75 patients with normal OGTT but were followed up in protocol similar to the GDM group in view of borderline OGTT or progressive foetal risk factors. Comparison between these groups were shown in Table 1.

Conclusion: Between pregnancies with gestational diabetes and those with normal OGTT, no significant difference between maternal age, BMI, foetal birthweight and gestational age of delivery was observed. While no difference in postnatal fasting glucose was demonstrated, those who had normal OGTT were less likely to require antenatal metformin or

insulin treatment compared to the GDM group. Despite this, a considerably large proportion (45.7%) of those with normal OGTT in this selective group developed abnormal glycaemic profile during antenatal follow-up and eventually required treatment with metformin or insulin. This suggests that an individualised approach may be required to identify who would otherwise miss the opportunity for treatment if we relied solely on the normal OGTT as a risk stratifying tool. A larger prospective study may be required to justify risk factors to consider for such individualised approach.

	GDM OGTT (N=415)	Normal OGTT (N=75)	
Fasting Glucose (mmol/L)	4.96±0.84	4.40±0.41	P<0.001[a]
OGTT: 2-hour glucose (mmol/L)	8.91±1.54	5.82±2.16	p<0.001[a]
Maternal age (year)	32.10±5.33	30.66±5.99	p=0.040[a]
Maternal BMI (kg/m²)	29.59±7.56	28.43±7.06	p=0.247[a]
Fetal birthweight (gm)	3352.95±487.953	3457.96±446.93	p=0.091[a]
Delivery gestational age (week)	38.98±1.45	38.67±4.85	P=0.286[a]
Post-natal fasting glucose (mmol/L)	3.97±1.69	3.97±1.69	p=0.317[a]
Diabetic treatment required antenatally			p=0.032[b]
Diet control only	153 (37.7%)	38 (54.3%)	
Diet control and metformin only	155 (38.2%)	20 (28.6%)	
Diet control and Insulin ± metformin	98 (24.1%)	12 (17.1%)	

Table 1
Data are presented as mean± standard deviation or n (%) (percentage are in column)
[a] Independent t-test
[b] Chi Square test

Disclosure: **S.M. Krishnan:** None.

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Gestational diabetes without risk factors: A group with increased risk of small for gestational age babies? Results from a retrospective case-controlled study

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Background and aims: Gestational diabetes mellitus (GDM) affects approximately 9% of pregnancies in France in 2013. GDM without risk factors represents 10% of all GDM. The prevalence of small for gestational age (SGA) is estimated at 4 to 12% according to a European meta-analysis, around 5.1% of births in France. The aim of our retrospective case-control study in a cohort of women with GDM was to evaluate the number of SGA and their potential links with the conventional criteria well known in GDM.
Materials and methods: We conducted a retrospective study in 1,464 women with GDM admitted to a university hospital ambulatory diabetology unit between January 2011 and November 2016. After randomly drawing files of GDM with and without risk factors (RF), we analyzed SGA and different parameters in the two different populations. Uni- and multivariate analyses were performed with stata V14 software, using Rank, Chi-2, Kruskal-Wallis and covariance tests; p values < 0.05 were considered significant.
Results: We analyzed 179 births: 63 births in the "cases" group =1 (GDM without RF) and 116 births in the "control" group= 0 (GDM with RF). The SGA rate was significantly higher in group 1 than in group 0: 25.4% vs. 11.6% (p= 0.029). The risk of SGA was higher in those with the lowest HbA1c: OR: 0.26, 95%CI [0.1; 0.7] (p= 0.008) and in smokers: 0R: 4, 69; 95%CI [1.4; 15.7] (p= 0.017). For birth weight in multivariate analysis, we found a positive correlation with maternal BMI in all GDM (r= 0.8) and a significant negative correlation with being in group 1 (p= 0.009).
Conclusion: In our cohort, SGA was significantly higher among women with GDM without RF than among those with RF. SGA correlated significantly with lower HbA1c (p= 0.008) and higher tobacco consumption (p= 0.017). There was a positive correlation between maternal BMI and birth weight (r= 0.8). Having GDM without RF was associated with a higher risk of lower birth weight. We need to confirm these preliminary data in a prospective study in women with GDM, in particular to better understand this high proportion of SGA.
Disclosure: **R. Jouini:** None.

PS 079 Risk and follow-up in patients with gestational diabetes

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Risk factors of early pregnancy loss in women with type 1 diabetes: 17 year one centre observation

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Background and aims: Macrosomia and congenital defects are well-defined complications of pregnancy in women with type 1 diabetes (T1DM). Less data are available for spontaneous abortions and early pregnancy losses. In this 17-year one-centre observation, we examined risk factors of early loss, defined as the spontaneous loss of a pregnancy before 21 weeks' gestation, in T1DM women.
Materials and methods: We analysed medical records of 649 singleton pregnancies in T1DM women treated in a regional tertiary reference diabetes centre. The women received diabetes care during pregnancy between 1998 and 2015. We assessed details of patients' characteristics, use of various therapeutic tools, glycaemic control measured by the HbA1c level and daily mean blood glycaemia (MBG).
Results: Pregnancy ending was identified for 544 women; a total 47 cases (8.6%) lost early pregnancy (mean 11.4 week of pregnancy). Both group of women with pregnancy loss and the rest of the study group did not differ by age (29±5.4 vs 28.2±6.8 years; p=0.4, respectively), T1DM duration (11.9±7.8 vs 12.2±7.7 years; p=0.8), pregnancy planning or not-planning (p=0.8), HbA1c on admission (7.1±1.5 vs 7.0±1.4 %; p=0.7). Women who lost pregnancy had higher weight (pre-pregnancy BMI 24.8±4.6 vs 23.7±3.7 kg/m^2; p=0.049), were earlier admitted at 1st pregnancy visit (6.4±2.4 vs 8.6±4.3 week of gestation; p=0.0001). At the 1st pregnancy visit women with pregnancy loss had a higher systolic (128 ±12.2 vs 121±14.1 mmHg; p=0.001) and diastolic (79.7±8.2 vs 75.8±8.4 mmHg; p=0.0047) blood pressure and MBG (118±42 vs 108±25 mg/dl; p=0.037). In multiple logistic regression analysis, the following variables were identified as risk factors of early pregnancy loss: T1DM duration (OR 0.9 95% CI 8.7-0.9), early admission on the 1st pregnancy visit (OR 0.98 95% CI 7.0-0.9), higher systolic blood pressure (OR 1.0 95% CI 9.9-1.0) and higher MBG (OR 1.01 95% CI 9.9-1.0).
Conclusion: We identified a number of early pregnancy loss risk factors, such as longer T1DM duration, elevated systolic blood pressure and mean blood glucose level as well as early 1st pregnancy admission. Due to an observational nature of this study, we are not able to distinguish between causative factors and incidental associations.
Disclosure: **K. Cyganek:** None.

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The effects of insulin resistance and insulin secretion at mid-pregnancy on postpartum glucose intolerance in women with gestational diabetes

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Background and aims: Gestational diabetes mellitus (GDM) has implications for postpartum glucose intolerance, as it is characterized by underlying defects in β-cell response due to increased insulin resistance during pregnancy. However, few studies have examined the effects of insulin resistance and insulin secretion on postpartum glucose intolerance in women with GDM. The aim of this study was to evaluate the effects of

insulin resistance and insulin secretion at mid-pregnancy on postpartum glucose intolerance at 6-12 weeks after delivery in women with GDM.
Materials and methods: We enrolled 913 pregnant women diagnosed with GDM from October 2005 to June 2015. Homeostasis model assessment-insulin resistance (HOMA-IR) and homeostasis model assessment β-cell function (HOMA-B) were calculated during 24-32 gestational weeks and 75-g oral glucose tolerance test (OGTT) was performed at 6-12 weeks after delivery. Insulin resistance was defined by more than the median value of HOMA-IR and low insulin secretion was defined by less than the median value of HOMA-B. Patients were classified into four groups: 1) neither low insulin secretion nor insulin resistance, 2) low insulin secretion but no insulin resistance, 3) insulin resistance without low insulin secretion, and 4) both low insulin secretion and insulin resistance. Postpartum glucose intolerance was defined as fasting plasma glucose ≥ 100 mg/dL or 2-h plasma glucose ≥ 140 mg/dL using 75-g OGTT at 6-12 weeks after delivery.
Results: Mean age was 33.3 years and mean pre-pregnancy BMI was 22.3 kg/m^2. The prevalence of postpartum glucose intolerance was 50.8 % (n = 464). Compared to women without insulin resistance, women with insulin resistance had higher postpartum glucose intolerance (60.0% vs. 41.7%, P < 0.001). However, there was no difference between women with and without low insulin secretion. The prevalence of postpartum glucose intolerance was the highest in women with both low insulin secretion and insulin resistance (group 1 = 34.8%, group 2 = 44.4%, group 3 = 56.3%, and group 4 = 68.9%, P for trend < 0.001). Group 3 and group 4 were 2.41 times (95% CI 1.58-3.67) and 4.15 times (95% CI 2.48-6.94) more likely to have postpartum glucose intolerance than group 1.
Conclusion: In women with GDM, insulin resistance at mid-pregnancy is stronger effect on postpartum glucose intolerance than insulin secretion.
Disclosure: **K. Kim:** None.

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Health care providers' perspectives on pregnancy related care for type 1 diabetes women in China: an online questionnaire based study

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Background and aims: Pregnancy related care (PRC) could improve adverse pregnancy outcomes of type 1 diabetes (T1D) women. Little was known on the current status of PRC for T1D women in China. We aimed to investigate health care providers (HCPs)' perspectives on PRC for T1D women in China.
Materials and methods: It was a cross-sectional study by an online questionnaire designed based on results of previously conducted in-depth interview of HCPs. Doctors and nurses involved in PRC for T1D women were included. The questionnaire was released online and advertised via doctor unions in China. The survey ended when estimated sample size was reached. The data collected were analyzed in SPSS v.19.0.
Results: The survey was conducted from Nov 13 to Dec 30, 2016. 991 responses were received (100% response rate) from 31 provinces of China, including 77.3% doctors and 22.7% nurses. Among them, 69.1% were female; 62.7% from endocrinology department and and 9.5% from the department of gynecology and obstetrics; 63.0% from general hospitals and 37% from primary care facilities. 52.9% had over 10 years of working experience. The results could be summarized as below. 1) On the attitude towards T1D with pregnancy, over 90% HCPs supported T1D women should have to right to get pregnant if well planned and could perceive T1D women's worries on it. 2) On the knowledge of PRC for T1D women, 63.8% reported to have some knowledge of PRC, but 62.5% reported to treat only a small number of T1D women annually. Only 16.6% HCPs thought they provided proper PRC. 3) On the content of PRC, over 90% suggested PRC include diet, exercise, adjustment of insulin regimen, glucose monitoring and T1D examinations (such as diabetic complication monitoring). 4) On the attitude towards social-psychological support, 80.6% considered it important, but only 48.4% thought they had the skill. 5) On the implementation of PRC, 82.0% thought PRC should be done by endocrinologists with the aid of department of gynecology/obstetrics and other related departments. 6) On the sufficiency of communication between T1D women and HCPs regarding PRC, in practice 51.4% considered the HCP-patient communication for PRC was very inadequate. 56.6% reported less than 10 minutes per visit, while 58.2% thought at least 20 minutes was required for sufficient communication. 7) On future perspective and suggestions on PRC for T1D women, investigated HCPs thought it the principal to train related HCPs on knowledge and social-psychological support skills (64.2%) of PRC for T1D women.
Conclusion: Although HCPs in China were aware of the importance of PRC for T1D women and could perceive the patients' worries, the current situation was dissatisfactory, largely due to inadequate experience, knowledge and limited communication time. It is necessary to accelerate and reinforce the training and appeal for more social forces to improve PRC for T1D women.
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Disclosure: **S. Luo:** None.

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Macrosomia in pregestational diabetes: associated factors

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Background and aims: Foetal macrosomia is one of the most frequent complications of diabetes in pregnancy, occurring in 20-50% of pregnancies, and is associated with increased risk of obstetric and perinatal complications. The aim of this study was to assess the factors associated with macrosomia in women with pregestational diabetes.
Materials and methods: The clinical records of women with pregestational diabetes, followed during pregnancy at a reference centre and with delivery between January 2011 and December 2016, were reviewed. Only singleton pregnancies and term (>37 weeks' gestation) deliveries were included. Macrosomia was defined as birth-weight of 4000g or more. Group comparison and logistic regression were performed to find factors associated with macrosomia. A two-tailed p<0.05 was considered significant.
Results: A total of 302 women fulfilled the study criteria. Their age was 33 (18-45) years, baseline BMI 28.6 (17.3-60.1) Kg/m2, 45.8% were nulliparous and 46.7% had type 1 and 52% class B (White) diabetes. Their HbA1c was 6.6 (4.6-11), 5.85 (4.3-9.4) and 6.0 (4.5-9.5)% in the 1st, 2nd and 3rd trimesters, respectively. Of the newborns, delivered at 39 (37-42) weeks' gestation, 91 (30.1%) had macrosomia. Third trimester HbA1c [6.3 (4.8-9.5) vs 5.95 (4.5-8.9)% p=0.006] and maternal weight at baseline [78.4 (44-150) vs 73 (41-126) Kg, p=0.044] and in the third trimester [91 (66.5-155) vs 84 (51-134) Kg, p=0.002] were higher and weight gain during pregnancy tended to be higher (13.2 (6.5) vs 11.6 (6.4) Kg, p=0.065] in the group with macrosomia. There were no significant differences between groups in type of diabetes, parity, gestational age, preconceptional care or earlier HbA1c. In multivariate, logistic regression, only maternal, third trimester weight and HbA1c remained significantly associated with macrosomia.

Conclusion: The frequency of macrosomia in singleton, term deliveries of women with pregestational diabetes is 30% and is associated with maternal third trimester HbA1c and weight.
Disclosure: **A. González Lleó:** None.

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Weight gain during pregnancy in women with diabetes: the pattern differs by diabetes type

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Background and aims: Extremes of weight gain during pregnancy (WG) are associated with adverse pregnancy outcomes. Institute of Medicine (IOM) recommendations differ by prepregnancy body mass index (BMI). We aimed to analyze WG according to IOM in women with gestational (GDM), type 2 (T2DM), and type 1 diabetes mellitus (T1DM).
Materials and methods: Subjects: Three cohorts of women with diabetes and singleton pregnancies (2770 GDM; 100 T2DM and 469 T1DM). Maternal characteristics analyzed: Prepregnancy age, weight, height, BMI, insulin treatment during pregnancy and WG according to IOM. Statistics: Descriptive statistics; Chi-square to compare WG categories.
Results: Maternal characteristics in women with GDM, T2DM and T1DM were: GDM: age 33 years, height 160 cm, weight 60 kg, BMI 23.4 kg/m2, insulin treatment 47.4%; T2DM: age 34 years, height 159 cm, weight 70 kg, BMI 28.0 kg/m2, insulin treatment 100%; T1DM: age 30 years, height 161 cm, weight 60 kg, BMI 23.0 kg/m2, insulin treatment 100%. WG according to IOM in the three cohorts were: GDM: 52.5% insufficient, 31.4% adequate, 16.1% excessive ($p < 0.001$ *vs* recommendations), T2DM and T1DM: T2DM: 29.8% insufficient, 25.5% adequate, 44.7% excessive ($p < 0.001$ *vs* recommendations and GDM, $p < 0.01$ *vs* T1DM); T1DM: 16.1% insufficient, 35.3% adequate, 48.6% excessive ($p < 0.001$ *vs* recommendations and GDM, $p < 0.01$ *vs* T2DM)
Conclusion: WG distribution in the three cohorts differs markedly vs IOM recommendations and between them. We propose that differences are driven by diet restriction in GDM and by insulin treatment in women with T2DM and T1DM. The knowledge of the current patterns could be of help in clinical practice.
Disclosure: **R. Corcoy Pla:** None.

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Birth weight of infants of type 1 diabetic and control women in the Tauffer Obstetric Database between 1996 and 2011

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Background and aims: Parallel to the epidemic spread of obesity a gradual increase in birth weight has been observed in the general population in recent decades. This increase has flattened out according to data from high income countries in the last 4-5 years. There is a lack of information however whether similar changes has occurred in the newborns of type 1 diabetic (T1DM) women. The present study investigated changes in infant birth weight in Hungary stratified by the mothers T1DM status based on the Tauffer Obstetric Database between 1996 and 2011.
Materials and methods: Our analysis was based on data from the compulsory Tauffer Obstetric Database (collected by the National Institute of Obstetrics and Gynecology) between 1996 and 2011. After excluding twin pregnancies and premature or postmature deliveries (<37 or >41 weeks of gestation), we analyzed changes in birth weight of n=1,263,919 control and n=1,152 T1DM newborns separately for males and females using multiple linear regression adjusted for maternal age and gestational week at delivery.
Results: T1DM women were older (29.4±5.3 vs 27.5±5.4 years), had shorter gestation at delivery (38.4±1.0 vs. 39.2±1.1 weeks) and had heavier newborns adjusted for gestational age at delivery (76.0±26.3 vs. 62.2±28.0 percentile, all $p<0.05$). Based on multivariably adjusted models, birth weight of both male and female control infants increased following a negative quadratic curve (female/male) from 3255 [SE 2]/3395 [2] g to 3279 [2]/3420 [2]g (p[quadratic term]<0,0001, increase in 1996 8.0 [0.5]/6.5 [0.5], in 2011 -4.8 [0.5]/-3.0 [0.5]g). Temporal changes of birth weight of female infants of T1DM mothers was similar to controls however babies of T1DM mothers were 299 [99]g heavier than controls throughout follow-up. Male infants of T1DM mothers were heavier than controls (weight difference in 2003 265 [18] g), moreover the weight difference increased throughout follow up (10.8 [3.8]g/year). Furthermore the increase of the birth weight of male infants has not yet finished at the end of follow up.
Conclusion: According to our descriptive epidemiological analysis of the Tauffer Obstetric Database, neonatal birth weights have changed similarly in Hungary and other high income countries. Temporal changes in birth weight of female infants of T1DM mothers was similar to controls, however the increase in birth weight of male infants has not finished during follow-up. The differing trajectories of male and female birth weights requires further investigations both in more recent data of the Tauffer database and prospective collection of potential correlates of birth weight (maternal weight, maternal insulin doses, gestational complications).
Disclosure: **A.G. Tabák:** None.

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Perinatal outcomes of pregnencies of type 1 diabetic women in Hungary: an analysis of the Hungarian Tauffer Database 1996-2011

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Background and aims: During the last decades the outcomes of pregnancies complicated by type 1 diabetes has improved however most studies still report worse outcomes compared to the general population. As there is no report on the outcomes of pregnancies complicated by type 1 diabetes in Hungary, we aimed to assess outcomes of pregnancies complicated by type 1 diabetes mellitus compared to a control population using data from the Hungarian Tauffer Obstertric Database.
Materials and methods: The Tauffer Database is a compulsory report of each delivery()24-43 weeks of gestation) in Hungary that has records on n=1,401,184 deliveries between 1996 and 2011. Fort the present analysis we excluded multiple pregnancies (n=22,291). Type 1 diabetes was defined by ICD-10 codes (n=1,485). Multiple logistic regression with pregnancy outcomes (stillbirth, perinatal mortality, neonatal mortality, congenital malformations, oligohydramonion, polyhydramnion, small for gestational age (SGA), large for GA (LGA), caesarian section, perinatal intensive centre treatment, 5-minute Apgar <7) as outcomes with type 1 diabetes as a predictor and adjusted for maternal and gestational age at delivery, and further adjusted for obstetrical and medical history were investigated.
Results: Type 1 diabetic mothers were older (29.5[SD 5.3] vs. 27.5[5.5] years), had a lower gestational age at delivery (37.5[2.1] vs. 38.8[2.0] weeks), larger newborn (3387[692] vs. 3275[573] g), and more

frequently had a pregnancy complicated by hypertension (3.4 vs. 0.6%, all p<0.0001). In multiple adjusted models, type 1 diabetic mothers had an extremely increased risk of polyhydramnion (OR 19.0, 95%CI 10.6-33.9), while there was a 2 to 4 fold increased risk of all other outcomes except for SGA (OR 0.45, 95%CI 0.28-0.74) and neonatal mortality (OR 1.08, 95%CI 0.44-2.66).

Conclusion: We found significantly increased risk of several pathological pregnancy outcomes that were robust to multivariate adjustment. However neonatal mortality was similar between pregnancies complicated by type 1 diabetes and controls.

Disclosure: **B. Domján:** None.

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Implementing a reminder system to stimulate postpartum screening for glucose intolerance in women with gestational diabetes: the 'Sweet Pregnancy' project

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Background and aims: Women with a history of gestational diabetes (GDM) are at increased risk to develop type 2 diabetes (T2DM). Timely detection of glucose intolerance postpartum is important since progression to T2DM can be prevented with 50% by lifestyle intervention. However, postpartum testing rates in routine clinical practice are often very low. We aimed therefore to evaluate the feasibility and efficacy of a GDM recall register on the long-term screening uptake postpartum and to evaluate the prevalence of (pre)diabetes postpartum.

Materials and methods: Evaluation of the registration and responses of women with a history of GDM in a recall register implemented in 66 obstetrical centers in the Northern part of Belgium from 2009-2016. Registrants receive yearly reminders (postal, email, SMS and/or telephone) to have a fasting plasma glucose (FPG) test in primary care to timely detect (pre)diabetes. The cumulative risk of diabetes was estimated using the Kaplan-Meier method, whereas for prediabetes, the estimation was based on the cumulative incidence function.

Results: Over a 7 year period, 7269 women have registered in the GDM recall register. Of all registrants, 84.4% (5465) responded to the letter sent three months after the delivery and 58.8% (3215) of responders indicated that they had received a screening test to detect glucose intolerance in early postpartum. The yearly response rates varied from 74.4% after the first year to 61.8% after the fifth year and the number of women who reported a screeningtest varied from 67.4% after the first year to 71.9% after the fifth year. Of all women who received a yearly follow-up letter (1157) and were 5 year in follow-up in the register, 75.0% (868) received at least once a screening test, 60.6% (701) received at least twice a screening test, 46.6% (539) received at least three times a screening test, 34.0% (393) received at least four times a screening test and 18.3% (212) of women received yearly a screening test over the 5 year period. Compared to women who responded at least once to a reminder, women who never responded were more often < 30 years (41.4% vs. 33.9%, p <0.001) and were more often obese (29.3% vs. 20.8%, p=<0.001). Over a period of 6 years, 27.4% (CI 23.9%-31.0%) developed impaired fasting glycaemia and 7.3% (CI 6.0%-8.4%) developed diabetes. Independent predictors for diabetes were age and BMI and for prediabetes independent predictors were age, BMI and waist circumference.

Conclusion: We show now the long-term feasibility and efficacy of a GDM recall register to stimulate screening postpartum. However, it remains challenging to stimulate women to get a yearly screening test since only 18.3% of women with 5 years of follow-up in the register, reported a screening test every year. On third of women developed (pre)diabetes within 6 years.

Disclosure: **K. Benhalima:** None.

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Adherence to a follow-up programme after gestational diabetes in an Italian population

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Background and aims: Women with Gestational Diabetes Mellitus (GDM) are at high risk of developing type 2 diabetes mellitus, metabolic syndrome and cardiovascular diseases. The aim was to identify the factors that characterize the adherence to postpartum follow-up of patients with previous GDM.

Materials and methods: Data on 1573 women with previous GDM were retrospectively collected. In pregnancy: age, family history of diabetes, ethnicity, prepregnancy BMI, weight gain, gestational week at diagnosis of GDM, timing and mode of delivery, metabolic parameters (fasting plasma glucose, glycated heaemoglobin, total cholesterol, HDL, LDL, triglycerides). After delivery: glucose and insulin values at the OGTT 75g performed after at least 6 weeks from delivery, total cholesterol, HDL, LDL, triglycerides and postpartum weight. Based on OGTT results, women were categorized into two groups: NGT (Normal Glucose Tolerance) and AGT (Altered Glucose Tolerance). Furthermore, factors that could encourage women to undergo postpartum glucose testing were evaluated.

Results: 889 (56,52%) women underwent follow-up after delivery: they had higher frequencies of maternal age > 35 years (44,8 vs 40,8% p 0,000), first degree family history of diabetes mellitus (46 vs 36 % p 0,000), insulin treatment during pregnancy (29,3 vs 18,1% p 0,000) than non-screened women. The adherence to follow-up was higher among Italians than migrants (59,4 vs 51,4 % p < 0,05): by differentiating in more specific ethnic groups, the frequency of follow-up was the highest in Italians and in women from Subsaharian Africa (59%) and the lowest in Asians and Arabs (57% and 55% respectively). At the follow-up, 741 (83,35%) were NGT, 148 (16,65%) were AGT. During pregnancy, women with AGT at postpartum screening had a higher prepregnancy BMI (25,6 ± 5,2 vs 24,5 ± 4,9 kg/m^2 p 0,016), received an earlier dignosis of GDM (22,5 ± 5,5 vs 24 ± 4,3gw p 0,001) and underwent the postpartum follow-up later (10,9 ± 4,5 vs 9,7 ± 3,6 weeks from delivery p 0,004) than women with NGT. Among women with AGT, there were more migrants (45,27 vs 29,96%, p 0,000), more first degree family history of diabetes (54,7 vs 44,3 % p 0,018) and a higher need for insulin therapy(43,2 vs 26,5%, p 0,000)than in women with NGT. Women with postpartum AGT had significantly higher values of first fasting glycaemia (92,1 ± 12,7 vs 86,7 ± 10,8 mg/dl p 0,000), HbA1c at the diagnosis of GDM (5,5 ± 0,5 vs 5,2± 0,4 % p0,000), HbA1c at the third trimester (5,5 ± 0,5 vs 5,3 ± 0,4 % p0,000), 2h-OGTT value(159,9 ± 27,7 vs 147,5 ± 30,4 mg/dl p0,000) and AUC_{glu} (18382,3 ± 2523,5 vs 17377,3 ± 2591,1 mg/ml x min p 0,000) at the diagnostic OGTT than women with NGT. After pregnancy, women with postpartum AGT had significantly higher values of all glycaemic points measured at postpartum OGTT 75g (in particular, 1h-OGTT value: 170,8 ± 45,9 vs 122,9 ± 31,4 mg/dl, p 0,000), of insulin at 1h-(71,1 ± 53,4 vs 57,2 ± 36,4 mU/ml p 0,022) and 2h-(85,8 ± 85,5 vs 41,4 ± 27,8 mU/ml p 0,000) during OGTT, of postpartum triglycerides(104,5 ± 67,7vs 83,1 ± 47,9 mg/dl p 0,001) and lower values of HDL (60,9 ± 18,8vs 66,02 ± 16,34 mg/dl) than women with NGT. Finally, women with NGT were more likely to breast-feed than women with AGT (45,6 vs 34,5 % p 0,009).

Conclusion: Factors that characterize the adherence of postpartum follow-up in patients with previous GDM are age, family history of diabetes, insulin therapy in pregnancy and ethnicity, but the rates of screening are still too low. Need more educational programs to increase the awareness of the risk in GDM women.

Disclosure: **M. Dalfrà:** None.

PS 080 Diagnosis and treatment of neuropathy

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The relationship between sudomotor function and insulin resistance in adults with type 1 diabetes

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Background and aims: The occurrence of insulin resistance in type 1 diabetic patients is one of the risk factors of chronic complications of diabetes. Dysfunction of small fibers is an early clinical manifestation of diabetic microangiopathy. SUDOSCAN+ is a device used to noninvasively assess the function of the sweat glands, which are innervated by C-fibers, on the basis of electrochemical skin conductance (ESC). The aim of the study was to evaluate the association between sudomotor function and insulin resistance in adult patients with type 1 diabetes (DM1).
Materials and methods: The study included 494 adult patients with DM1 (253 men), aged 41 (IQR: 33-52) years, with disease duration of 24 (IQR: 19-32) years and HbA1c level of 7.9 (IQR: 7.1-8.9) %. The sudomotor function was evaluated using SUDOSCAN device on the basis of the electrochemical reaction of chlorine ions secreted in sweat due to low voltage current. Insulin resistance was assessed by calculating the estimated glucose disposal rate (eGDR) according to the formula based on HbA1c, WHR and presence of hypertension. The study group was subdivided into 3 groups based on quartiles of eGDR (<5.5, 5.5-9.5, >9.5 mg/kg/min).
Results: Patients with lower eGDR (higher insulin resistance) had lower Feet ESC [71 (IQR:50-81) vs 79 (IQR:63-85) vs 83 (IQR:74-86) μS; p<0.0001 and Hands ESC (58 (IQR:40-70) vs 61 (IQR:50-72) vs 69 (IQR:60-77) μS; p<0.001)]. We found positive correlation between Feet and Hands ESC and eGDR (Rs=0.28, p<0.001 and Rs=0.21, p<0.001 respectively). In a multiple linear regression model which takes into account age, sex, duration of diabetes, Feet ESC was independently associated with eGDR (β=0.14, p=0.001).
Conclusion: In adult patients with DM1 the higher insulin resistance the greater sudomotor dysfunction is.
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Neuroretinal dysfunction is associated with early peripheral motor unit loss in type 1 diabetes

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Background and aims: It is already accepted that early retinal neurodegeneration has a predictive role in the development of clinically detectable microvascular alterations in diabetic retinopathy (DR). However, no data are available about the relationship between retinal neurodegeneration and diabetic peripheral neuropathy. The aim of the study was, therefore, to investigate the hypothesis that retinal neurodegeneration could be considered as a marker of early diabetic neuropathy.
Materials and methods: 19 subjects affected by Type 1 Diabetes Mellitus (T1DM) with no symptoms/signs of peripheral polyneuropathy, without DR or with very mild non-proliferative DR, and 14 healthy controls (C) matched for age and gender were enrolled. All subjects underwent the following electrophysiological tests: standard nerve conduction studies (NCS), incremental motor unit number estimation (MUNE) from abductor hallucis (AH) and abductor digiti minimi (ADM) with assessment of AH and ADM average single motor unit potential (SMUP) size. The retinal functional analysis was performed by multifocal electroretinogram (mfERG) measuring amplitude density (Amp) and implicit time (IT), for 3 concentric annular rings (Ring 1, R1= 0-2.5°; R2= 2.5-5°; R3= 5-10° degrees of foveal eccentricity), of nasal (N)/ temporal (T)/superior (S)/ inferior (I) macular quadrants.
Results: Amp was significantly decreased in T1DM vs. C (p<0.05), in each ring and quadrant. ADM MUNE and AH MUNE were significantly decreased in T1DM (p 0.039; p <0.0001, respectively), and AH-SMUP significantly increased (p 0.002), vs. C. No abnormalities of NCS were found in any subject. A positive correlation between Amp in N and I quadrant and AH MUNE (Pearson correlations: r^2 0.368, p 0.01; r^2 0.288, p 0.03, respectively) was observed in T1DM subjects.
Conclusion: Retinal neurodegeneration is already present in T1DM patients without peripheral neuropathy. MUNE proved to be a reliable instrument to detect precocious and subclinical peripheral neuropathic damage, representing by the motor unit loss and collateral re-innervation process. The observed relationship between neuroretinal dysfunction and early peripheral motor unit decline supports the hypothesis that neuroretina can be considered as a potential "window" to track the early neuropathic process in diabetes.
Disclosure: **D. Ylli:** None.

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Dorsolateral prefrontal cortex activity towards fatigue of type 2 diabetes patients with macro-angiopathy and peripheral neuropathy (pilot study)

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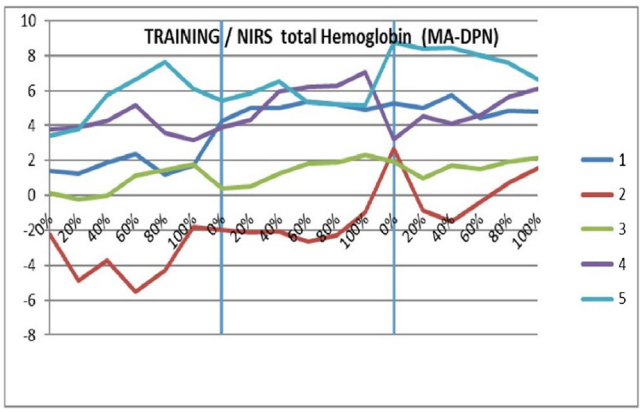
Background and aims: Patients with chronic type 2 Diabetes Mellitus (T2DM) show a decreased neuromuscular activation and increased fatigue depicted by a raised postural central of pressure (COP) kinetics during standing tasks. The dorsolateral prefrontal cortex (DLPFC) is responsible for many cognitive and motor functions and is believed to be involved in the overriding mechanism of fatigue. It is unclear, however, if there are differences in COP and DLPFC activity between T2DM patients who present macro-angiopathy (MA) without diabetic peripheral neuropathy (DPN) and those with both MA and DPN. The aim of the present study is to determine whether differences in COP kinetics between patients with MA alone and both MA-DPN exist and if these differences correlate with DLPFC activity.
Materials and methods: 15 participants, both males and females, (mean age 66±5,7), divided in 3 groups of 5 [5 healthy controls (HC group), 5 T2DM patients with MA alone (MA group) and 5 T2DM patients with both MA and DPN (MA-DPN group)] were included in the study. They performed 3x2 minutes trials of COP Antero/Posterior (A/P) displacements, with a visual feedback, controlled by a metronome in 20bits/min, moving their weight between 2 points, on the top and the bottom of a screen. Pre-Post Data collection: Near Infrared spectroscopy for DLPFC, Tibialis Anterior isometric strength, COP sway velocity, COP area (mm^2) and COP A/P amplitude (mm) were also measured.
Results: Repeated measures and matched pair T-test analysis was performed. During every 2' trial, the COP Sway area and the avegCOP A/P

displacements amplitude decreased, in both MA group and MA-DPN group, but not in HC group. Moreover, after each 2' trial the average COP sway velocity increased only in MA-DPN group and not in the other 2 groups. The Maximal Voluntary Isometric Contraction of the Tibialis Anterior decreased in MA group and MA-DPN group, but not in HC group, between baseline and immediately after the last bout. Finally, there was an increase trend in total Hemoglobin (tHB, blood volume) at the DLPFC, in MA-DPN group, but not in the other 2 groups, during and at the end of the trials.
Conclusion: T2DM patients with MA alone and both MA and DPN demonstrated decreased mobility during the exercise, but only the MA and DPN patients demonstrated increased DLPFC activity. This can be probably explained by the greater cognitive dependence showed by the MA-DPN group, in comparison with the other two groups, in order to anticipate the peripheral fatigue and follow the task.



Disclosure: **K. Kotsa:** None.

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Enhanced subclinical inflammation in painful compared to painless diabetic polyneuropathy. A multimarker approach

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Background and aims: The determinants and mechanisms contributing to the phenotype of diabetic sensorimotor polyneuropathy as a painful (DSPN+p) or painless (DSPN-p) entity remain unclear. Since inflammation has been implicated in the pathogenesis of neuropathic pain, we hypothesized that pro- and/or anti-inflammatory processes could be more relevant in DSPN+p than DSPN-p.
Materials and methods: Using a new multimarker assay (Proseek Multiplex INF I assay, OLINK Proteomics), we measured 92 serum biomarkers including pro- and anti-inflammatory cytokines and chemokines as well as vascular and growth factors in 193 patients with DSPN+p and 171 with DSPN-p from the PROPANE study (DSPN+p/DSPN-p [mean±SD]: age: 65.7±9.9/68.0±10.3 years, male: 68/82%, BMI: 31.3±5.5/29.3±5.1 kg/m²; type 2: 86/83%, diabetes duration: 16.1±12.1/16.1±12.6 years, HbA1c: 7.6±1.4/7.3±1.2%). DSPN was diagnosed using modified Toronto Consensus (2011) criteria, while DSPN+p and DSPN-p were stratified using a cutpoint of 4 points on the Likert scale for chronic pain lasting >1 year in the distal lower limbs.
Results: After adjustment for sex, age, BMI, smoking, diabetes type, diabetes duration, and HbA1c, compared to patients with DSPN-p those with DSPN+p showed increased levels (normalized protein expression values) of the pro-inflammatory markers CUB domain-containing protein 1 (CDCP1: 3.29±0.64 vs 3.16±0.58), signaling lymphocytic activation molecule 1 (SLAMF1: 2.57±0.58 vs 2.71±0.57), and chemokine (C-C motif) ligand 20 (CCL20: 5.40±1.07 vs 5.74±1.31) as well as the anti-inflammatory markers interleukin 10 (IL-10: 2.37±0.43 vs 2.53±0.64) and osteoprotegerin (OPG: 10.23±0.39 vs 10.13±0.38) (all P<0.05). In patients with DSPN+p, several inflammatory markers correlated with diminished quantitative sensory tests (e.g. CDCP1 with cold thermal detection threshold on the hand: ß=-0.241; P=0.002) and nerve conduction velocity (NCV) (e.g. SLAMF1 with peroneal motor NCV: ß=-0.262; P=0.0005), whereas in those with DSPN-p these associations were either absent or markedly weaker.
Conclusion: Patients with painful DSPN show higher systemic levels of pro- and anti-inflammatory mediators than those with painless DSPN, pointing to a role of inflammatory processes in painful diabetic neuropathy.
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Disclosure: **D. Ziegler:** None.

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Effects of epalrestat therapy for diabetic neuropathy evaluated using pain threshold

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Background and aims: The aldose reductase inhibitor, epalrestat, was approved in Japan after a three-month double-blind trial showed that it improved symptoms and nerve function. Epalrestat effects were more obvious in patients with early neuropathy and modestly elevated levels of glycated hemoglobin. However, the effects of epalrestat in individual patients are still difficult to assess. In annual neuropathy checkups, we found that epalrestat lowered the pain threshold in some patients. Therefore the present study used intraepidermal electrical stimulation (IES) to investigate variation in pain threshold in patients with type 2 diabetes treated with epalrestat.
Materials and methods: This retrospective study investigated pain thresholds in patients selected from among 647 patients (mean age, 61.2 ± 12.2 years; male, n = 428) with type 2 diabetes who were administered epalrestat and who had undergone three consecutive annual neuropathy examinations at Takahashi Family Clinic. We also compared pain thresholds in seven symptomatic patients (mean age, 71.6 ± 5.7 years; male, n = 6) with type 2 diabetes before and three months after starting epalrestat therapy. We determined the pain threshold by stimulating the dorsal skin of the feet using IES electrodes at an initial intensity that was sufficient for patients to feel a pricking sensation, and then reduced the current in 0.05-mA increments until sensation was indiscernible.
Results: The mean pain thresholds in each year were 0.21 ± 0.17, 0.19 ± 0.18 and 0.16 ± 0.13 mA ($F = 3.0$, $p < 0.01$). The mean pain threshold of eleven patients before taking epalrestat therapy were 0.25 ± 0.17 mA and 0.13 ± 0.05 mA ($p < 0.05$), respectively. Three months after taking epalrestat, the mean pain thresholds in these patients were lowered from 0.55 ± 0.34 mA to 0.14±0.03 mA, respectively ($p < 0.05$). Furthermore, symptoms related peripheral neuropathy improved.
Conclusion: The present findings suggest that measuring pain threshold is useful for assessing the effects of epalrestat in patients with neuropathy caused by type 2 diabetes.
Disclosure: **N. Takahashi:** None.

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Prevalence of diabetic neuropathy in adolescents with type 1 diabetes and the association to insulin pump therapy

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Background and aims: Two of the most prevalent subgroups of diabetic neuropathy are diabetic peripheral neuropathy (DPN) and cardiovascular autonomic neuropathy (CAN), but data on the prevalence of theses

complications in adolescents with type 1 diabetes are scarce. Since early stages of CAN and DPN may be reversible, screening adolescents with type 1 diabetes may be important. Furthermore, it is unknown whether insulin pump reduces development of diabetic neuropathy. The aim of this study was to investigate the prevalence of DPN and CAN in a Danish population of adolescent patients with type 1 diabetes and to assess the association between treatment with insulin pump and these complications.
Materials and methods: Adolescents with type 1 diabetes underwent examination of CAN and DPN. CAN was assessed by the cardiovascular autonomic reflex tests (CARTs). The diagnosis 'early CAN' was given if one out of the three CARTs was abnormal and 'definite CAN' if two or three out of the three tests were abnormal. DPN was assessed by light pressure perception, pain perception, vibration perception threshold (VPT), Brief Pain Inventory and Michigan Neuropathy Screening Instrument questionnaire, electrochemical skin conductance (ESC), sural nerve conduction velocity (SNCV) and sural nerve action potential (SNAP). Distribution of outcome measures is presented in table 1. The associations between insulin pump therapy and the various measures of CAN and DPN were analysed in logistic and linear regression models.
Results: The 156 adolescents (42% were males) with a mean age of 22 years (SD 1.6), a mean diabetes duration of 11.3 years (SD 5.1) and a median HbA_{1c} of 66.5 mmol/mol (IQR 58;77) (8.3% (IQR 7.4;9.2)) had a prevalence of CAN and early CAN of 9% and 26.1%, respectively. The composite prevalence of DPN was 55.8% encompassing the prevalence of abnormal SNAP of 23.8%, SNCV of 37.1%, ESC on the hands and feet of 4% and 8%, respectively, VPT of 1.3% and BPI questionnaire of 1.9%. No statistically significant association was found between insulin pump therapy and the various measures of DPN and CAN.
Conclusion: DPN and CAN are prevalent in adolescents with type 1 diabetes with no association found with insulin pump treatment. The use of novel measuring modalities in the present study identified a higher number of subjects with DPN compared to established measures. Screening for diabetic neuropathy in adolescents may be beneficial in order to detect and prevent nerve damages at early stages.

Table 1 Distribution of outcome measures and prevalences of abnormal results in a population of adolescents with type 1 diabetes.

Neuropathy outcome measure	n	Mean/median (SD)/[IQR]	Prevalence n (%)
CAN	154	NA	14 (9)
Early CAN	154	NA	40 (26.1)
Symmetric peripheral neuropathy	156	NA	87 (55.8)
Monofilament (≥ 1 missing response)	156	NA	0
Pin prick (≥ 1 missing response)	156	NA	0
VPT (V)	156	4.5 [3.5;5.6]	2 (1.3)
DPN check, symmetric abnormalities	152	NA	80 (53)
SNAP (μV)	152	10.7 [8.3;14.6]	36 (23.8)
SNCV (m/s)	152	50.5 (4.7)	56 (37.1)
ESC - hands (μS)	156	77.5 [69.9;83.5]	6 (3.8)
ESC - feet (μS)	155	82.5 [78.5;86]	12 (7.7)

Data are given in means (SD), medians [IQR] or proportions. *NA:* Not applicable *CAN:* Cardiovascular autonomic neuropathy, *VPT:* vibration perception threshold, *SNAP:* sural nerve action potential, *SNCV:* sural nerve conduction velocity, *ESC:* Electrochemical skin conduction

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Disclosure: **M.M.B. Christensen:** None.

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Simultaneous pancreas kidney transplantation in type 1 diabetes is associated with an early improvement in small fibres

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Background and aims: Simultaneous pancreas kidney transplantation (SPK) can normalise glucose levels and improve the lipid profile and blood pressure in patients with type 1 diabetes (T1DM). The effect on microvacsular complications, in particular neuropathy needs to be defined further. The aim is to determine the effect of SPK on neuropathy in patients with T1DM.
Materials and methods: A detailed assessment of neuropathy was undertaken at baseline, 6 months and annually over 3 years following SPK in 36 patients with T1DM.
Results: HbA1c improved significantly (69·1±16·9 v 39.0±3.0, p<0.0001). Corneal confocal microscopy (CCM) parameters (corneal nerve fibre density (CNFD) (9.4±1.0 v 12.2±1.7, p=0.005), corneal nerve branch density (CNBD) (9.8±0.05 v 13.2±2.7, p=0.05), corneal nerve fibre length (CNFL) (7.2±0.5 v 8.2±0.8, p=0.05)) and mean dendrite length (MDL) (10.9±0.8 v 15.6±1.4, p=0.02) in skin biopsies significantly improved at 12 months. Intra-epidermal nerve fibre density did not improve as well as no early change in neuropathic symptoms, quantitative sensory testing or neurophysiology. However, at 36 months there was a significant improvement in the neuropathy symptom profile (5.3±0.9 v 3.1±1.2, p=0.04) and peroneal nerve conduction velocity (31.1±1.8 v 38.7±2.7, p=0.05) as well as a further improvement in CNFD (9.4±1.0 v 14.4±1.6, p=0.01), CNFL (7.2±0.5 v 10.3±0.6, p=0.001) and MDL (10.9±0.8 v 18.0±1.3, p=0.02).
Conclusion: CCM and skin biopsy demonstrates an improvement in small fibres within 12 months of SPK, followed by an improvement in neuropathic symptoms and neurophysiology at 36 months. These data provide further support for the use of CCM as an early surrogate endpoint in clinical trials of diabetic neuropathy.
Disclosure: **S. Azmi:** None.

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Oral alpha lipoic acid adjunctive treatment for symptomatic diabetic peripheral neuropathy: a prospective, double blind, placebo controlled study

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Background and aims: Differential efficacy of alpha-lipoic acid treatment on different sensory modalities had been reported. These differences could be attributed to the heterogeneous nature of neuropathy, study designs, routes and duration of administration of alpha lipoic acid (ALA). The aim of this work was to assess the efficacy of oral ALA 600 mg twice daily for 6 months in the treatment of patients with type 2 diabetes and peripheral neuropathy. The primary outcome was to the study the effect of the intervention on pain perception evaluated by neuropathy symptom score (NSS) and visual analogue pain scale (VAS). The secondary outcome was to study the effect of the intervention on vibration perception threshold (VPT) and neuropathy disability score (NDS).
Materials and methods: A total of 200 patients with diabetes and symptomatic peripheral neuropathy were randomly assigned into add on treatment with either ALA (n = 100) or placebo (n = 100) (Batch 1 or Batch 2) for 6 months. Batches were unknown for patients and investigators. Patients followed their usual treatment regimens during the conduct of the study. VPT was quantified with a biothesiometer (Arnold Horwell, UK). NSS, VAS, NDS

and VPT were scored at baseline and at each visit (1, 3 and 6 months).

Results: At randomization, no significant differences between the ALA and placebo groups were noted for age (54.1± 8.2 vs 52.7 ±7.1 years), sex (M/F 38/62 vs 40/60), BMI (33.7± 4.7 vs 32.2±5.7), diabetes duration (11.1± 6.1 vs 11.3±5.8 years), HbA1c (8.3± 1.2 vs 8.1±1.6), VAS (5.7 ±2.5 vs 4.88±2.1) and NSS (7.7±1.2 vs 7.47±1). Significant reduction in the percent change between baseline and Visit 4 (Month 6) was found in NSS (60.9±32.9% for ALA vs 23.2±14.1% for placebo, p <0.001). Whereas, non-significant difference in the percent change of VAS at visit 4 was found (8.4±108.7% for ALA vs 6±103.3% for placebo, p = 0.879). Significant reductions in NDS were noted in the ALA group in comparison to the placebo group after 1month (6.2±2.8 vs 8±2.1, p <0.001), 3 months (3.9±2.4 vs 6.5±1.2 P <0.001), and 6 months (3.5±2.4 vs 6.7±1.7 P <0.001). Thermal perception was the most significantly improved parameter in ALA group in comparison to the placebo group (p < 0.001). Significant differences in VPT favoring ALA over placebo were observed after 1 month (17±7.7 vs 19.83±6.9 volts, p 0.008), 3 months (14.1±6.4 vs 18.92±7.9 volts, p< 0.001) and 6 months (12.6±5.3 vs 16.95±9.7 volts, p < 0.001).

Conclusion: Improvement in patient reported pain as assessed by NSS were observed only after 6 months of oral ALA treatment. However, the reduction of pain was not evident when VAS was used for the assessment. Improvements in NDS and VPT were observed after 1month of ALA treatment and the difference remain significant thereafter. Thermal perception was the most significantly improved parameter. We suggest that various assessment scales should be considered in the interpretation of data.

Clinical Trial Registration Number: RP/52

Supported by: alpha lipoic acid and placebo are supplied by Eva Pharma

Disclosure: **M.R.R. EL-Nahas:** Non-financial support; Alpha Lipoic acid and Placebo supplies.

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Bariatric surgery improves neuropathic symptoms, deficits and corneal nerve morphology in obese patients with diabetes

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Background and aims: Whilst bariatric surgery can lead to remission of type 2 diabetes mellitus, the effect on microvascular complications, in particular neuropathy is unclear. The aim is to determine the effect of bariatric surgery on neuropathy in obese patients.

Materials and methods: 25 morbidly obese patients with diabetes underwent a comprehensive assessment of neuropathy at baseline, 6 and 12 months after bariatric surgery.

Results: There was a reduction in BMI (49.6±1.8 v 37.5±1.7 v 34.4±1.2, P<0.0001), HbA1c (57.3±3.4 v 41.2±2.9 v 38.4±1.9, P<0.0001), systolic (135.7±3.3 v 121.9±3.8 v 118.7±3.5, P=0.002) and diastolic (73.6±3.6 v 66.6±2.9 v 69.0±2.6, P=0.008) blood pressure, 6 and 12 months after bariatric surgery. There was a significant and progressive improvement in neuropathy symptom profile (4.6±0.9 v 2.7±1.1 v 0.7±0.3, P=0.001), neuropathy disability score (2.1±0.4 v 1.4±0.6 P=0.02), corneal nerve fibre density (24.2±1.4 v 25.5±2.3 v 28.1±1.3, P=0.019), corneal nerve branch density (34.1±3.5 v 39.5±4.7 v 42.3±3.7, P=0.048) and corneal nerve fibre length (14.9±0.8 v 16.4±1.0 v 16.9±0.7, P<0.009), with no change in quantitative sensory testing or neurophysiology.

Conclusion: Bariatric surgery improves neuropathic symptoms and deficits and small nerve fibre structure using corneal confocal microscopy as early as 6 months after surgery.

Disclosure: **M. Ferdousi:** None.

PS 081 Understanding diabetic neuropathy

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Three question set from Michigan Neuropathy Screening Instrument add independent prognostic information on cardiovascular outcomes: post-hoc analysis of ALTITUDE trial

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Background and aims: The Michigan Neuropathy Screening Instrument (MNSI) is used to diagnose diabetic peripheral neuropathy. We investigated whether the MNSI (a 15-item self-administered questionnaire) also offers prognostic information concerning risk of death and major CV events in patients with type 2 diabetes mellitus (T2DM) and chronic kidney disease (CKD) and/or cardiovascular (CV) disease from ALTITUDE.

Materials and methods: We divided the cohort of 8561 patients into independent training (n=3300) and validation sets (n=5261). In the training set, stepwise selection techniques identified specific questions that were independently associated with CV composite [CV death, resuscitated cardiac arrest, non-fatal myocardial infarction/stroke, heart failure hospitalization (HF)]. Selected questions were then evaluated in the validation set in adjusted models to assess their potential independent associations with clinical outcomes.

Results: In the training data set, 3 questions (Are your legs numb? Have you ever had an open sore on your foot? Do your legs hurt when you walk?) were significantly associated with the CV outcome composite. In the validation set, 3076 patients (59%) answered "yes" to at least 1 of these 3 questions. After adjustment for other key covariates, these patients demonstrated increased risk for CV composite (HR 1.54, 95% CI 1.28-1.85, p<0.001), all cause death (HR 1.23, 95% CI 0.98-1.54, p=0.072), CV death (HR 1.24, 95% CI 0.93-1.66, p=0.15), HF (HR 1.73, 95%CI 1.28-2.33, p<0.001), myocardial infarction (HR 1.86, 95%CI 1.25-2.76, p=0.002) and stroke (HR 1.74, 95%CI 1.19-2.53, p= 0.004) relative to those who answered "no" to all of the questions (Table). These associations were even stronger if patients answered positively to all of these 3 questions (n=552, 11%). Addition of these questions to existing models significantly improved Harrell's C statistic (0.71 vs 0.72, p=0.011) and continuous net reclassification improvement (+22%, 95%CI 7%-31%, p=0.013).

Conclusion: We identified three straightforward questions which add incremental prognostic information concerning risk of all cause death and major adverse CV events in patients with T2DM and CKD and/or CV disease. If externally validated these questions may be integrated into the clinical history to augment the prediction of CV events in high risk T2DM patients.

Table. Association of the 3- question set and outcomes in validation set (adjusted*)

	Any Yes (vs all No)		All Yes (vs All No)	
Event	**HR, 95 % CI**	**p**	**HR, 95 % CI**	**P**
CV outcome composite 587 events	**1.54 (1.28-1.85)**	**<0.001**	**1.70 (1.30-2.23)**	**<0.001**
All cause death 366 events	**1.23 (0.98-1.54)**	**0.072**	**1.58 (1.14-2.19)**	**0.006**
CV death 220 events	**1.24 (0.93-1.66)**	**0.15**	**1.62 (1.06-2.46)**	**0.025**
Heart failure hospitalization 232 events	**1.73 (1.28-2.33)**	**<0.001**	**1.75 (1.13-2.71)**	**0.013**
Myocardial infarction 143 events	**1.86 (1.25-2.76)**	**0.002**	**2.19 (1.26-3.80)**	**0.005**
Stroke 149 events	**1.74 (1.19-2.53)**	**0.004**	**1.42 (0.78-2.59)**	**0.25**

*Model adjusted for age, sex, race, smoking status, systolic blood pressure, eGFR, urinary albumin to creatinine ratio, HF history, myocardial infarction, stroke, atrial fibrillation, diabetic nephropathy, diabetic retinopathy, amputation, claudication, unstable angina, coronary revascularization, duration of diabetes, HbA1c, and randomized treatment.

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Disclosure: **J.P. Seferovic:** Grants; Institution received funding from Novartis in relation to the ALTITUDE trial.

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Evaluation of clinical diagnostic markers for early detection of diabetic neuropathy

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Background and aims: Diabetic neuropathy is among the most distressing and costly of all the chronic complications of diabetes and is a cause of significant disability and poor quality of life. Early detection, risk stratification and timely intervention to prevent long-term complications by maintaining good metabolic control is a key goal of diabetes management. Therefore identification of subjects with early subclinical neuropathy may provide an opportunity to identify the high-risk individuals who merit more aggressive intervention. The aim of present study was to determine the optimal diagnostic operating characteristics for diabetic neuropathy in cross-sectional analysis for a series of standard clinical neurological examinations.
Materials and methods: 274 diabetic patients and 84 healthy control subjects underwent detailed examinations for assessing symptoms, electrophysiology, quantitative sensory testing, autonomic neuropathy with assessing heart rate, punch skin biopsy and corneal confocal microscopy.Patients classified for presence of neuropathy based on NDS score more than 3.
Results: 274 Diabetic patients including 131 T1DM (60% male; Average age 46.5; Duration of Diabetes: 26.8; BMI 26.2 (4.3); HbA1c: 8.3) and 143 T2DM ((64% male; Average age 63; Duration of Diabetes: 13.1; BMI 31.7 (5.4); HbA1c: 7.7 (SD: 1.4)) and 84 age and sex matched healthy control subjects studied in details for battery of neurological examinations including symptoms profile (NSP), neuropathy deficit (NDS), electrophysiology (Sural and peroneal nerves), quantitative sensory testing (Vibration, hot and cold perception thresholds), autonomic neuropathy with assessing heart rate and deep breathing, punch skin biopsy (IENFD), corneal sensitivity (NCCA) and corneal confocal microscopy for assessing small c-nerve fibres in cornea including CNFD, CNBD, CNFL, CNFT which measured manually. 133 (48%) patients had neuropathy (NDS >3) and that was comparable between type 1 (63 patients, 48%) and type 2 diabetic patients (70 subjects, 49%). All markers except for NCCA showed a statistically significant diagnostic ability with AUC ranging from 0.58 to 0.81. The best marker was VPT. Using a diagnostic criterion VPT > 11 gave sensitivity and specificity values of 75% and 71% respectively, with a PPV of 70% and NPV of 75%. IENFD <6 gave sensitivity and specificity values of 79% and 44% respectively, with a PPV of 54% and NPV of 70%. From corneal nerves parameters, CNFD gave sensitivity and specificity values of 72% and 45% respectively, with a PPV of 55% and NPV of 63%. Corneal nerves fibre length (CNFL) showed sensitivity and specificity values of 71% and 39% respectively, with a PPV of 52% and NPV of 59%.
Conclusion: Evaluation of tested neurological markers in this study showed difference performance in T1DM and T2DM. However we validated the diagnostic thresholds for each of these markers that may be used regardless of diabetes type. To our knowledge this is one of the most complete cohort of subjects that compared the sensitivity of several diagnostic markers for early detection of diabetic neuropathy. Relatively poor performance of all these neurological markers maybe related to lack of standard reference point for diabetic neuropathy. There is a need for longitudinal studies to evaluate the predictive validity of these clinical markers.
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Disclosure: **M. Tavakoli:** None.

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Patterns of small and large fiber dysfunction in painful and painless diabetic polyneuropathy

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Background and aims: The distinguishing features contributing to the phenotype of diabetic sensorimotor polyneuropathy (DSPN) as a painful (+p) or painless (-p) entity are poorly understood. We hypothesized that DSPN+p and DSPN-p are characterized by predominant small and large nerve fiber dysfunction, respectively.
Materials and methods: We assessed somatic, cardiac autonomic, and sudomotor function (Sudoscan, Neuropad) in 332 patients with DSPN from the PROPANE study, 179 of whom had DSPN+p and 153 had DSPN-p and 54 diabetes patients without DSPN (DM) from the German Diabetes Study (DSPN-p/DSPN+p/DM [mean±SD]: age: 68.4 ±10.4/66.1±10.0/63.2±5.2 years, BMI: 29.5±5.3/30.9±5.6/30.1±4.9 kg/m²; T2D: 83/86/87%, diabetes duration: 16.4±12.4/16.1±12.1/5.1±3.2 years, HbA1c: 7.3±1.1/7.7±3.3/6.8±0.9%). DSPN was diagnosed using modified Toronto Consensus (2011) criteria, while DSPN+p and DSPN-p were stratified using a cutpoint of 4 points on the Likert scale for chronic pain lasting for >1 year in the distal lower limbs.
Results: After adjustment for sex, age, BMI, smoking, diabetes type, diabetes duration, and HbA1c, compared to patients with DSPN-p those with DSPN+p showed impaired warmth detection thresholds (TDT-w) (foot: 46.0±4.4 vs 45.3±4.4 °C), coefficient of R-R interval variation during deep breathing (5.18±3.95 vs 6.71±4.86%), and electrochemical skin conductance (ESC: hand: 56.1±17.8 vs 60.9±17.3 μS, foot: 57.8 ±20.7 vs 62.8±19.9 μS) (all $P<0.05$). Large fiber function tests (nerve conduction, vibration threshold) did not differ between the DSPN groups. In both DSPN groups, ESC (foot) correlated with peroneal motor and sural sensory nerve conduction, sural sensory nerve action potential, TDT-w (foot), intraepidermal nerve fiber density (IENFD), and Neuropad (all $r=|0.2|-|0.5|$; $P<0.05$). ROC analyses showed that both small and large fiber function tests were useful to detect DSPN+p (AUC vs DM: 0.7-0.9) and DSPN-p, albeit to a slightly lesser degree (AUC vs DM: 0.6-0.8).
Conclusion: Compared to painless DSPN, painful DSPN is characterized by more pronounced small fiber dysfunction involving sudomotor, cardiac autonomic, and cutaneous C fibers, while large fiber dysfunction is common to both entities.
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Disclosure: **G.J. Bönhof:** None.

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Determinants of incident distal sensorimotor polyneuropathy in a cohort with screen-detected type 2 diabetes followed for 13 years, the ADDITION Denmark Study

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Background and aims: Cross-sectional studies indicate that determinants beyond hyperglycemia, such as obesity, dyslipidemia and cardiovascular disease are important particularly in type 2 diabetes (T2DM) in the development of distal sensorimotor polyneuropathy (DPN).

Methylglyoxal is a marker of oxidative stress and has been proposed as being involved in the damage of nerve fibers. We aimed to study the incidence of DPN prospectively during the first 13 years after a screening-based diagnosis of T2DM and to identify determinants present at the time of T2DM diagnosis associated with DPN development.

Materials and methods: From the Danish arm of the Anglo-Danish-Dutch study of Intensive Treatment in People with Screen-Detected Diabetes in Primary Care (the ADDITION trial), 1256 participants were eligible for this study. Symptoms of DPN were assessed by the Michigan Neuropathy Screening Instrument questionnaire (MNSI) at four time-points and DPN was defined by a MNSI score of 4 or above. We evaluated the cumulative incidence of DPN and used Cox proportional hazard models to calculate hazard ratios (HR) to assess the association of various determinants and the development of incident DPN. Models were stepwise adjusted for trial intervention group, age, sex, lipid-lowering treatment and anti-hypertensive treatment at the 5 year follow-up of ADDITION.

Results: The median age of the study population was 60.8 years (p25;p75: 55.6;65.6), 59% were men and the median baseline HbA1c was 6.3 % (6.0;6.9) or 45.4 mmol/mol (42.1;51.9). The cumulative incidence of DPN during 13 years of follow-up was 10%. The risk of incident neuropathy was higher in older participants with a HR of 1.03 (95%CI: 1.00;1.07) per year, while sex was not associated with incident DPN with a HR of 0.67 (95% CI;0.43;1.05) for men compared to women. In Table 1 the results of Cox proportional regression models are presented.

Conclusion: This study demonstrates a fairly low cumulative incidence of DPN defined by the MNSI in people with screen-detected T2DM. Our study suggests that macrovascular disease, obesity and higher levels of methylglyoxal present at the time of diagnosis of T2DM are determinants for the development of incident DPN. The association between LDL cholesterol and incident DPN needs to be further elucidated.

Table 1. Risk factors for incident DPN (during a 13-year follow-up) present at the time of diagnosis of screen-detected type 2 diabetes expressed as hazard ratios (HR) and 95% confidence intervals (CI) at two levels of adjustment.

	Model A HR (95 % CI)	*Model B* HR (95 % CI)
HbA_{1c} (%)	0.93 (0.75;1.15)	0.93 (0.75;1.15)
Systolic BP (mmHg)	1.00 (0.99;1.02)	1.00 (0.99;1.02)
Waist circumference (cm)	1.03 (1.01;1.04) *	1.03 (1.01;1.04) *
BMI (kg/m^2)	1.07 (1.03;1.11) *	1.07 (1.03;1.11) *
Log albumin-creatinine ratio	1.10 (0.96;1.28)	1.12 (0.97;1.29)
Total cholesterol (mmol/L)	0.81 (0.64;1.02)	0.83 (0.66;1.04)
LDL (mmol/L)	0.70 (0.55;0.91) *	0.70 (0.55;0.91) *
HDL (mmol/L)	0.46 (0.23;0.94) *	0.46 (0.23;0.95) *
Triglycerides (mmol/L)	1.07 (0.96;1.20)	1.07 (0.97;1.18)
History of cardiovascular disease #	3.32 (1.51;7.31) *	3.22 (1.44;7.22) *
Log^2 methylglyoxal (nmol/L)	1.05 (1.02;1.08) *	1.05 (1.01;1.08) *

* Indicates statistical significance with a p-value<0.05
# Non-fatal myocardial infarction or stroke in the 10-year period prior to the diagnosis of type 2 diabetes
HRs are expressed per one unit increase in each continuous risk factor and for dichotomous risk factors the unexposed group is the reference group

Model A Adjusted for intervention group, sex and age
Model B Adjusted for intervention group, sex, age, use of lipid-lowering medication and anti-hypertensive medication at the 5-year follow-up

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Disclosure: **S.T. Andersen:** None.

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The association between pulse wave velocity and peripheral neuropathy in patients with type 2 diabetes

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Background and aims: Diabetic peripheral neuropathy (DPN) is the most common diabetic complication, affecting up to half of the patients with type 2 diabetes mellitus (T2DM). Increased aortic stiffness, measured with the carotid-femoral pulse wave velocity (PWV), has been associated with incidence of cardiovascular disease independently of traditional risk factors. Previous data showed associations between risk factors for macroangiopathy and DPN in diabetes. However, the association between PWV and DPN is unknown. In this study we examined the association between PWV and presence as well as severity of DPN in subjects with T2DM.

Materials and methods: A total of 381 patients with T2DM were recruited. Participants were classified as having DPN (107) and not having DPN (274). PWV was measured at the carotid-femoral segment with a non-invasive method using applanation tonometry. DPN was assessed by determination of vibration perception threshold (VPT), the Neuropathy Symptom Score and the Neuropathy Disability Score (NDS).

Results: Patients with DPN were significantly more often male and older, had longer duration of diabetes, higher height, larger waist circumference, higher peripheral and central systolic arterial blood pressure (SBP) and higher PWV (all $P < 0.05$). Furthermore, participants with DPN had lower low density lipoprotein cholesterol because they were treated more often with statins; in addition, they were treated more often with antiplatelets, b-blockers and insulin than those without DPN. Univariative logistic regression analysis demonstrated that there was significant association between the presence of DPN and age, gender, diabetes duration, height, waist circumference, peripheral and central SBP, PWV, dyslipidemia, HbA1c, retinopathy and nephropathy. Multivariate logistic regression analysis, after adjustment for age, gender, waist circumference, peripheral and central SBP and nephropathy demonstrated that the odds [OR (95% confidence intervals)] of peripheral neuropathy were associated significantly and independently only with height [1.070 (1.038 - 1.103), $P < 0.001$], diabetes duration [1.051 (1.017 - 1.087), $P = 0.003$], HbA1c [1.579 (1.261 - 1.978), $P < 0.001$], PWV [1.202 (1.081 - 1.337), $P < 0.001$], dyslipidemia [2.425 (1.311 - 4.488), $P = 0.005$] and retinopathy [4.589 (2.361 - 8.918), $P < 0.001$]. In addition, multivariate linear regression, after controlling for age, gender, peripheral and central SBP and nephropathy, analysis demonstrated that an increased NDS was significantly and independently associated with height [standardized regression coefficient (beta) = 0.247, $P < 0.001$], diabetes duration (beta = 0.118, $P = 0.042$), HbA1c (beta = 0.112, $P = 0.038$), PWV (beta = 0.232, $P < 0.001$) and retinopathy (beta = 0.286, $P < 0.001$).

Conclusion: This study has shown that in patients with T2DM beyond the known factors associated with DPN, increased PWV emerged as a new factor associated strongly and independently with the presence and severity of DPN.

Disclosure: **A. Tentolouris:** None.

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Impact of glycaemic variability on Müller cell activation

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Background and aims: In our recent work, 37 patients affected by type 1 diabetes mellitus (DM1), without diabetic retinopathy (DR) or very mild non-proliferative DR underwent a spectral domain optical coherence tomography (SD-OCT), obtaining individual retinal layer thickness measurements, and a 72-hours continuous glucose monitoring, from which indices of glycemic variability (GV) were measured. From SD-OCT, we found a significant increase of inner nuclear layer (INL), that includes mainly the nuclei of bipolar and Müller cells. A significant positive

correlation between INL e GV was also found. We assumed that the increase of INL may represent a clinical sign of Müller cell activation, due to glycemic excursions. We, therefore, carried out an in vitro study in order to confirm our hypothesis of activation of Müller cells, in response to glycemic variability.

Materials and methods: The effect of glucose was evaluated on the immortalized retinal Müller Cell Line, rMC-1 (Kerafast). rMC-1 cells (1x104cells/cm2) were grown 4 days in DMEM-10% FBS medium and different glucose conditions: continuous normal glucose medium (25 mmol/l) (NG); continuous low-glucose medium (5 mmol/l) (LG); 30-min episodes of "hypoglucose" (to mimic the hypoglycemic condition), twice a day (30' LG); continuous high-glucose medium (45 mmol/l) (HG); alternating normal and high-glucose media every 24 h (NG/HG); alternating low and high-glucose media every 24 h (LG/HG). Mannitol (20 mmol/l) was used in control cultures to exclude a hyperosmolar effect. Control groups were grown in continuous mannitol medium (20 mmol/l) (M), alternating normal glucose and mannitol media (NG/M) or low-glucose and mannitol media every 24 h (LG/M). Changes of the cell culture medium with fresh medium were performed, in each group, every 24h. Müller cells activation was evaluated by measuring glial fibrillar acidic protein (GFAP) expression using Western Blot analysis.

Results: Continuous HG medium administration for 4 days and alternating (NG/HG) media every 24 h did not induce activation of rMC-1. In contrast, a significant increase in GFAP was detected in rMC-1 exposed to alternating LG/HG media every 24 h ($p<0.05$) versus control groups.

Conclusion: These results demonstrate that GV, characterized by hypoglycemic and hyperglycemic excursions, rather than chronic hyperglycemia, is able to activate Müller cells, thus confirming what observed in our previous study in humans.

Disclosure: **F. Picconi:** None.

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Skin autofluorescence: correlation with measures of diabetic sensorimotor neuropathy

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Background and aims: The production and accumulation of Advanced Glycation Endproducts (AGEs) is exacerbated in subjects with diabetes. AGEs are involved in the pathogenesis of diabetic complications in general, and the pathogenesis of diabetic neuropathy in particular. Skin accumulation of AGEs can be measured quickly (results are displayed within seconds) and non-invasively by assessing skin autofluorescence (SAF). The aim of our study was to investigate the correlation between SAF and measures of diabetic sensorimotor neuropathy (DNP).

Materials and methods: In this multicenter study (8 centers), 497 consecutive subjects with diabetes mellitus (6.6% with type 1, 93.4% with type 2 diabetes, 51% female, 49% male, age: 60.5±9.1 years , HbA1c 7.28±1.02%, mean±SD) were investigated. SAF was assessed using the AGE Reader (Groningen, The Netherlands), measurements were performed at the forearm, and results of 3 measurements were averaged. For the diagnosis of DNP we used the Toronto Clinical Neuropathy Score (TCNS) as a score integrating symptoms and deficits, as well as the Neuropathy Symptoms Score (NSS) and the Neuropathy Disability Score (NDS) that assess separately symptoms and deficits.

Results: (mean±SD):According to the TCNS, SAF (measured in arbitrary units- AU) was significantly higher in subjects with DNP (TCNS>5, n=237 subjects): 2.59 ±0.56 AU compared with patients without DNP (TCNS≤5, n=260): 2.45±0.53 AU, (p=0.04) and significantly increased with the severity of DNP: 2.58±0.53 AU (TCNS:6-8, n=176 subjects), 2.61±0.64 AU (TCNS: 9-11, n=53), 2.76±0.57 AU (TCNS: 12-15, n=8), (p=0.028). SAF was higher in subjects with neuropathic deficits (NDS>2, n=261 subjects): 2.58 ±0.56 AU compared with patients without deficits (NDS≤2, n=236): 2.45±0.53 AU, (p=0.009) and significantly increased with the severity: 2.50±0.48 AU (NDS: 3-5, n=152 subjects), 2.69±0.64 AU (NDS: 6-8, n=107), 2.47±0.85 (NDS: 9-10, n=2), (p=0.002). Higher SAF existed in subjects with neuropathic symptoms (NSS>2, n=410): 2.54±0.56 AU compared to patients without symptoms (NSS≤2, n=87): 2.40±0.47 AU, (p=0.022) and increased numerically with the severity of symptoms: 2.51±0.54 AU (NSS:3-4, n=62 subjects), 2.53±0.57 AU (NSS: 5-6,n=148), 2.56±0.56 AU (NSS: 7-10, n=200), p=0,126. SAF significantly correlated with the duration of DNP (r=0.19, p=0.004) and diabetes duration (type 1 diabetes r=0.38, p=0.03; type 2 diabetes r=0.15, p=0.001) as well as with the HbA1c (r=0.18, p<0.001).

Conclusion: Skin AGEs accumulation assessed by measurement of SAF is increased in subjects with DNP and gradually increases with the severity of DNP. Since SAF measurement represents an easy-to-use, quick and non-invasive method, it might help to identify subjects at high risk for DNP. Especially in subjects without typical neuropathic symptoms, increased SAF could be an indicator for the presence of DNP and thus trigger further confirmatory investigations.

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Endothelial progenitor cells are increased in patients with type 2 diabetes and peripheral neuropathy

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Background and aims: Endothelial progenitor cells (EPCs) are a population of adult stem cells with the ability to differentiate into epithelial cells and to promote endothelial regeneration and neo-vascularization in response to tissue ischemia. Several studies have already reported an association between EPCs reduction/dysfunction and diabetic macrovascular complications. Although peripheral neuropathy (PN) has been associated with changes in the microcirculation and reduced endothelial-dependent and endothelial-independent vasodilation, regardless of the presence of macrovascular disease, data about its association with EPCs are scarce. The aim of the present study is to evaluate the relationship between PN and EPCs in patients with type 2 diabetes mellitus (DM).

Materials and methods: A total of 59 patients with DM (29 without PN and 30 with PN) and 20 healthy controls were recruited. Participants were non-smokers and had no clinical macrovascular disease. After venipuncture peripheral blood mononuclear cells (PBMCs) were obtained and stained with monoclonal antibodies against CD45, CD34, CD309 and CD133. A hierarchical gating strategy was employed to count low expressing CD45 cells ($CD45^{dim}$) and $1x10^6$ events per subject was acquired and analyzed using the six-color flowcytometer BD FACSCanto. EPCs were defined as cells expressing the $CD45^{dim}/CD34^+/CD309^+/CD133^+$ phenotype.

Results: The number of EPCs differed significantly between the 3 groups of participants (p=0.015). The sub-analysis showed that patients with PN had significantly higher number of EPCs when compared with patients

without PN (p=0.020) and participants without DM (p=0.012). No significant difference was observed in the EPCs number between patients without PN and participants without DM (p=0.476).

Conclusion: The number of EPCs was significantly higher in patients with PN in comparison with patients without PN and participants without DM. This finding may imply that there is an effort for restoration of the damaged peripheral nerves and more research is warranted to clarify the role of EPCs in diabetic PN.

Table 1.	controls	without PN	with PN	p
n	20	29	30	
Gender (female/male)	15/5	14/15	13/17	0.072
Age (years)	58.5 ± 9.4	61.2 ± 7.9	64.4 ± 7.9	0.056
DM duration (years)	-	13.0 (5.5, 20.0)	16.5 (10.0, 20.0)	<0.001
Retinopathy n (%)	0	4 (13.8)	10 (33.3)	0.304
ACR (mg/gr)	4.8 (3.0, 9.9)	6.0 (3.0, 30.4)	15.4 (7.7, 36.1)	0.005
Hypertension n (%)	2 (10.0)	19 (65.5)	25 (83.3)	<0.001
Dyslipidemia n (%)	4 (20.0)	21 (72.4)	19 (63.3)	0.001
BMI (kg/m^2)	26.5 ± 4.4	30.5 ± 7.9	32.3 ± 5.5	0.013
HbA1c (%)	5.3 (5.2, 5.4)	6.7 (6.1, 7.4)	6.6 (6.2, 7.1)	<0.001
EPCs	18 (12, 38)	23 (13, 35)	39 (28, 59)	0.015

Disclosure: **I. Eleftheriadou:** None.

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Protective effect of Nerve Growth Factor (NGF) on retinal ganglion cells degeneration in early stage of diabetic retinopathy

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Background and aims: Diabetic Retinopathy (DR) is an ocular microangiopatic disease that affects up to 70% of patients with both type 1 and type 2 diabetes. It takes several years of diabetes before the development of DR, suggesting that subclinical dysfunctions might characterize this "silent" phase. Recent studies show that retinal ganglion cell (RGC) death and retinal thinning precede the onset of DR suggesting an early neurodegeneration of the retina as a prodrome of DR. NGF is an endogenous neurotrophin active on RGC, bipolar neuron and glial cells with a well demonstrated protective effect on RGC in animal models of glaucoma. We tested the effect of NGF treatment on RGC loss and on the prevention of the development the vascular stage of DR. Ins2Akita mouse develops spontaneous diabetes at about 3 weeks of age. At 8 weeks of age we found, by Optical Coherence Tomography (OCT) analysis, a significant thinning of retinal nerve fiber layer (RNFL) and of the total retina compared to controls.

Materials and methods: Based on these observations we performed a study were C57Bl6/J and Ins2Akita were treated twice a day with one drop per eye of NGF (200 μg/ml) or Vehicle (16 C57 Vehicle, 20 Akita Vehicle, 15 C57 NGF, 14 Akita NGF). Treatment started at 3 weeks of age up to 8 weeks of age. Retinal layers thickness was evaluated in vivo by OCT at 3, 5 and 8 weeks. Electroretinogram (ERG) was performed at 8 weeks before sacrifice and RGCs numbers was evaluated by histological analysis.

Results: Weight and glycemia were comparable between the diabetic animals and the controls. At 3 and 5 weeks we did not found significant differences between the different groups, while at 8 weeks NGF was able to counteract the diabetes-induced thinning of RNFL (Akita Vehicle (19.1 ±1.2) Vs Akita NGF (21.6±1.0), Vs C57 Vehicle (22..2±1.1), Vs C57 NGF (22.1±1.0) (mean(μm)±SD): p<0.0001) and of the total retina (Akita Vehicle (182.4±5.2) Vs C57 Vehicle (189.7±4.5), Vs C57 NGF (189.0±4.7): p<0.001; Akita Vehicle Vs Akita NGF (186.2±4,7): N.S.; Akita NGF Vs C57 Vehicle, Vs C57 NGF: N.S.). Rescue induced by NGF was confirmed also by ERG a-wave amplitude (Akita Vehicle (111.0±23.3) Vs Akita NGF (142.4±15.0), Vs C57 NGF (144.0±8.4) (mean(μV)±SD): p<0.001; Akita Vehicle Vs C57 Vehicle (136.5±8.5): p=0.007) and ERG b-wave amplitude (Akita Vehicle (271.9±40.6) Vs Akita NGF (318.2±9.8), Vs C57 NGF (320.1±11.5) (mean(μuV)±SD): p<0.001 and Akita Vehicle Vs C57 Vehicle (310.8±12.9): p=0.019). Both ERG parameters were improved in treated animals. Interestingly, the analysis performed on histological samples showed no difference in RGC and in retinal fiber number.

Conclusion: These data suggest that OCT and ERG can detect the neuroretinal suffering before substantial anatomic alteration (i.e cell death) occurs. Taken together these data are in line with the hypothesis that topic treatment with NGF prevents the early, diabetes driven, retinal neurodegeneration. Further studies are now warranted to clarify whether, on the long run, this same pharmacological approach will allow to avoid RGC death and the progression of DR toward its sight-threatening vascular stage.

Disclosure: **S. Maestroni:** None.

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Metabolic shift from glycolysis towards lipid oxidation in Schwann cells in response to hyperglycaemia

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Background and aims: Diabetic neuropathy (DN) is characterized by hypersensitivity in the early stage of the disease, and hyposensitivity at later stages. However, hyperglycemia alone is insufficient to account for the development and the progression of diabetic complications. Metabolomic and proteomic analysis has revealed that a failure of energy homeostasis, particularly within the Schwann cell-rich sciatic nerve may be a driving force in the pathogenesis of DN. The aim of this study was to investigate the effect of hyperglycemia on energy homeostasis in Schwann cells.

Materials and methods: Schwann cells (SW10) were cultured in low glucose (LG, 5mmol/l) DMEM and high glucose (HG, 25mmol/l) DMEM. Mitochondrial properties and oxidative stress were assessed by flow cytometry. Glycolysis, mitochondrial respiration and fatty acid metabolism were measured by Seahorse Bioanalyzer. Transcription and expression of the key regulators involved in energy homeostasis were assessed by quantitative PCR and western blotting. Metabolomic analysis was performed by LC-MS/MS. Experimental DN was studied in streptozotocin-induced diabetes in C57BL/6 mice.

Results: In response to chronic hyperglycemia, Schwann cells showed a down-regulation of both glycolysis and mitochondrial respiration, in particular a decrease in maximal respiration (LG 301.3 -/+ 19.7 vs. HG 216.2 -/+ 9.4; p=0.0014) and glycolytic capacity (LG 86.6 -/+ 5.4 vs. HG 73.3 -/+ 6; p=0.0352). The loss of glucose-dependent metabolism was compensated by a shift towards fatty acid metabolism, as indicated by increased expression of ATP-citrate lyase and acyl-CoA synthase. However, this shift was towards fatty acid oxidation, as indicated by increased phosphorylation of acetyl-CoA carboxylase. This was associated with activation of ERK-dependent endoplasmic reticulum stress response leading to an increased the accumulation and/or secretion of neurotoxic metabolic intermediates, such as methylglyoxal (wt 290.3 -/+ 50.4 vs. diabetes mean 584.6 -/+ 47,6; p=0.0074).

Conclusion: This study shows that hyperglycemia induces a shift in metabolism from glycolysis to fatty acid oxidation in Schwann cells. This shift, driven by ER stress, is ultimately detrimental to the cell.

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Disclosure: **T.T.A. Smit:** None.

PS 082 Neuropathy everywhere

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A short period of slow breathing is able to reveal obstructive sleep apnoea syndrome in type 2 diabetes and obese patients

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Background and aims: Obstructive sleep apnoea syndrome (OSAS) has multifactorial causes and it is characterised by cardio-respiratory reflex imbalance. It heavily worsens cardiovascular prognosis in diabetes and obesity. Accurate diagnosis by polysomnography is expensive and requires night hospitalisation. A brief period of slow breathing (SLB), by transiently improving baro-chemoreflex interaction and oxygen saturation (SAT), could shift an ill-balanced cardiovascular/respiratory reflex interconnection, and acutely trigger OSAS-related respiratory abnormalities.
Materials and methods: In 90 patients (59 diabetics: age 55±13yrs, 30 males, BMI 33.4±7.3 kg/m^2, HbA$_1$c 8.03±1.56%; and 31 obese: 50 ±13yrs, 8 males, BMI 39.2±5.3 kg/m^2) and 20 healthy subjects (55 ±15yrs, 7 males, BMI 22.0±2.3 kg/m^2) we continuously monitored SAT, respiration (inductance plethysmography) and baroreflex sensitivity (BRS) during spontaneous respiration (5min), 5-min SLB at 6 cycles/min and 10-min follow-up under spontaneous breathing (POST-SLB). So far 56 patients underwent standard polysomnography (PSG).
Results: 55/90 patients (36 diabetics and 19 obese) developed apnoeas/hypopneas during POST-SLB (POST-SLB+); comparing SLB test to PSG, 40/41 were true positives, 1/41 was false positive and 10/15 true negatives (5 false negatives, but these patients had only mild OSAS at PSG, apnoeas/hypopneas index<15 per hour), PPV: 98%, NPV: 67%, sensitivity: 89%, specificity: 91%. At baseline POST-SLB+ patients had lower SAT (p=0.003) and BRS (p<0.001) compared to healthy subjects. During SLB all subjects increased SAT (p<0.001) and BRS (p<0.01) compared to baseline. During POST-SLB period, in patients who developed apnoeas/hypopneas SAT fell below (p<0.0001) and BRS returned to baseline, whereas in the other patients SAT and BRS returned to baseline values.
Conclusion: In diabetic and obese patients a short (20min), simple and inexpensive clinical test based upon analysis of cardio-respiratory reflex imbalance can unmask underlying OSAS, due to the post-effects of transient relief of subclinical hypoxia.
Disclosure: **L. Bianchi:** None.

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Small fiber neuropathy, suggested by clinical examination, is objectively linked to autonomic neuropathy and other complications in type 2 diabetes

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Background and aims: Early detection of distal diabetic neuropathy (DDN) is essential to slow down its progression. Making an objective diagnosis of Small Fiber Neuropathy (SFN) is nevertheless very challenging in daily practice. All recommendations and validated scores allow the diagnosis, once DDN becomes irreversible. We examined the characteristics of type 2 diabetics with SFN, suggested by a basic clinical examination, and compared them with diabetics who have normal sensory tests and those with evident neuropathy.
Materials and methods: Between 2009 and 2013, we recruited 327 newly diagnosed type 2 diabetics, aged from 40 to 70 years. We collected clinical and biological data and screened for chronic complications for all patients. Foot sensory examination included vibration perception with 128 Hz tuning fork, light touch, heat-pain and cold perception; we tested the ankle reflex and muscle strength (quadriceps and tibialis anterior) symmetrically. We used Michigan Neuropathy Screening Instrument (MNSI) for the diagnosis of DDN, and considered as a SFN the abolition of at least heat and pain perception of the feet and a MNSI within the range of 0-2, with or without distal pain sensation. Statistical analysis was performed with Epi-Info 6.04.
Results: We analyzed 317 patients after excluding 10 patients for having a neuropathy with another etiology than diabetes. We found DDN, SFN and normal neurological examination, respectively in 98 (30.9%), 92 (29.0%) and 127 (40.1%) diabetics. There were no statistical differences between the group of patients with SFN and those with a normal neurological examination, on age, height, smoking status, hypertension prevalence, renal function, glycemic and lipid status, but patients with SFN were less educated (p=0.006), had a higher BMI (p=0.039), higher prevalence of distal pain (p<10-5), diabetic retinopathy (p=0.032), autonomic neuropathy (p=0.021), specially sudoral dysfunction (p=0. 019) and minor distal trophic disorders (p=0.004). Diabetics with DDN were four years older (p=0.001) and had much more ischemic heart disease (p=0.002), dysautonomy (p=0.0002), erectile dysfunction (p=0.001) than the two other groups of patients.
Conclusion: We suggest, in our study, that a simple clinical examination could help to determine a distinguishable group of patients that presents an early stage of diabetic neuropathy. Without specific recommendations, these patients are too often considered as "free of neuropathy" in daily practice, and may not benefit from an appropriate therapeutic intervention to reverse the course of this complication.
Clinical Trial Registration Number: NCT02002091
Disclosure: **W. Nibouche-Hattab:** None.

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Association of endothelial dysfunction with autonomic neuropathy in Japanese type 2 diabetes

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Background and aims: Assessment of endothelium mediated vascular response in the finger tips after occlusion of the brachial artery is reflecting a hyperemic response, which is a measure for arterial endothelial function. Sympathetic nerve function plays a critical role in regulating vascular function. Although the vascular function of diabetes was known to be impaired more seriously than non-diabetic subjects, it is still unclear whether vascular hyperemic response were impaired by autonomic neuropathy in type 2 diabetes (T2DM) or not. To clarify the relationship between endothelial function and diabetic autonomic neuropathy, we measured reactive hyperemia peripheral arterial tonometry (RH-PAT) for evaluation of endothelial function, and heart rate variability (Coefficient of variation of R-R intervals: CV_{R-R}) as an indicator of autonomic function in type 2 diabetes.
Materials and methods: We assessed T2DM with RH-PAT for evaluation of endothelial function, CV_{R-R} for evaluation of autonomic nerve function by 5-min ECG recordings, and vibratory perception threshold for evaluation of diabetic peripheral neuropathy by using the C128 quantitative tuning fork. The results of RH-PAT were expressed as reactive hyperemia index (RHI) for evaluating endothelial function. Plasma VEGF concentration was measured using ELISA as a marker of vascular endothelial marker. Participants with CV_{R-R} less than 2.0 were

determined as impaired autonomic function, or with vibration perception threshold less than 9-seconds were as impaired vibration sensation. Patients with history of heart failure or arrhythmias were excluded.
Results: 281 T2DM were included. Mean age was 68.7±9.1 years (mean ±SD), duration of diabetes 15.5±9.9 years, HbA1c 7.4±1.1%, Plasma VEGF concentration 132.5±40.5 pg/ml, prevalence of albuminuria 40.0%, retinopathy 52%, mean RHI 1.56±0.38, 48% patients have low $CV_{R\text{-}R}$, and 64% patients have impaired vibration sensation. We compared RHI dependent on low $CV_{R\text{-}R}$ or normal $CV_{R\text{-}R,}$ and low vibration sensation or normal vibratory sensation. Our preliminary results show there was no significant difference between groups regarding age, BMI, duration of diabetes or hypertension. The RHI in low $CV_{R\text{-}R}$ group was significantly lower compared with normal $CV_{R\text{-}R}$ group (1.70±0.39 vs. 1.43±0.33, p<0.01). Whereas there was no significant difference in RHI between low vibration sensation group and normal vibration sensation group (1.56±0.31 VS. 1.49±0.45, p=0.09). Plasma level of VEGF were not significant difference in low $CV_{R\text{-}R}$ group compared with normal $CV_{R\text{-}R}$ group (139.7±32.0 vs. 128.5±28.3, p=0.20). RHI was significantly correlated with $CV_{R\text{-}R}$ (p=0.01) after adjustment for age, gender, blood pressure, albuminuria, LDL cholesterol, plasma VEGF concentration, and prevalence of retinopathy.
Conclusion: According to our study, endothelial function was more impaired in T2DM with autonomic neuropathy compared to T2DM without autonomic neuropathy. Our findings support the hypothesis that endothelial dysfunction was independently correlated with autonomic neuropathy in Japanese type 2 diabetes.
Disclosure: **M. Furuta:** None.

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The effect of autonomic dysfunction in haemodynamic parameters in patients with type 2 diabetes

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Background and aims: Recent studies have reported that central blood pressure hemodynamics, such as pulse pressure (PP) amplification (PPA), augmentation index (AIx) and aortic pulse wave velocity (PWV) may better reflect overall cardiovascular risk compared to traditional risk factors in patients with and without type 2 diabetes mellitus (T2DM). Cardiac autonomic dysfunction (CAD) is a chronic complication of diabetes associated with increased cardiovascular risk. The effect of CAD on central blood pressure hemodynamic is not yet clear. The aim of our study was to investigate the association of CAD with blood pressure hemodynamics in patients with T2DM.
Materials and methods: A total of 142 patients with T2DM were recruited. Hemodynamic parameters, such as PPA, AIx and PWV were determined by applanation tonometry. Cardiac autonomic nervous system activity was assessed with short term analysis of heart rate variability (HRV) and baroreflex sensitivity (BRS).
Results: Univariate linear regression analysis showed that PPA was significantly associated with age, male gender, height, waist circumference, aortic systolic blood pressure (SBP), heart rate, AIx, PWV, central BRS, total power, low frequency power of HRV and use of b-blockers, while there was a trend for association with diabetes duration and high frequency power. No associations were found with smoking, dyslipidemia, HbA1c and renal function. Multivariate analysis, after adjustment for age, diabetes duration, height, waist, PWV, use of b-blockers and central BRS, demonstrated that male gender [standardized regression coefficient (β)=0.154, p=0.008], aortic SBP (β=-0.221, p<0.001), heart rate (β=0.521, p<0.001), AIx (β=-0.441, p<0.001), and total power of HRV (β=-0.156, p=0.005) were associated independently with PPA. No associations in the univariate analysis were observed between AIx and parameters of HRV or central BRS. Similar, no associations were observed between PWV and parameters of HRV or central BRS.
Conclusion: In patients with T2DM, PPA is associated with cardiac autonomic activity irrespective of known factors related to pressure pulsatility, such as age, gender, heart rate, wave reflections and traditional cardiovascular risk factors. However, CAD is not associated with AIx and PWV. CAD may attenuate pressure wave reflections and contribute to higher PPA observed in patients with T2DM.
Disclosure: **M. Nikoloudi:** None.

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Benfotiamine in the treatment of diabetic cardiovascular autonomic neuropathy

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Background and aims: Cardiac autonomic neuropathy (CAN) represents a serious complication with approximately five-fold risk of mortality. Development of hyperinsulinemia, insulin resistance and chronic hyperglycemia in patients with type 2 diabetes mellitus (DM) have a negative impact on the metabolism of thiamine. Benfotiamine is a relative of the vitamin B_1 that appears to have a therapeutic role in reduction of diabetic complications development and progression. This study was aimed to investigate the effect of benfotiamine on insulin resistance parameters, some markers of chronic inflammation and heart rate variability (HRV) parameters.
Materials and methods: The study involved 32 patients with type 2 DM and moderate CAN of median age 54.13±0.28, median BMI: 27.9±0.26 kg/m^2 and HbA_{1c} level 7.0±0.13%. The diagnosis of CAN was based on the results of five standard cardiovascular tests, 24-hour HRV during the ECG recording (ECG "EC-3H" ("Labtech", Hungary). Patients with CAN were allocated to two groups: control (n=15) received standard therapy and treatment (n=17) received in addition benfotiamine 300 mg/day. We investigated lipid profile, levels of immunoreactive insulin (IRI), leptin, tumor necrosis factor alpha (TNF-α), high-sensitivity C-reactive protein (hsCRP) and interleukine-6 (IL-6). The duration of the study was three month. Statistics: ANOVA.
Results: We found out that the HbA_{1c} of patients with type 2 DM and CAN was not statistically significant influenced by the treatment (p>0.05). Benfotiamine prescription to patients with type 2 DM and CAN did not cause any significant changes in lipid profile and leptin levels (p>0.05), while it probably helped reduce the IRI concentration [26.8±1.37 mcIU/ml (before treatment) and 23.2±0.89 mcIU/ml (after treatment), Δ=-12.7±1.4%, p<0.05]. The use of benfotiamine in the comprehensive treatment of type 2 DM helped reducing hsCRP [2.93±0.16 mg/l (before treatment) and 2.5±0.1 mg/l (after treatment), Δ=-13.3 ±2.1%, p<0.05], TNF-α [5.39±0.24 pg/ml (before treatment) and 4.76 ±0.14 pg/ml (after treatment), Δ=-10.2±1.4%, p<0.05] and IL-6 [5.71±0,3 pg/ml (before treatment) and 4.78±0.2 pg/ml (after treatment), Δ=-15.4 ±2.04%, p<0,05)] concentrations. After 3 month of treatment we found out that there was a increase the percentage of differences between successive NN intervals over 24 hours that are greater than 50 ms (pNN50) [4.94±0.55% (before treatment) and 7.41±1.06% (after treatment), Δ=+45.9±7.91%, p<0.05)], high-frequency (HF) activity parameters during the active [267.7±17.61 ms^2 (before treatment) and 326.4±16.96 ms^2 (after treatment), Δ=+25.8±5.58%, p<0.05] and passive [267.7±17.61 ms^2 (before treatment) and 326.4±16.96 ms^2 (after treatment), Δ=+21,1 ±4,17%, p<0.05] periods of the day.
Conclusion: The administration of benfotiamine for three month promotes reduction of chronic inflammation markers and increase of parasympathetic activity, that allows to recommend prescription of this drug to patients with type 2 DM and CAN.
Disclosure: **V.A. Serhiyenko:** None.

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Longitudinal evaluation of gastric emptying in type 2 diabetes

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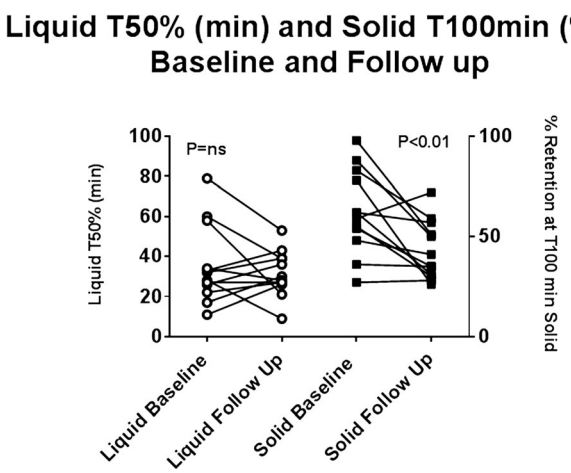
Background and aims: The rate of gastric emptying is a key determinant of postprandial glycaemia which can be manipulated for therapeutic gain in type 2 diabetes (T2DM). Measurements of gastric emptying using scintigraphy show good reproducibility within healthy individuals in the short term, although little is known about the natural history of gastric emptying in patients with T2DM.
Materials and methods: We examined the records of patients with diabetes who had scintigraphic measurement of gastric emptying for research purposes in our laboratory between 2000 and 2005. Of the 167 subjects with diabetes identified, 42 had type 1 diabetes, 45 were deceased, 16 did not respond to the letter of invitation, 27 did not have a known address, 21 declined to participate, and 4 were excluded due to age, iron deficiency, opiate use, and bariatric surgery. 12 subjects with T2DM [7 female; age 51.6 ± 1.2 years; body mass index 30.4 ± 1.0 kg/m^2; duration of diabetes 8.9 ± 1.6 years at the time of the initial study] were able to return for repeat measurements of gastric emptying over 2 hr using the same dual-radioisotope labelled solid (100g minced beef) and liquid (150mL 10% glucose) meal, a mean of 14.0 ± 0.5 years after their initial study. On each study day, glycated haemoglobin (HbA1c) was measured, blood glucose was monitored before and after meal ingestion, and autonomic nerve function evaluated using standardised cardiovascular reflex tests. Data were evaluated using paired t-tests and repeated measures analysis of variance (ANOVA) and are shown as mean values ± SEM.
Results: Six patients were insulin-treated at follow up, vs. 2 at baseline. Six subjects had cardiovascular autonomic dysfunction at follow up, vs. 7 at baseline. Mean HbA1c was higher at follow up (9.0 ± 0.3 % [73.5 ± 3.5 mmol/mol] vs. 7.4 ± 0.4% [56.8 ± 4.5 mmol/mol], P= 0.03). However, fasting blood glucose (10.4 ± 0.8 mmol/L vs. 9.7 ± 0.8mmol/L, P=0.6) and postprandial blood glucose excursions (incremental area under the curve [0-120 min] 295 ± 55.8 vs. 213.0 ± 37.3 mmol/L.min, P=0.09) did not differ. Gastric emptying of solids was more rapid at follow up (retention at 100 min 42.3 ± 4.3%, vs. 64.1 ± 5.9% at baseline, P<0.01), whereas liquid emptying was unchanged (half-emptying time 31.5 ± 3.3 min vs. 35.6 ± 5.7 min at baseline, P=0.4). Solid emptying was abnormally slow in 6 patients at baseline, but only in one at follow up, while liquid emptying was delayed in 6 patients initially, and 5 at follow up (4 of whom were delayed at baseline). (Figure 1). None had abnormally rapid gastric emptying on either study.
Conclusion: In patients with long-term type 2 diabetes, gastric emptying of solids and liquids does not usually become more delayed over time, and abnormally slow gastric emptying of solids may improve.



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Disclosure: **L.K. Phillips:** Grants; National Health and Medical Research Council (NHMRC), Australia, Royal Australasian College of Physicians.

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Gastric electrical stimulation device decreases inpatient days in the population with diabetes

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Background and aims: Diabetic gastroparesis is increasingly recognised as a frequent and clinically important complication of diabetes. The efficacy of medical treatment is limited, and gastric electrical stimulation (GES) offers a potential alternative treatment. We performed a retrospective cohort study to determine the effect of gastric pacemaker insertion on all-cause and gastroparesis-related admissions, readmission following post-operative complications, and duration of inpatient stay in patients with diabetic gastroparesis.
Materials and methods: A total of 13 patients with gastroparesis secondary to type 1 diabetes mellitus underwent gastric pacemaker insertion between January 2011 and November 2014 at a single centre in Glasgow. All had the same type of GES device placed by one surgeon. Data was collected retrospectively using the Scottish Care Information Diabetes Collaboration (SCI-DC) database and the Clinical Portal system (electronic patient record) for a period of 24 months pre-operatively, and 24 months post-operatively.
Results: This cohort had a mean age of 37 years. Nine of the 13 patients were female. Mean duration of diabetes was 22.7 years. Mean HbA1c at time of surgery was 88.6 mmol/mol. In terms of microvascular complications, 7 of the 13 (54%) patients had diabetic eye disease, defined as presence of pre-proliferative retinopathy, proliferative retinopathy or maculopathy. Of the 7 patients with eye disease, 6 had undergone laser therapy. Four of the 13 patients (31%) had diabetic nephropathy, with two of these having dialysis-dependent end stage renal disease. Five of the 13 patients suffered from diabetic neuropathy. Two patients in this cohort died out-with the 24-month follow up period - at 28 months and 61 months after pacemaker insertion respectively. In the 24 months prior to gastric pacemaker insertion, this cohort had a mean of 7 admissions from any cause. In the 24 months following pacemaker insertion, this fell to 6.3. The number of gastroparesis-related admissions fell from a mean of 5.5 to 3.8 admissions per patient over the same period. This corresponds to a fall in mean length of stay from 33.5 days to 22.3 days following pacemaker insertion. Inpatient days for gastroparesis-related admissions for the entire cohort fell from a total of 435 days in the 24-month period prior to insertion, to 290 days in the 24-month period following pacemaker insertion. Mean duration of admission for surgery was 3.8 days. Two of the 16 patients required re-admission due to complications following pacemaker insertion. This accounts for 5 of the 82 post-operative admissions for this cohort.
Conclusion: These results demonstrate a reduction in the number of all-cause and gastroparesis-related admissions following gastric pacemaker insertion. Duration of gastroparesis-related inpatient stay fell by 11.2 days, a reduction of 33.4% from baseline, in those who underwent pacemaker implantation. Rate of re-admission with post-operative complications from this procedure was acceptably low. Gastric pacemaker insertion is a safe and effective therapy that has been demonstrated to reduce numbers of diabetic inpatient days in our retrospective cohort study.
Disclosure: **Z.M. Chong:** None.

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Correlation between symptoms of gastroparesis and ^{13}C-octanoic acid breath test in patients with type 2 diabetes

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Background and aims: Gastroparesis, one of the commonest gastrointestinal (GI) complications of diabetes mellitus (DM), produces symptoms of gastric retention in the absence of physical obstruction. The aim of this study was to correlate symptoms using the GCSI with delayed gastric emptying in patients with type 2 DM referred for ^{13}C - octanoic acid breath test (^{13}C-OBT).
Materials and methods: We studied 180 subjects with type 2 DM duration of 9.9±6.4 years, mean value of HbA_1C was 8.6±1.7% and control group: 30 healthy volunteers. In results of GCSI all diabetics divided into 2 groups: I group consisted of 94 (52.2%) participants (56 male/38 female, mean age was 54.7±6.7 years) without GI symptoms and II group: 86 (47.8%) patients (48 male/38 female, mean age was 59.8±8.3 years) with diabetic gastroparesis (DG). All subjects had negative markers of other disorders of GI tract. GCSI questionnaire for assessment of GI symptom severity which consists of 9 questions answered by use of a six-point Likert response scale, ranging from 0 (none) to 5 (very severe). The severity of DG was assessed by GCSI questionnaire and Gastric emptying rate (GER) with ^{13}C-OBT.
Results: From the patients of type 2 DM, all subjects of II group were reported one or more GI symptoms from the GCSI scale: 50% reported fullness and/or early satiety from very mild to severe, 30% reported bloating from very mild to severe, 19% reported vomiting or nausea from very mild to moderate, other participants and volunteers without GI symptoms. GCSI total score in the II group was 16.7±0.7, mean GCSI nausea/vomiting score - 0.4±.0.2 and mean GCSI bloating score - 1.1 ±0.4. Low gastric motility has been diagnosed in patients with the help of ^{13}C-OBT: in the II group T ½ - 99.5±13.7 min, but in the I group T ½ - 60.1±5.4 min and control group that result is T ½ - 50.4±6.5 min. There was a strong correlation between ^{13}C-OBT and the GCSI total (r=.85, p=0.001), nausea/vomiting (r = .80, p=0.001) and bloating scores (r = .79, p=0.001).
Conclusion: Gastropaesis is quite a common complication of type 2 DM and use of the questionnaire GCSI allows to screen patients for DG symptoms which is confirmed by the strong correlation between GER and severity symptoms of questionnaire revealed in our study.
Disclosure: **I.O. Kostitska:** None.

PS 083 Do not miss the diagnosis of autonomic neuropathy

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Diagnostic value of different autonomic symptoms assessed by COMPASS 31 for cardiovascular autonomic neuropathy and diabetic polyneuropathy

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Background and aims: We recently validated the questionnaire Composite Autonomic Symptom Score (COMPASS) 31 for autonomic symptoms of diabetic neuropathy. In a wider population from the same diabetes centre, we aimed to further investigate the diagnostic performance of different autonomic symptoms (i.e., orthostatic intolerance, vasomotor, secretomotor, gastrointestinal, bladder, and pupillomotor domains of COMPASS 31) for diabetic cardiovascular autonomic neuropathy (CAN) and diabetic polyneuropathy (DPN).
Materials and methods: A total of 93 participants with diabetes (age 54 ±14 years, diabetes duration 13±10 years) completed the COMPASS 31 questionnaire before undergoing cardiovascular reflex tests and assessment of neuropathic symptoms (using the Michigan Neuropathy Screening Instrument Questionnaire), signs (using the Michigan Diabetic Neuropathy Score), vibration, and thermal thresholds.
Results: As expected, the COMPASS 31 total weighted score was higher in the presence of CAN (26.7±19.5 Vs. 12.5±14.3; P=0.0044) and DPN (26.9±17.8 Vs. 12.5±11.3; P=0.0000). Among the 6 domains of COMPASS 31, the highest differences were seen in gastrointestinal (P=0.0004) and orthostatic intolerance weighted scores (P=0.0188) according to the presence of CAN, and in secretomotor (P=0.0000), gastrointestinal (P=0.0000), pupillomotor (P=0.0001), and orthostatic intolerance weighted scores (P=0.0005) according to the presence of DPN. Receiver-operating curve analysis confirmed a fair diagnostic accuracy of total weighted score for CAN [area under the curve (AUC) 0.685 ±0.067, 95% CI 0.583-0.782] and DPN (AUC: 0.755±0.050, 95% CI 0.652-0.836). Among the six COMPASS 31 domains, a fair diagnostic accuracy for CAN was reached only by gastrointestinal domain (AUC: 0.728±0.066, 95% CI 0.630-0.821), whereas it was achieved for DPN by secretomotor (AUC: 0.758±0.050, 95% CI 0.652-0.836), gastrointestinal (AUC: 0.728±0.066, 95% CI 0.630-0.821) and pupillomotor domains (AUC: 0.705±0.056, 95% CI 0.606-0.799). Secretomotor weighted score at the cut-off of 4.28 had a sensitivity of 61.5% (95% CI 46.3-76.8) and a specificity of 81.5% (95% CI 71.1-91.8) for DPN. Vasomotor and bladder domains showed the worst diagnostic performance.
Conclusion: Among autonomic symptoms as assessed using COMPASS 31, the best diagnostic performances were provided by the secremotor domain (exploring sweating changes, dry eyes and dry mouth) for DPN and by the gastrointestinal domain for both CAN and DPN. These findings expand previous observations, give insights into the relationship between autonomic and sensorimotor neuropathy, and further support the inclusion of COMPASS 31 in a comprehensive evaluation of diabetic neuropathy.
Disclosure: **C. Greco:** None.

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Should we avoid the handgrip test in the assessment of cardiovascular autonomic neuropathy in diabetic patients? Exploratory factor analysis

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Background and aims: Historically, a set of five cardiovascular autonomic reflex tests (CARTs) was considered to be the gold standard of cardiovascular autonomic neuropathy (CAN) assessment. Current guidelines however suggest only the use of four with the omission of the diastolic blood pressure response to sustained handgrip. Thus we aimed to assess the association between the handgrip and the other tests.
Materials and methods: We recruited 353 diabetes patients (age: 60.2 ±7.4 years; female: 57.2%; BMI: 29.3±2.1 kg/m^2; diabetes duration: 15.6 ±9.9 years; HbA1c: 8.2±1.9%; type 1 diabetes: 18.1%). We measured the following CARTs: deep breathing test, Valsalva ratio, handgrip test, and orthostatic hypotension test. Definite CAN was defined as ≥2 abnormal CARTs excluding the handgrip test.
Results: The handgrip test had a sensitivity of 24.6% (95%CI 17.7%-33.1%) and a specificity of 79.4% (95%CI 73.3-84.4%) for the diagnosis of definite CAN. According to exploratory factor analysis, the four examined CARTs showed a 2-factor structure with the handgrip test loading to one factor (factor loading: 0.98) and the deep-breathing test, Valsalva ratio and orthostatic hypotension test clustered on another component with factor loadings 0.68, 0.77 and 0.66, respectively. Handgrip test abnormality showed an independent association with higher initial diastolic blood pressure values (OR: 1.05, p=0.0009) and an independent inverse association with the presence of hypertension (OR=0.42, p=0.006).
Conclusion: According to the exploratory factor analysis, there is an independent factor underlying the results of the handgrip test differing from that underlying the results of the other cardiovascular reflex tests. Potential factors influencing handgrip test results could be the presence of hypertension and baseline diastolic blood pressure.
Disclosure: **A.E. Körei:** None.

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Valsalva test alone is the best and economically valued tool for diagnosing cardiac autonomic neuropathy

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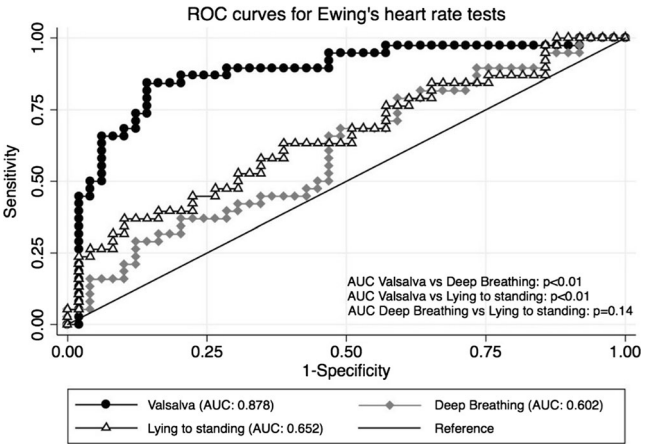
Background and aims: Cardiac autonomic neuropathy (CAN) is a life-threatening diabetes complication. It often goes ignored as the recommended Ewing's battery for CAN diagnosis is time-consuming and difficult to perform.
Materials and methods: To evaluate if the number of required tests could be reduced, we compared each test included in the Ewing's battery and their combinations in 115 diabetic subjects with a median age of 58 [interquartile range: 48-67] years.
Results: According to the Toronto criteria, CAN was diagnosed in 53 subjects. After multivariable adjustments, CAN was associated with higher HbA1c and triglycerides levels, lower HDL cholesterol and higher retinopathy rates (all p-values<0.05). No subject had positive orthostatic hypotension test. The age-adjusted area under the receiver operating characteristic curve of Valsalva maneuver was significantly higher than deep breathing and lying to standing tests alone (Figure) and in combination (p<0.01). Performing a complete Ewing's battery did not increase sensitivity and specificity for CAN diagnosis than performing Valsalva test alone (p= 0.48): Youden's J statistic showed the best age-adjusted sensitivity and specificity for CAN diagnosis were 80.5% and 86.3% respectively with Valsalva vs 85.0% and 85.7% with the full battery.
Conclusion: CAN can be efficiently diagnosed by using Valsalva test alone, thus allowing a fast, economically valued and easy screening for a frequently unrecognised deadly diabetes complication.



Disclosure: **R. Del Toro:** None.

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Analysis of the relationship between glycaemic variability and autonomic neuropathy in type 1 diabetes

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Background and aims: A relationship is suspected between autonomic neuropathy (AN) and glucose variability (GV) as variability is associated with oxidative stress responsible for neuronal damage while the impaired autonomic function has a detrimental effect on metabolism. The aim of our study was to find relationship between the parameters of AN and GV in type 1 diabetic patients.
Materials and methods: 21 type 1 DM patients were involved (age: 39.5 ±3.4 years, duration of DM: 17.5±2.5 years; HbA1c: 8.1±0.2%, mean ±SE). Autonomic neuropathy (AN) was assessed by the cardiovascular reflex tests (CRT). The interstitial glucose levels were determined following insertion of a subcutaneous electrode during the continuous glucose monitoring (CGM) method on 6 consecutive days. GV was characterized by calculation of 4 parameters.
Results: Standard deviation of interstitial glucose values (SD) correlated positively with the overall AN score and the degree of the orthostatic reduction of systolic blood pressure (AN-score-SD r=0.47, p<0.05; orthostasis- SD: r=0.51, p<0.05). Mean Absolute Glucose: (MAG) correlated with 3 parameters of AN (AN-score-MAG: r=0.62, p<0.01; 30/15 ratio-MAG: r= -0.50, p<0.05; orthostasis- MAG: r=0.59, p<0.01). The HbA1c also correlated with 2 parameters of GV (HbA1c - Continuous Overlapping Net Glycaemic Action (CONGA): r=0.56, p<0.05; HbA1c- MAG: r=0.45, p<0.05). The frequency of hypoglycemia did not exhibit any correlation with characteristics of GV.
Conclusion: Severity of glycemic variability correlates with both parasympathetic and sympathetic dysfunctions in long-standing type 1 diabetes. Higher HbA1c is associated with more severe glucose variability. The relationship of increased glucose variability and autonomic neuropathy might be explained by the higher frequency of hypoglycemia and postprandial hyperglycemia in patients with neuropathy.
Disclosure: **S. Nyiraty:** None.

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The beneficial effect of intracavernosal mirabegron, a selective β$_3$-adrenoceptor agonist, on erectile dysfunction in streptozotocin-induced diabetic rats

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Background and aims: Diabetes mellitus is one of the common risk factors for erectile dysfunction (ED). Phosphodiesterase type 5 inhibitors (PDE5i), which are the first-line treatment for ED, are not effective in the management of diabetes-associated ED. Mirabegron, a recent selective β_3-adrenoceptor (β_3-AR) agonist, has been approved for the treatment of overactive bladder. The aimed of this study is to investigate the possible beneficial effect of mirabegron treatment on ED in streptozotocin-induced diabetic rats.
Materials and methods: A total of 20 adult male Sprague-Dawley rats were equally divided into control and diabetic groups. Diabetes was induced by single intraperitoneal injection of 45 mg/kg of streptozotocin. In vivo erectile responses were evaluated by the stimulation of cavernosal nerves and repeated after intracavernosal injection of mirabegron (dose of 0.4 mg/kg) in anaesthetised rats. The relaxant responses of corpus cavernosum (CC) strips were examined in the presence or absence of mirabegron (10 μM). β_3-ARs expression and localization were determined by Western blot analysis and immunohistochemistry in CC tissue.
Results: In vivo erectile responses in diabetic rats were lower than in control rats, which were partially restored after intracavernosal administration of mirabegron. Basal intracavernosal pressure (8.3±0.9 mmHg) in diabetic rats was markedly increased after mirabegron (39.5±6.9 mmHg, $p<0.001$). Mirabegron induced the marked relaxation after precontraction with phenylephrine in diabetic rat CC (100%). The nitrergic relaxation response to electrical field stimulation (10 Hz) in diabetic CC was lower than in control CC, which was increased in the presence of mirabegron (untreated 10.3%; treated 69.3%, $p<0.001$). Mirabegron markedly enhanced sodium nitroprusside-induced (10nM) relaxation in diabetic CC. The relaxant response to sildenafil (1μM) in the diabetic group was reduced as compared to the control group, which was potentiated after incubation with mirabegron. The expression and immunoreactivity of β_3-ARs localized to the smooth muscle cells of CC were observed in diabetic and control rats.
Conclusion: This study firstly revealed the beneficial effect of intracavernosal administration of mirabegron in partially improving erectile function in diabetic rats. This agent completely enhanced neurogenic relaxation of CC in diabetic rats. These results may be supported further by studies using combinations of mirabegron and PDE5i for the treatment of diabetic ED, especially in patients who do not respond to PDE5i therapy.
Disclosure: **D. Yilmaz-Oral:** None.

PS 084 Diagnosis and treatment of the diabetic foot syndrome

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Implementation of IWGDF risk classification in predicting the development of diabetic foot in type 2 diabetic patients admitted in the hospital: a three-year follow-up study

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Background and aims: Diabetic foot is one of the key areas of morbidity associated with diabetes, which is not only represent a major personal tragedy but also place a substantial financial burden on healthcare systems and society in general. Therefore, preventing the development of diabetic foot in patients with high risk is of important clinical significance. This study aimed to investigate the predictive value of International Working Group on the Diabetic Foot (IWGDF) risk classification on development of diabetic foot in type 2 diabetic patients adimitted in the hospital.
Materials and methods: Consecutive type 2 diabetic patients admitted to the endocrinology department of our hospital who were ≥18 years or older had been enrolled. Those already suffered from diabetic foot, peripheral neuropathy caused by any diseases, hypothyroidism and diseases affecting life span were excluded. Patients fulfilled the above criteria underwent a standardized general medical examination and detailed foot assessment. IWGDF risk classification were evaluated in these patients. Thereafter, patients were regularly followed up for three years. During the follow-up period, information of the patients suffered from diabetic foot were collected and analyzed. Receiver-operating characteristic (ROC) curve was used to evaluate the predictive value of the IWGDF risk classification on diabetic foot development in these patients.
Results: 254 patients (Male 59.4%) with the mean age of 54.7±12.8 years old, and HbA1c of 9.9±2.8% were recruited between September, 2012 and January, 2013. Based on the IWGDF risk classification, 177 patients (69.7%) were graded as risk group 0, 31 patients (12.2%) as risk group 1, 26 patients (10.2%) as risk group 2, and 20 patients (7.9%) as risk group 3. During the three-year follow-up period, 32 patients among the 254 patients (12.6%) experienced diabetic foot, with 12 patients (4.7%) ended up with amputation. It was found that using the combination of risk group 2 and risk group 3 had better value than using risk group 3 alone or the combination of risk group 1, risk group 2 and risk group 3 in IWGDF risk classification when considering the sensitivity and specificity to predict the development of diabetic foot (Table 1). Further analysis also revealed that the ROC curve for patients in risk group 2 and risk group 3 (0.919, 95% CI: 0.893~0.945, $p<0.01$) was superior to that for patients in risk group 0 and risk group 1 evaluated with IWGDF risk classification.
Conclusion: The IWGDF risk classification is an effective tool for predicting the risk of diabetic foot in type 2 diabetic patients admitted to the hospital. Patients who were in risk group 2 and risk group 3 evaluated with IWGDF risk classification may have higher risk to develop diabetic foot in the future.

Table 1. The sensitivity and specificity to predict the development of diabetic foot with different risk groups evaluated with IWGDF risk classification

	IWGDF risk classification		
	Risk group 3	Risk group 2 + Risk group 3	Risk group 1 + Risk group 2 + Risk group 3
Sensitivity	0.593	0.926	1.00
Specificity	0.945	0.799	0.612

Disclosure: **X. Hu:** None.

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Diabetic patients with foot ulcer express lower educational support needs and display more cognitive dysfunction

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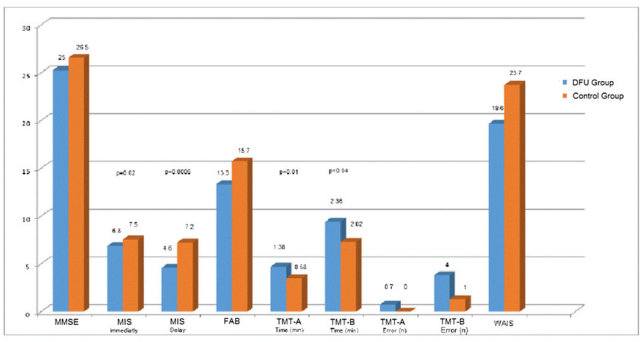
Background and aims: Therapeutic patient education (TPE) is essential for the prevention of diabetic foot ulcer (DFU). In this study, we explored the neuropsychological abilities of diabetic patients (DP) displaying DFU and their perception of their TPE needs.
Materials and methods: In this proof of concept study, we selected 22 consecutive adult DP with DFU, and a control group (CG) of 22 DP without active or past ulcer history, matched for age (± 5 years). Psycho-sensorial status was assessed by the Hospital Anxiety and Depression Scale and the Saint-Antoine Pain Questionnaire. TPE needs were estimated with a 4-point Likert Scale based on 9 items completed by 4 open questions. The interviews explored different aspects of diabetic self-management, knowledge, understanding and health priorities. Neuropsychological functioning was assessed using standardized tests: Mini Mental State Exam (MMSE), Memory Impairment Screen (MIS), Frontal Assessment Battery (FAB), Trail-making Test A and B (TMT-A and -B) and Wechsler Adult Intelligence Scale (WAIS). Both groups were compared using Wilcoxon's signed rank test for quantitative variables and conditional logistic regression models for qualitative variables.
Results: DP of the DFU group tended to have a longer disease duration (mean: 15 years *vs* 11) and more diabetes-related complications, compared to the CG. No significant difference was found for BMI, HbA1c, anxiety, depression or pain, TPE needs evaluated by Likert Scale, between the two groups. In open questionnaires, DP with DFU tended to declare less medical needs compared to the CG (8/22 *vs* 12/22). Needs were focused on material difficulties, none of them concerned educational skills, in contrast to 5 DP in the CG ($p<0.05$). Moreover, only 15 (68%) DP in the DFU group did consider health as a priority in their lives, whereas 21 (95%) in the CG did ($p= 0.06$). Cognitive abilities in the DFU group seemed to be lower as shown by the lower score obtained in different neuropsychological tests (Graph 1). MIS and TMT scores were significantly lower in the DFU group, reflecting a lower ability to memorize and treat information.
Conclusion: Although DP with DFU displayed more diabetic complications they expressed lower educational needs and less heath priorities. Their cognitive scores are in agreement with lower learning abilities and may suggest need to develop more medical help support to better health care. Whether this association is related to cerebral micro and/or macroangiopathy remains to be investigated.



Graph 1: Mean Scores obtain at the different neurocognitive tests in DP of DFU compare to the CG.

Disclosure: **C. Bouché:** None.

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Sudomotor examination should be regularly performed in patients from predialysis stage (CKD4) but also after transplantation to detect nerve regeneration

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Background and aims: Diabetes(DM) or hypertension are listed as the primary causes for 70% of new cases of end stage renal disease(ESRD). The aim of this prospective 5-year study was to examine the incidence of foot morbidity among diabetic and non-diabetic patients in different stages of chronic kidney disease(CKD).
Materials and methods: Overall, 199 patients during the preceding 5 years were enrolled. We included 108 diabetic patients (18 with type 1DM, 90 with type 2 DM) with the following conditions: 25 with glomerular filtration rate(GFR) 30-59mL/min /1.73 m^2- stage 3 CKD(G1); 27 on HD(G2b); 56 with GFR $\geq$90 mL/min/ 1.73m^2(G3b). We also included non-diabetic patients with the following conditions: 35 with nephroangiosclerosis on HD(G2a); 30 with other causes HD(G2c); and 26 transplant recipients (5 nephroangiosclerosis, 5 T1DM, 1 T2DM, 15 with other diseases) (G3a). Before 5 years, we had graded severity of foot pathology by Neuropathy Disability Score(NDS) plus Neuropad test (evaluated as time to total colour change), colour doppler, ulcer and/or amputation. Of the entire 199 patient population, 79 had meanwhile died.
Results: Five-year mortality rates were: 68% in G1(CKD3+DM); 57.1% in G2a(HD+NAScl); 70.4% in G2b(HD+DM); 36.7% in G2c(HD+Other); 3.84% in G3a(Transplanted); 19.6% in G3b(No-CKD+DM) ($p<00.1$). Despite the similar mortality, patients in G1 were older (71.12±7.8 years) compared with G2b(HD+DM) (60.3 ±13.1 years) and G3b(59.9±7.75yrs) ($p<0.01$). Comparing G2a(HD+NAScl) vs. G2b(HD+DM) among those who had died, male sex was more prevalent among diabetic patients, (45.7 vs. 70.4%, $p<0.05$); duration of HD was shorter in diabetic patients (5.2±2.5 vs. 4.6±2.5years, $p=0.03$). Prevalence of arterial hypertension in G1 vs. G2b vs.G3b was: 13(52%) vs. 26(96.3%) vs. 9(16.1%) ($p<0.01$) and duration of diabetes was 17.9±6.2 vs.23 ±10 vs. 15.9±7.8 years($p<0.01$). Neuropad time until colour change was: G1 8.9±5.8 min., G2a-c 26.8±8.2min., G3a 9.1±7.6min., G3b 11.3±7.4min. ($p<0.01$). Multivariable logistic regression analysis including Categories 1-3 diabetic foot (IWGDF) and Neuropad test found significant association of Neuropad time with CKD III-V (OR: 1.14, 95% Cl: 1.09-1.19, $p=0.000$) and mortality (OR: 1.05, 95% Cl: 1.02-1.08, $p=0.001$). Peripheral arterial disease among dead patients was: G1 8 (47.1%), G2b 15 (78.9%), G3b 7 (63.6%). No patient without diabetes developed ulceration or amputation in the entire study period. Ulcerations and/or minor amputations were present in G2b 5 (18.5%) and G3b 13 (23.2%). Major amputations were: 1 in G1, 1 in G3b and 6 in G2b(HD+DM) ($p=0.003$).
Conclusion: Category 3 diabetic foot (according to the Interantional Working Group on the Diabetic Foot classification) is exclusively seen in diabetic patients (whether on HD or not). Categories 1 and 2 may be present even in non-diabetic patients. We reccomend prospective monitoring of sudomotor dysfunction from CKD stage 4 onwards, regardless of the presence of diabetes. Indeed, this dysfunction is not only strongly associated with end-stage diabetic foot pathology but also with end-stage renal insufficiency, regardless of the presence of diabetes.
Disclosure: **D.S. Tesic:** None.

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Toe-brachial index is associated more strongly with healing and recurrence of chronic diabetic foot ulcers than ankle-brachial index in patients with type 2 diabetes

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Background and aims: Fifteen percent of people with diabetes develop an ulcer in the course of their lifetime. Eighty-five percent of the major lower-leg amputations in diabetes are preceded by an ulcer. Management of ulcers and preventing their recurrence is important for the quality of life. Both micro- and macro-angiopathy strongly contribute to the development and delayed healing of diabetic wounds. Peripheral arterial disease can be diagnosed noninvasively by segmental blood pressure measurement and calculating an ankle-brachial index (ABI) or toe-brachial index (TBI). The objective of this study was to determine whether ABI or TBI influences healing of chronic diabetic foot ulcers in patients with type 2 diabetes, and to determine whether TBI influences recurrence of ulcer in patients with healed foot ulcers.
Materials and methods: We recruited a total of 102 type 2 diabetic patients (63 men and 39 women) with chronic non-healing diabetic foot ulcers with Wagner grade 1 or 2 ulcers that are ≥2 cm in largest diameter at diagnosis for more than 1-month duration. The age was 69.2±15.8 years, and the diabetes duration was 16.2±11.4 years. Anthropometric, clinical, and laboratory data were measured. All patients were seen bi-weekly for debridement, offloading, and other treatments during the initial 8 weeks. All patients subsequently underwent ABI and TBI testing. ABI and TBI measurements were performed with the subject in a supine position, and were determined as the ratio of ankle or toe systolic blood pressure to the brachial systolic blood pressure, with both determined using an automatic device. To investigate recurrence status in patients with healed foot ulcers, Healed patients were followed with telephone interview after a minimum of 1 year of follow-up [median 2.3 (range 1.1-2.8) years].
Results: At 8 weeks, 64 of the 102 ulcers had completely healed. The patients were assigned into healed group (n = 64) or unhealed group (n = 38) according to clinical outcome of ulcer healing at 8 weeks. There were not significantly different in age, duration of diabetes, HbA1c, or initial wound size in diameter of the ulcer between the healed and unhealed groups. The healing time of foot ulcers in healed group was 6.2±2.4 weeks (range 4.0-8.0). The TBI was significantly ($P<0.05$) lower in the unhealed group (0.59±0.22) as compared with the healed group (0.74±0.21). ABI was not significantly different between unhealed and healed group. Age ($r = 0.481$; $p< 0.01$), duration of diabetes ($r = 0.460$; $p< 0.05$) and ABI ($r = 0.711$; $p< 0.001$) were significantly correlated with TBI. But BMI, cholesterol levels, HbA1c, and systolic and diastolic BP were not correlated with TBI. Univariate analysis revealed that TBI was significantly correlated with healing rate of ulcers ($r = 0.287$, $p < 0.05$) and duration of diabetes ($r = 0.410$, $p < 0.05$). At the end of follow-up (median 2.1 years), 21/64 patients (32.8%) had recurrence of diabetic foot ulcers. The recurrence rate in group with lower TBI (< 0.7) was significantly higher than that in the normal TBI (> 0.7) group (48.1% vs 21.6%; $P < 0.05$), but not correlated with ABI.
Conclusion: This study demonstrated that toe-brachial index is more strongly associated with the healing and recurrence rate of diabetic ulcers than ankle-brachial index. We suggest that the measurement of toe-brachial index may help to identify ulcers at risk of poor healing or recurrences in chronic diabetic foot ulcers in patients with type 2 diabetes.
Disclosure: **D. Cho:** None.

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Bedside blind bone biopsy for suspected diabetic foot osteitis: feasible, simple and useful

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Background and aims: To shorten therapeutic decision for suspected diabetic foot osteitis, Bedside Blind Bone Biopsy (B4) performed by a diabetologist might challenge biopsy usually performed by either an orthopaedic surgeon or a radiologist.
Materials and methods: Observational, monocentric study of B4 feasibility assessment in a diabetes inpatient setting. Inclusion criteria: foot ulcer (area >2 cm^2, lasting ≥4 weeks), suspected osteitis (clinically and/or X-ray), no antibiotics. B4 performed through clean skin with trocart (Madison^{TM} KDP13/6) following local and light systemic anaesthesia. Primary endpoint: collection of a suitable bone specimen. Adverse events: local (provoked ulcer/inflammation/necrosis, bleeding), general (fever, positive blood cultures) within 72h. Assessment of pain by the face appearance scale during B4 and by evaluating pain reliever consumption within 24h. Systematic blood cultures (1/hour) within 3 hours or in case of fever. Microbiological examinations were completed by histological examinations (HE) in the last ten B4 (table).
Results: 28 consecutive patients with type 2 diabetes. Male: 82%, age: 68 ±12yrs (Mean±SD), diabetes duration: 17±8yrs, HbA1c: 8,5±2%. Ulcer University of Texas scale: Grading 0/1/2/3: 3,5/3,5/3,5/89,5%, Stages A/B/C/D 25/43/11/21%. International scale: Grading 1/2/3/4: 4/0/46/50%. Severe proximal and distal peripheral arteritis: 14% and no arteritis in 35%. Systolic pressure index <0,9: 57%. TcPO2 <30mmHg: 57%. B4 performed within 5 days following indication in every patient. Procedure duration: 60±10mn. B4 sites: metatarsal (65%), proximal phalange (14%), distal phalange (14%), calcaneus (7%). Primary success: 96,5% (2±1 specimens/patient). Positive cultures: 50%. No local complication. Fever occurrence: 21%. Bacteriemia: 11%. Per-B4 pain: 7%. Post-B4 pain: 50% (step 2 relievers prescribed in 7%).

	M+/H+	M-/H-	M+/H-	M-/H+
***n* (%)**	2 (20%)	6 (60%)	2 (20%)	0 (0%)

Table: Results of combined microbiological (M) and histological (H) examinations (10/28). M+: positive culture (contaminated bone not included), H+: presence of neutrophils
Conclusion: For suspected diabetic foot osteitis, B4 is easy and quick to perform. It is informative (notably if completed by HE) and safe with rare major related events. Its daily clinical practice by a diabetologist might shorten therapeutic decision. Its cost-effectiveness remains to be evaluated.
Disclosure: **F. Féron:** None.

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Incidence and predictors of recurrent and new diabetic foot ulcers

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Background and aims: Patients with diabetic foot ulcers (DFU) have a high risk of amputations and increased mortality compared to patients without DFU. Studies among patients with a healed DFU have shown that two-thirds develop a recurrent (at the same location) or a new (at another location) DFU within 5 years. These relatively small studies with up to 83 patients have found the following risk factors or indicators for recurrent/new DFU: neuropathy, plantar ulcer location, Charcot feet, foot deformities, peripheral vascular disease, osteomyelitis, high HbA1c, macrovascular complications and non-adherence with foot-wear and -care. Some of these studies are restricted to only neuropathic patients

and by not calculating progression rates. Aims: 1) among patients with a healed DFU to estimate the progression rate to recurrent/new diabetic DFU, 2) to compare the progression rates depending on whether the healed ulcer was neuropathic, neuro-ischemic or ischemic, and 3) to study selected risk factors rate ratios (RR) for progression to recurrent/new DFU.

Materials and methods: A retrospective cohort study including all patients with a healed diabetic foot ulcer at our centre from January 2010 to October 2016. Patients were followed to an outcome (a recurrent/new DFU), to end of contacts with our centre, to death or to study end. All information was extracted from our electronic patient record system. Poisson regression analyses were made.

Results: Among 780 patients with a healed DFU, (489 (63%) neuropathic, 202 (26%) neuro-ischemic and 89 (11%) ischemic), 53% (33%/person year) progressed to a recurrent/new DFU during follow-up. The patients were followed for 1249 years in total (median 1.04 (Q1= 0.38 - Q3=2.46) years pr. patient). When adjusted for age and gender, the RR for neuro-ischemic vs. neuropathic was 1.29 (95% confidence interval (CI) 1.03-1.61) and ischemic vs. neuropathic was 1.42 (95% CI 1.04-1.95). A quarter of the patients (26%) died during follow-up. A majority of the DFU seen during follow-up were new (88 %). Men - RR 1.26 (95% CI 1.01-1.56), patients with lost sense of vibration (greater than 50 Volt) - RR 1.31 (95% CI 1.08-1.59), patients with Charcot feet - RR 1.66 (95% CI 1.20-2.28) and patients with foot deformities - RR 1.26 (95% CI1.00-1.59) had higher progression rates to recurrent/new DFU compared to patients without these risk factors. Patients with type 1 diabetes - RR 0.81 (95% CI 1.01-1.56), non-smokers - RR 0.76 (95% CI 0.63-0.93) and patients with a creatinine level less than 90 μmol/l - RR 0.76 (95% CI 0.63-0.93) had lower risk of progression to a recurrent/new DFU compared to patients without these characteristics.

Conclusion: Per year one third of the patients progressed to a recurrent/new DFU which is comparable to other studies. Patients with a neuro-ischemic or ischemic DFU progressed to recurrent/new DFU at a higher rate than patients with neuropathic DFUs. Male gender, type 2 diabetes, smoking, lost sense of vibration, Charcot feet and foot deformities were risk factors for recurrent/new DFU.

Disclosure: **S. Engberg:** None.

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Prevalence of distal symmetric polyneuropathy in hospitalised diabetic patients

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Background and aims: Distal symmetric polyneuropathy (DSPN) is one of the most prevalent chronic complications of diabetes but is largely underdiagnosed. It's frequently painful and if undiagnosed can lead to diabetic foot ulceration. Screening for DSPN enables early intervention, prevention of complications and appropriate treatment of symptoms. The Semmes-Weinstein Monofilament Examination (SWME) is currently the method of choice to screen DSPN, but ideally two neurologic tests should be used. The Michigan Neuropathy Screening Instrument (MNSI), which evaluates vibratory sensation and ankle reflex, has been proposed as a useful screening test of DSPN. DN4 is a scale that evaluates the presence of neuropathic pain. The objective of this study was to determine the prevalence of DSPN in hospitalized diabetic patients by both methods (MNSI and SWME) and the percentage of painful neuropathy.

Materials and methods: Diabetic patients admitted to internal medicine wards of two different Portuguese hospitals within a 5 months period were evaluated using the MNSI and the SWME after exclusion of other causes of polyneuropathy. A score > 2 in the clinical examination of MNSI or a ≤ 7 positive answers in a total of 10 in SWME were considered diagnostic of DSPN. Patients were also evaluated for the presence of neuropathic pain with the DN4 scale. A score ≥ 4 was positive for painful neuropathy.

Results: A total 88 patients were evaluated: 74.7 ± 13.2 years; 54.5% female; 93.2% type 2 diabetic patients with 12.0 ± 8.8 years of disease duration and HbA1c 7.57 ± 1.79 %. Macrovascular complications were present in 40.2% of the patients and microvascular in 26.4% of them. Only 6 (6.8%) patients had a previously diagnosis of DSPN. The MNSI was positive in 66 (75%) of the patients while SWME was positive in 33 (37.5%) of patients. The rate of undiagnosed patients was 93.8% using MNSI and 93.9% using SWME. The agreement rate between MNSI e SWME was 44.3%: 25 patients were positive in both instruments and 14 negative. Using both instruments would classify 74 out of 88 (84.1%) patients with DSPN and painful neuropathy was present in 28 (38.9%) of these.

Conclusion: This study detected a high prevalence of undiagnosed DSPN among diabetic hospitalized patients, suggesting that hospitalization is an excellent opportunity to screen for this complication. Our data indicates that using both MNSI and SWME increases the detection of DSPN.

Disclosure: **H.S.S. Gonçalves:** None.

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Multidrug resistant bacteria: an increasing complication of diabetic foot

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Background and aims: Multigrug resistant (MDR) infections complicate diabetic foot ulcers (DFU) severely worsening their prognosis. The aim of this study was to assess the prevalence of MDR phenotypes of different bacterial strains in diabetic patients followed by our diabetic foot clinic from January 2001 until December 2014.

Materials and methods: We retrospectively analysed 7.826 culture results from deep wound specimens in diabetic patients (M/F: 6.065/1.761. Age: 63.2±11.7 yrs) followed by our outpatient Clinic for DFU. From all bacterial strains we selected those more prevalent and we analysed antimicrobial sensitivity pattern in relation to the more widespread antibiotic resistance phenotypes. In particular, we evaluated the prevalence of Staphylococcus aureus (SA), Pseudomonas aeruginosa (PA) and Enterobacteriae (EB), sorting out Methicillin-Resistant SA (MRSA), PA resistant to Ciprofloxacin (CiproRPA) and Carbapenem (CRPA), EB resistant to Ciprofloxacin (CiproRE) or Extended Spectrum Beta Lactamase producers (ESBL). To test if the MDR pattern changed overtime, we divided the obtained results in two groups: the first (Group A) included those from 2001 until 2007 while the second (Group B) from 2008 until 2014.

Results: SA was detected in 2.483 specimens in Group A and in 2.131 in Group B (NS), the presence of MRSA was 58.7% in Group A and 51.2% in Group B (NS). PA was observed in 1.428 specimens in Group A and 1.783 in Group B ($p < 0.03$): in particular, CiproRPA was detected in 45.1% of cultures in Group A and 64.1% in Group B ($p < 0.04$) while CRPA in 32.7% in Group A and 34.2% in Group B (ns). The presence of EB was detected in 1.516 specimens in Group A and 2.032 in Group B ($p < 0.001$); CiproRE prevalence was 28.0% in Group A and 47.7% in Group B ($p < 0.02$) while ESBL prevalence was 23.0% in Group A and 39.7% in Group B ($p < 0.05$).

Conclusion: In conclusion, our data confirmed the high prevalence of MDR bacteria infections in DFU and their increasing overtime, stressing the importance of a close monitoring of antimicrobial drugs susceptibility.

Disclosure: **E. Iacopi:** None.

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Assessment of pain sensitivity and speed of wound healing in rats with streptozotocin-induced diabetes

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Background and aims: Diabetes mellitus is often accompanied by peripheral neuropathy and various disorders of reparation processes. However, the relationship between neural disorders and pathological wound healing in DM remains unclear. Our aim was to assess the relationship between the development of peripheral neuropathy and wound healing rate in rats with streptozotocin-induced DM.
Materials and methods: The study was performed in rats, diabetes was induced by a single injection of streptozotocin in 0.1 M citrate buffer in a dose of 65 mcg/kg. In control group an injection of 0.1 M CB was performed. 42 days later, the wound on the rats' back was inflicted. The observation of wounds lasted for 8,16 or 24 days. During whole period of experiment DM rat group received maintenance therapy with insulin detemir 1 IU/kg/day. The assessment of the development of peripheral neuropathy was performed by the pain test with the tail immersed in hot water every 7th day. The evaluation of wound healing was based on wound's area measurement every 3rd day. Skin samples were taken at 0, 8, 16 and 24 days of wound healing. The samples were stained with hematoxylin and, also immunohistochemical staining on ß2 - adrenergic receptors (ß2-AR) and Ki67 were conducted.
Results: To 42 day of DM the time of withdrawal of the tail in rats with diabetes has almost doubled in comparison with the group of CB ($p<0.05$), which allows to establish the presence of sensory neuropathy. At the same time, a significant gap in the rate of wound healing in the group of rats with DM was noticed. According to the results of histological staining it was found that the intact skin was the same in all groups. On 8 day after modeling of the wound in the region of the edges of the wound, the expression of Ki67 was significantly less than in intact areas ($p=0.004$). On the 16th day, the expression of Ki67 in the area of the wound increased and did not differ significantly from the remote areas of the skin. On the 24th day, Ki67 staining was again significantly weaker in the area of the wound edges. The density of ß2-AR in diabetic group at the wound edge was lower than in intact areas.
Conclusion: STZ-induced DM in rats is accompanied by development of peripheral sensory neuropathy and a decrease in the rate of wound healing already to 42 day of the disease. Morphologically, the decrease in the rate of wound healing is manifested by decrease of square cone area and the rate of appearance of keratinocytes in the wound area despite the fact that the intact skin of diabetics did not differ from the intact skin of healthy animals. According to immunohistochemical analysis, time and zone factors were significant for Ki67 expression, and only time factor was significant for ß2-AR expression.
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Disclosure: **A.M. Gorbacheva:** Grants; Russian Science Foundation project № 16-15-10365.

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Transcutaneous oxygen pressure as a predictor for short term survival in patients with diabetic foot ulcers

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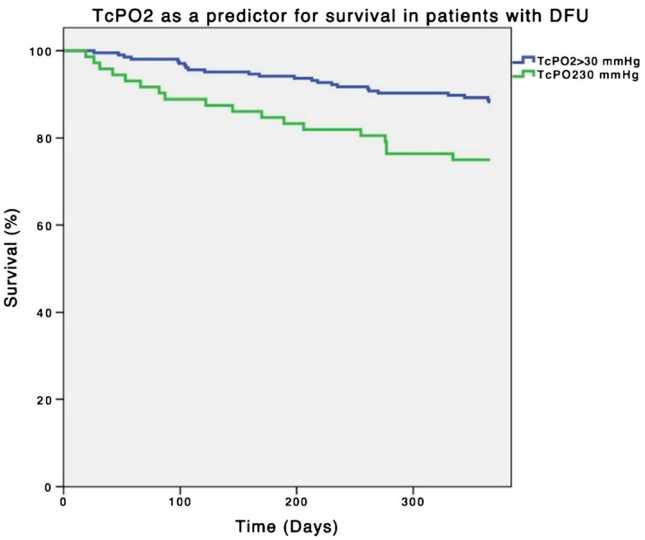
Background and aims: Ankle-brachial index (ABI) is the most commonly used test for the diagnosis of peripheral vascular disease (PVD), and is considered a marker for increased cardiovascular risk both in the general and diabetic population. Transcutaneous oxygen pressure ($TcPO)_2$ is a test considered to better reflect the microvascular status and has in some studies shown better correlations with diabetic foot ulcer healing. Several studies have suggested a cut-point of $TcPO_2$ >30 mmHg as a threshold for acceptable oxygen supply for wound healing. Whether $TcPO_2$ could be a risk marker for mortality in diabetic foot ulcer (DFU) patients has to our knowledge not been studied. The aim of this study was to evaluate the predictive value of $TcPO_2$ ≤30 mmHg, arterial toe blood pressure (TBP, cut-off level ≤ 50 mmHg) and ABI (cut-off level ≤0.8 and ≥1.4) on 1-year mortality.
Materials and methods: We enrolled 277 consecutive patients aged < 90 years with diabetes and at least one DFU who attended our multi-disciplinary diabetic foot clinic. Patients were screened with transcutaneous oxygen pressure ($TcPO_2$) measurements at the dorsum of the foot, ankle-brachial index (ABI) and arterial toe blood pressure (TBP) with Perimed© diagnostic instrument. All patients were treated according to international guidelines and were evaluated for vascular intervention when needed. After one year mortality data was obtained from the national death registry in Sweden. Survival was analysed with Kaplan-Meier curves and log rank test and to adjust for age and gender a Cox regression analysis was performed.
Results: 277 patients (34% women) with a median age of 75 (67-82) years were evaluated in this study. 15% of the patients died during the first year of follow-up. $TcPO_2$ ≤30 mmHg was associated with a significantly higher 1-year mortality rate compared to TcPO2 >30 mmHg (25% vs 12%, p=0.005, figure 1). Also among patients with TBP ≤ 50 mmHg 1-year mortality was higher compared to patients with TBP>50 mmHg (23% vs. 12%, p=0.014). ABI did not influence 1-year mortality. After adjusting for age and gender in a Cox regression analysis, only age and $TcPO_2$ ≤30 mmHg were independently predicting 1-year mortality with a hazard ratio of 2.4 (1.3-4.4, 95% CI, p=0.006) for $TcPO_2$ ≤30 mmHg. TBP did not independently affect mortality in this analysis.
Conclusion: This study indicates that $TcPO_2$ ≤30 mmHg is an independent prognostic marker for short term survival among patients with diabetic foot ulcers.



Supported by: Skane county council's Research and Development Foundation
Disclosure: **K. Fagher:** None.

PS 085 Taking good care of the foot

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The impact of topical phenytoin loaded nanostructured lipid carriers in healing of neuropathic diabetic foot ulceration

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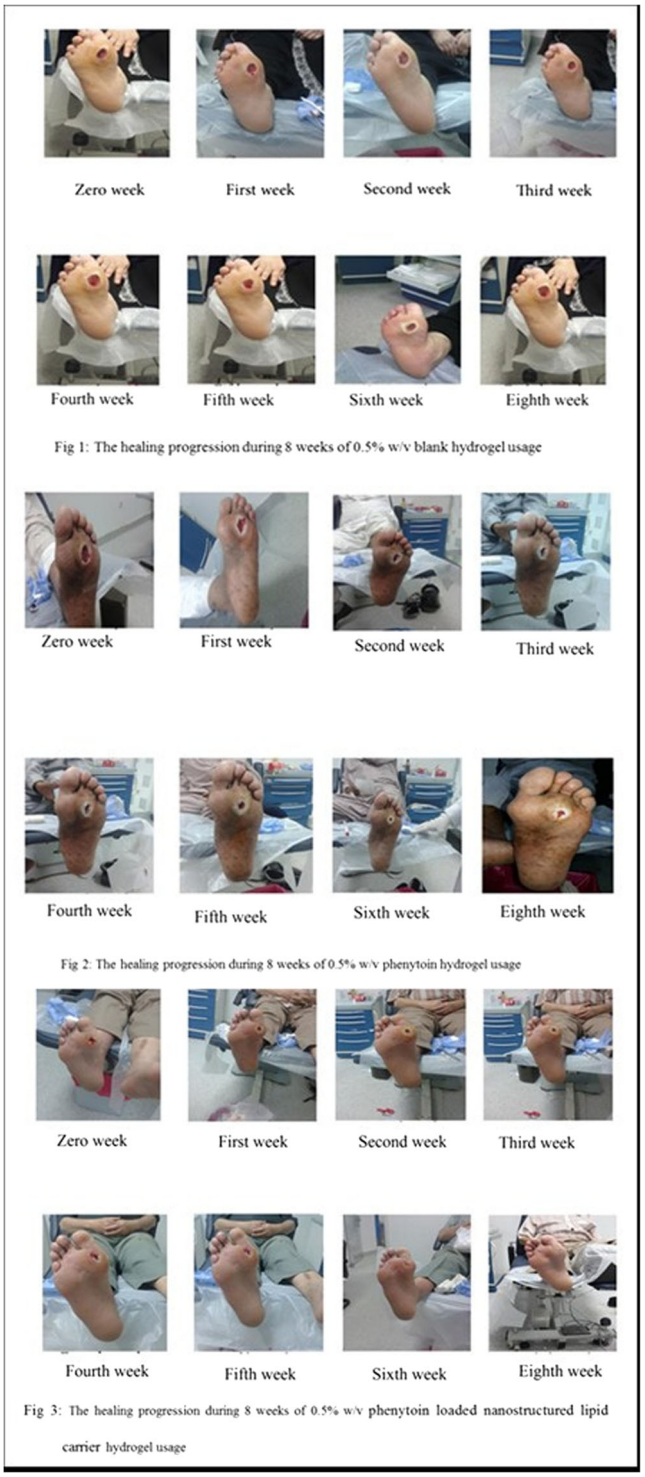
Background and aims: Many previous researches support various forms of topical phenytoin as an effective promoter for healing of diabetic foot ulceration. Recently, nanostructured lipid carrier was suggested to increase loading of topical drugs. We aim to study the impact of topical phenytoin loaded nanostructured lipid carrier in improving wound healing in patients with neuropathic diabetic foot ulceration.

Materials and methods: Twelve patients with neuropathic diabetic foot ulceration under the metatarsal-heads were enrolled in this study. Patients were comparable regarding ulcer size, grade & control of diabetes with no major deformity. All patients were managed by weekly sharp debridement if indicated & offloading with cast shoes done by the same hands. They were divided into 3 groups: phenytoin loaded nanostructured lipid carrier hydrogel 0.5% (Group 1), phenytoin hydrogel 0.5% (Group 2) & blank hydrogel (Group 3). Wound area was reassessed after 1, 4 & 8 weeks. Also, we investigated the in vitro release of phenytoin & phenytoin loaded nanostructured lipid carrier hydrogels by modified Franz diffusion cell at pH 7.4 up to 48h.

Results: Group 1 showed complete healing of 2 cases & smaller wound area compared to the other groups ($\rho < 0.05$). Baseline wound area of Group 1, 2 and 3 were 3.42±1.77, 3.62±1.98 & 3.87±2.39cm^2, respectively. The average wound area changed to 3.19±1.98, 3.51±1.75 & 3.60±1.63cm^2 after the first week; 0.86 ±0.40, 2.62±1.40 & 4.07±1.65cm^2 after the fourth week; 0.10 ±0.15, 1.69±0.78 & 4.06±1.93cm^2 after the eighth week, respectively. Overall reduction in ulcer size was 97.48±2.61% for Group 1, in comparison to 52.29±9.18% & -9.58±14.85% for Group 2 & Group 3 respectively ($\rho < 0.001$) (as shown in figures 1, 2, & 3). This may be attributed to small particle sizes of the nanostructured lipid carrier hydrogel with subsequent large surface area, its lipoid nature (acts as a reservoir), in addition to solubility & penetration enhancement that increase the skin delivery. Also, the in vitro release study clarified that, drug release from the nanostructured lipid carrier hydrogel displayed a biphasic release pattern with initial burst followed by sustained release. On the contrary, in phenytoin hydrogel drug release was very rapid & completed within 48h.

Conclusion: Phenytoin loaded nanostructured lipid carrier dressing is more effective than phenytoin hydrogel at the same concentration in healing of neuropathic diabetic foot ulceration. This effect may be assigned to its small particle sizes with consequent increase in its solubility, in addition to their biphasic release pattern that is recommended for topical products. These promising results encourage large scale trials for use of phenytoin loaded nanostructured lipid carrier in treatment of diabetic foot ulcers & other chronic wounds.



Disclosure: **M.M.M. Motawea:** None.

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Exploring the impact of liraglutide on diabetic foot ulcers on subjects with type 2 diabetes and increased risk of cardiovascular events: results from the LEADER trial

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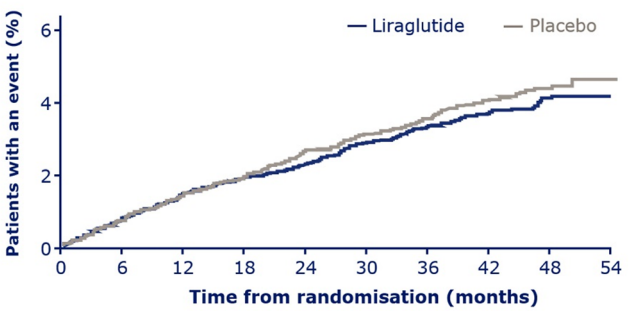
Background and aims: The risk of developing foot ulcer in people with type 2 diabetes (T2D) is increased due to various factors, including peripheral neuropathy and peripheral arterial disease. The LEADER trial (Liraglutide Effect and Action in Diabetes: Evaluation of cardiovascular outcome Results) reported cardiovascular (CV) risk reduction vs placebo in addition to standard of care (SoC). To date, limited information on the treatment effect of glucagon-like peptide-1 receptor agonists (GLP-1RAs) on diabetic foot ulcer (DFU) or their outcomes has been published. We investigated these measures with liraglutide vs placebo from the LEADER trial.
Materials and methods: LEADER was a randomised, double-blind, international, multicentre, placebo-controlled CV outcomes trial assessing the CV and long-term safety of liraglutide up to 1.8 mg/day vs placebo, both in addition to SoC for up to 5 years, in patients with high CV risk and T2D. Information on DFU was systematically collected in LEADER. Based on this, DFU complications were assessed post-hoc by the sponsor through review of the individual cases.
Results: DFU was reported as medical history at baseline in 4.5% and 4.2% of patients receiving liraglutide and placebo, respectively; 1.5% and 1.3% of patients, respectively, had DFU at screening. Proportions of patients reporting at least one episode of DFU during LEADER were similar between patients receiving liraglutide vs placebo (3.9% vs 4.2%, HR=0.91, 95% CI [0.75-1.12] p=0.38). A lower rate of DFU events was observed in the liraglutide group vs placebo from around month 18; this continued for the remainder of the trial, although not reaching statistical significance (Figure). Among patients with DFU during LEADER, proportions reporting complications of DFU included infection (60.8% [107/176] vs 68.6% [131/191], p=0.12), involvement of underlying structures (36.4% [64/176] vs 41.9% [80/191], p=0.28), any amputation (25.0% [44/176] vs 35.1% [67/191], p=0.04) and peripheral revascularisation (11.4% [20/176] vs 12.0% [23/191], p=0.84) with liraglutide and placebo, respectively. Of those in need of amputation, a lower proportion of amputation of the foot, lower leg or leg was reported for liraglutide vs placebo (29.5% [13/44] vs 44.8% [30/67], p=0.01).
Conclusion: LEADER is the first trial of GLP-1RAs in T2D to report DFU data. These findings may suggest a reduced risk of DFU and associated complications with liraglutide vs placebo in patients with T2D and increased risk of CV events.

Figure: Time to first DFU among all patients in LEADER



Kaplan-Meier plot. Full analysis set. Based on MedDRA search (version 18.0) of SAEs + non-serious MESIs. DFU, diabetic foot ulcer; MedDRA, Medical Dictionary for Regulatory Activities; MESI, medical event of special interest; SAE, serious adverse event

Clinical Trial Registration Number: NCT01179048
Supported by: Novo Nordisk A/S
Disclosure: **K. Dhatariya:** Other; K. Dhatariya is on the Clinical Endpoint Adjudication Committee for the Sotagliflozin trials implemented by Lexicon pharmaceuticals and has received consulting fees and honoraria from Novo Nordisk.

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Treatment of a new diabetic foot ulcer in a diabetic foot service: a one year follow up prospective study of 347 patients

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Background and aims: The objective of the study was to evaluate the 1 year outcome of the management of 347 new diabetic foot ulcers by the multidisciplinary team of our diabetic foot service (DFS)
Materials and methods: A single centre prospective cohort study of 347 patients with a new diabetic foot ulcer (DFU) between 1/07/2009 and 31/12/2010. We used university Texas (UT) and PEDIS classification for all the DFU Patients who needed parenteral antibiotic therapy, revascularization or surgical debridement were hospitalized in our DFS The follow up was 1 year après the date of inclusion Multiple logistic regression modeling was used to identify independent predictors of outcome (non healing, major amputations and deaths)
Results: At inclusion: Average age : 64.88 ± 13.7 years, 68% male, Type 2 Diabetes mellitus (D) in 87%, Age of DM: 18.7± 12.3 years Average age of the DFU : 187 ± 546 days According to PEDIS classification, 69% DFU were classified P2 or P3, infected in 55%, 82% had a surface > 1cm², and Depth 2 et 3 in 51% According to UT classification, 28% C, 33% class D, 14% class B, 52% (75%: B+C+D) Depth 2 and 3: 51% At M12 (12 months): 312 patients were reviewed (9% lost of follow up) Healing rate was 68%, average duration of healing 189 ± 160 days The univariable associations of the potential predictors of non-healing were hemodialysis (p =0.006), Peripheral arterial disease (PAD) (p=0.000001), Surface of DFU (p= 0.003) et DM age (p=0.05) The multivariable association with independent predictors of non-healing were PAD :OR 0.251 [95% CI 0.112-0.563], p =0.001, Extent of the DFU: OR 0.385 [95% CI 0.186-0.799] p= 0.01, hemodialysis OR 0.323 [95% CI 0.143-0.726]p= 0.006, Duration of DM: OR 1.025 [95% CI 1.002-1.049] p =0.032 Amputations : 7.1% major amputations (5.7% below-knee, 1.4% above-knee) ,11.5 % minor amputations (toes ; 11.5%, trans metatarsal 6.1%) In univariate analysis major amputations were associated with PAD (p= 0.03), Osteomyelitis (p= 0.01) and hospitalization in post acute department(p= 0.000) In multivariate analysis predictor was hospitalization in post acute department : OR 23.5 [95% CI 7.10-78.15] P=0.000 Mortality Death rate was 8.3% at M12, and alive healed patients without amputation: 60% In univariate analysis predictors of mortality were PAD (p= 0.0000), Age (p= 0.0009), Duration of DFU at inclusion (p=0.001), Body mass index (p= 0.008) and non healing at M12 (p= 0.00002). In multivariate analysis mortality was associated with PAD: OR 13.2 [95% CI 1.81-96.2]; p= 0.01 and non healing at M12 : OR 0.191 [95% CI 0.07-0.523] ; P=0.001
Conclusion: Despite an average age of 65 years, a duration of DFU of more of 6 months, and 75% of DFU with infection, ischemia (or both) the healing rate was 68 %, the death rate of 8.3% and the major amputation rate of 7.1%. In France according to a recommendation of the HAS (Health Authority) patients with DFU of at risks foot have to be addressed within 48 hours to a diabetic foot service This study showed that the results are not so bad and could probably have been even better with an earlier management. Ischemia is the main risk factor for healing, death and amputation
Disclosure: **G. Ha Van:** None.

1007

Long-term changes in BMD and bone turnover markers in diabetes patients with or without previous Charcot foot

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Background and aims: It remains controversial whether the bones in the diabetic Charcot foot is osteoporotic, and whether the skeleton in general in these patients is osteoporotic. In a previous study, diabetes patients with acute Charcot had increased markers of bone turnover, whereas patients with chronic Charcot had decreased BMD of the calcaneus in the Charcot foot, -otherwise there were no differences in BMD or markers of bone turnover between diabetes patients with acute or chronic Charcot or without Charcot. The aim of the present study was to investigate changes after 8.5 years in BMD and markers of bone turnover in diabetes patients with or without previous Charcot.
Materials and methods: An 8.5-year follow-up study of 49 individuals with diabetes mellitus, 24 of whom also had acute or chronic Charcot foot at the baseline visit in 2006. BMD (total body, hip, spine and calcaneus) by DXA (Lunar Prodigy) and standard biochemical markers of bone turnover (P1NP, CTX-1 and osteocalcin) were measured.
Results: 22 diabetes patients were included in the follow-up, whereas 12 had passed away, 4 were out of reach, 7 declined and 4 had had amputations. Of the 22 diabetes patients included, 11 had previously had a Charcot foot, while the other 11 did not. On average, the age of the included diabetes patients at the follow up was 69 yrs, diabetes duration 27 yrs, HbA1c 59 mmol/mol, 18 were males and 4 females, 7 with type 1 diabetes and 15 with type 2 diabetes, with no difference between those with or without previous Charcot ($p>0.05$). There was no difference in the change in BMD from baseline to follow-up between the diabetes patients with and without previous Charcot, in neither total-body, hips, lumbar spine, calcaneus ($p>0.3$) nor in calcaneal BMD between the previous Charcot foot or healthy foot in those patients with a previous Charcot foot ($p>0.3$). The mean annual change in BMD, over the 8.5 years, in the different skeletal sites ranged from -0.3% to +1.2%. At the follow up, 16 of the diabetes patients had normal T-score, 3 had osteopenia, and 4 had osteoporosis (according to WHO criteria, T-score < -2.5) with no difference in the distribution between those with or without previous Charcot ($p>0.05$). Furthermore, there were no differences between the diabetes patients with or without previous Charcot in changes from baseline to follow-up, nor at follow-up, in the markers of bone turnover ($p>0.05$).
Conclusion: After more than 8 years of follow up, diabetes patients with, or without, previous Charcot had similar BMD and biochemical markers of bone turnover. Thus, in diabetes patient, a Charcot foot does not seem to have long term negative impact on bone mineral or risk of osteoporosis.
Clinical Trial Registration Number: NCT02335931
Disclosure: **O.L. Svendsen:** None.

1008

Five year mortality following diabetic amputation has not changed over 14 years

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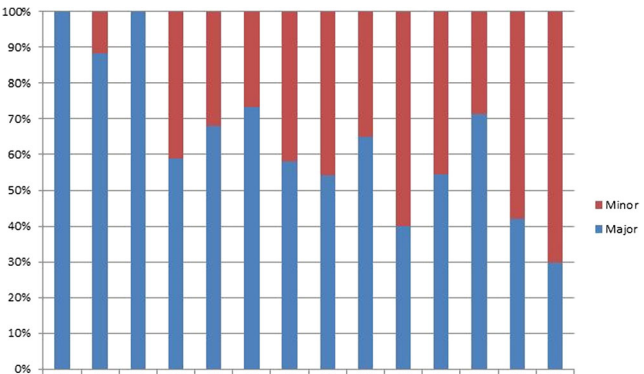
Background and aims: Diabetes is the most common cause of non-traumatic limb amputations. The consequences of undergoing an amputation remain significant. There is an increased risk of mortality compared to non - diabetic patients. Other factors such as the emotional impact and the associated rehabilitation programme also need to be taken into account. There is higher 5- year mortality following diabetes related amputations which is about 70% as per NICE literature review. We wanted to study if this has changed over the last 14 years in our cohort.
Materials and methods: This was a retrospective study on 356 patients who underwent amputation between 1997 and 2010. List was obtained from discharge summary and theatre record. Subjects who underwent amputation between 1997 - 2003 were 'Early' cohort and those between 2004 - 2010 were 'Late' cohort. Amputations were grouped at their first surgery into major (above ankle) or minor (below ankle). Electronic database was examined in 2016 to know the date of their death. The cause of death could not be ascertained.
Results: There was no difference in the mean number of total amputations performed annually between early and late cohort (25 +/- 8.8 vs 25.9 +/- 4.9; $p > 0.05$), however there was a significant reduction in major amputations performed annually (18.4 +/- 3.9 vs 12.9 +/- 3.2; $p = 0.01$) and a trend for a rise in minor amputations (6.6 +/- 6.3 vs 13.0 +/- 5.1; $p = 0.07$) between these cohorts. 5-year mortality was 59% which was significantly higher in major amputation group than minor (63.9% vs 51.1%; $p = 0.02$). There was no difference between 'Early' and 'Late' cohorts in 5-year total mortality (60.1% vs 57.4%; $p > 0.05$), major amputation mortality (63.2% vs 64.0; $p > 0.05$) or minor amputation mortality (43.9% vs 52.8%; $p > 0.05$).
Conclusion: There has been no change in total mortality with time in subjects with diabetes who needs lower limb amputations. There is significant reduction in major amputation with increase in minor amputation between these cohorts. The change in amputation level started with the introduction of multi-disciplinary diabetic foot clinic in 2001.



	97	98	99	00	01	02	03	04	05	06	07	08	09	10
Minor	0	2	0	16	7	8	13	11	7	15	15	6	18	19
Major	22	15	14	23	15	22	18	13	13	10	18	15	13	8

Disclosure: **B.P. Soo:** None.

1009

Care path of patients with diabetic foot wound in France: a multicentre observational study

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Background and aims: Prevalence of hospitalizations for a diabetic foot ulcer or amputation remains very high in France (912 per 100 000 diabetic patients in 2013). However, national and international recommendations on diabetic foot ulcers are clear: patients should be addressed to a multidisciplinary foot centre within 24 to 48 hours after wound

occurrence. The aim of this study was to describe the care pathway of patients with a diabetic foot ulcer before arriving in foot centers, and to look for factors, influencing pathways dysfunction.
Materials and methods: This is a multi center, observational study including 21 french foot centres. Each center included the ten successive patients attending for a new foot ulcer between May and July 2015. The only exclusion criteria was " French not spoken". Patient pathway was accessed by a pre-defined questionary. The primary outcome was " time between ulcer occurrence and consultation in the foot centre". Analysis was performed by Student or Man-Whitney test, and ANCOVA.
Results: 209 patients were included: 133 men, 76 women, 29 to 97 years old (mean: 68 years) ; 89% had type 2 diabetes ; 39% have had a previous wound ; 17% had an amputation and 31% had peripheral arterial disease. 26% have had a prescription of an offloading device before referring to the foot center but only 35 patients (17%) complied. Concerning wounds 84 (40%) were infected and 95 (45%) reached joint, tendon or bone. Time between ulcer occurrence and the first GP contact was 12 (+/- 2) days, median 5 days. Patients were addressed to the foot center after an average of 83 days (+ / -15), median 26 days. Factors that significantly influenced the delay before referring were " having had a prescription of offloading ($p = 0.043$)", "having had dressing by a nurse ($p = 0.01$)", or "being resident of Ile de France region ($p = 0.002$)". The significant statistical relationship for these criteria persisted in multivariate analysis.
Conclusion: The mean delay before referring patients with a diabetic foot wound to a foot center in France is major. Some regions are more concerned than others. The prescription of an offloading device by GP is rare. Partial care of the wound (dressing or offloading device) participate to this delay. Information on foot centers location and diffusion of international guidelines on diabetic foot care is urgently needed.
Disclosure: **C. Amouyal:** None.

1010

Effectiveness of implementing a joint diabetes-renal-microbiology initiative in individuals with active diabetic foot disease receiving dialysis

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Background and aims: Diabetic foot disease (DFD) and lower extremity amputation are considered to be 10 times higher in diabetic individuals receiving dialysis. Furthermore, they have disproportionately high rates of foot-related hospital admissions. Those on dialysis often lose contact with care outside the dialysis units and access to foot care is limited in dialysis units. Therefore, interventions and strategies aimed at reduction of DFD burden in dialysis patients are important. We report on our joint diabetes-renal-microbiology multidisciplinary team (JDRMDT), set up to address the challenge and facilitate cross-specialty care.
Materials and methods: Retrospective analysis of all the patients discussed at the JDRMDT meetings over a 24 month period. Patients were reviewed by the acute foot team while undergoing dialysis with liaison and referral to the hospital multidisciplinary foot unit when required. Where necessary a vascular or orthopaedic review was arranged whilst the patient was still in the dialysis unit. A summative team discussion was held at the end of each week comprising of a podiatrist, diabetologist, nephrologist and microbiologist to update and address any concerns. Our analysis was limited only to those with *active* DFD mandating closer surveillance.
Results: We discussed 37 patients with an average age 65±12 years (mean±SD), males 51%, with active DFD. Duration since dialysis start was 3.5±3.1 years. Glycated haemoglobin was 56±18 mmol/mol with BMI of 28.4±6.9 kg/m^2. In 24/37 (67%) of patients, there was evidence of significant peripheral vascular disease; they underwent 2.3±2.1 revascularisation procedures. There were 14 (38%) minor amputations (13/14 had CLI) and 4 (11%) major amputations (all had CLI). Microbiology was predominantly polymicrobial (44%) with gram-negative organisms predominating although pure MSSA (17%), MRSA (21%) VRE (10%) were also noted. Overall, 8/37 (22%) died during this period with a higher (50%) mortality observed among those with major amputations. While emergency hospital admissions were 6.0±5.1 episodes/patient; only 1 ±1.25 episodes/patient were foot-related.
Conclusion: The JDRMDT was an effective way of bridging the care gap in diabetic foot patients receiving dialysis. It allowed for complex diabetic foot care delivery within the dialysis unit reducing the dependency on the main foot clinic while demonstrating encouragingly low foot-related hospital admissions. In addition, our observed major amputation rate was lower than reported in the literature. Such an initiative may represent significant quality of care and cost benefits.
Disclosure: **P.R.J. Vas:** None.

1011

Should calcaneal ulcers be managed in a class of their own?

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Background and aims: Diabetic foot ulcers affecting the calcaneum are experientially the most challenging to manage, and automatically score a point on the SINBAD severity staging system. This study aims to interrogate the specific clinical features of this subpopulation of diabetic foot patients.
Materials and methods: 34 calcaneal ulcers in 28 patients presenting to our diabetic foot service between March 2010 and November 2016 were retrospectively studied. Clinical, laboratory and radiological data were collected on all patients. All episodes were assigned a SINBAD score. Outcomes of interest included mortality rates, average healing time, and major limb amputation. Data collection for an age-matched control group with non-calcaneal foot disease is in progress.
Results: The average age of our calcaneal cohort was 65 years and the mean diabetes mellitus duration was 25 years (which does not differ significantly from our overall diabetic foot disease population). However, the mortality rate in the calcaneal ulcer cohort was strikingly high at 43% (12/28) compared with 11% in previously published data for all patients with diabetic foot ulcers in our unit over the same 6-year time period. In 6 of the patients who died, the calcaneal ulcer was still an active problem at the time of death, with a mean preceding duration of 30 months. Only 1 calcaneal ulcer (3%) healed by 24 weeks. 10 ulcer episodes took longer than 24 weeks to heal, with an average healing time of 15 months. By comparison, National Foot Audit data reports that 49% of all diabetic foot ulcers can be expected to heal by 12 weeks. 7 calcaneal ulcer episodes were still ongoing at the time of data analysis, although all had been present for more than 12 weeks, with an average preceding duration of 12.7 months. A below knee amputation (BKA) was required in 21% (7/34) of calcaneal ulcer episodes. 2 episodes requiring BKA were due to chronic ulceration lasting a mean duration of 51 months. 94% (32/34) of episodes had a SINBAD score greater than or equal to 3. Chi squared testing reveals that, compared with our non-calcaneal ulcer population, heel ulcers are more significantly associated with peripheral vascular disease ($P<0.01$).
Conclusion: Our data confirms that diabetic calcaneal ulcers have a significantly poorer prognosis. They are associated with poor healing rates,

higher rates of amputation, and a 5-year mortality rate of close to 50%. We plan to interrogate predictors of healing in calcaneal diabetic foot disease, which may be a marker of other significant underlying co-morbidities beyond neuropathic and vascular disease. The diabetes foot community should consider revising current treatment guidelines for calcaneal disease, considering earlier, more aggressive interventions to prevent chronic and potentially life threatening longer-term sequelae.
Disclosure: **D. Hirani:** None.

1012

Is it reasonable to indicate autologous cell therapy of critical limb ischaemia in diabetic patients with chronic kidney disease?

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Background and aims: Autologous cell therapy (ACT) by bone marrow derived mononuclear cells is an alternative therapeutic option for diabetic patients with critical limb ischemia (CLI) not eligible for standard revascularization. The impact of severe stages of chronic kidney disease (CKD) on the outcome of ACT is rarely published, because these patients are usually excluded from clinical trials. In our previous studies we proved favorable clinical effect of ACT on amputation rate in patients with end-stage renal disease in comparison with conservative treatment. The aim of our current study was to compare the clinical effect of ACT in diabetic patients with regard to CKD stages.
Materials and methods: Eighty-seven patients with diabetic foot and no-option CLI who underwent ACT in our foot clinic between 2008-2016 were included into the study. Patients were divided in 3 groups in accordance with CKD stages. Fifty-four patients with CKD stages 1-2 (glomerular filtration rate [GFR] 60-90 ml/min/1.73 m^2 or higher) were included in group 1, 12 patients with CKD 3-4 (GFR 15-59 ml/min/1.73 m^2) were included in group 2 and 20 patients with CKD 5 treated by hemodialysis formed group 3. Survival and major amputation rate were assessed in the mean follow-up of 49 ± 29 months; the clinical effect of ACT measured by changes in transcutaneous oxygen pressure ($TcPO_2$) and wound healing was evaluated at 12 months after the procedure.
Results: $TcPO_2$ significantly increased in all study groups after 12 months compared to baseline ($p < 0.0001$, $p = 0.0043$ and $p = 0.0003$ in the first, second and third group), with no significant difference among them. Patients in group 1 had significantly higher survival rate during follow-up period compared with group 3 (87.1 vs 50 %, $p = 0.0006$), no other significant differences in survival were observed. Major amputation rate was without a significant difference among all study groups (27.8 vs 25 vs 35 %, NS). Ulcer healing after 12 months was also without any significant difference among the first, second and third study group (38.9 vs 41.7 vs 30 %, NS).
Conclusion: Our study showed that autologous cell therapy by bone marrow-derived mononuclear cells significantly improved CLI in patients with diabetic foot and enhanced ulcer healing regardless of CKD stages. There was a trend to a little higher frequency of major amputation in hemodialysis patients in comparison with other CKD groups, but comparable major amputation rates in patients in CKD 1-2 and 3-4 stages. Hemodialysis patients had significantly lower survival rate compared with patients with CKD stages 1-2. These results suggest that ACT is a reasonable therapy of CLI even in patients with severe CKD, but major amputation and survival rate are influenced by comprehensively different factors.
Supported by: Czech Ministry of Health, grant 16-27262A and MZO0023001.
Disclosure: **M. Dubsky:** None.

PS 086 Catch me if you can: diabetic foot and skin disorders

1013

Serum levels of angiogenic cytokines in the assessment of vasculogenesis after autologous cell therapy in diabetic patients with critical limb ischaemia

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Background and aims: Autologous cell therapy (ACT) is a promising method of revascularization for diabetic patients with no-option critical limb ischemia (CLI). Vasculogenesis, which is difficult to prove, is the likely mechanism of action of ACT, while percutaneous transluminal angioplasty (PTA) improves ischemia by different mechanism. The aim of our study was to evaluate the serum levels of angiogenic cytokines in diabetic patients with CLI treated by ACT in comparison to patients treated by PTA, and to assess the association of these cytokines with the clinical effect of ACT measured by transcutaneous oxygen pressure ($TcPO_2$) and with the number of injected angiogenic precursor cells (CD34+).
Materials and methods: Thirty-five patients with CLI treated by ACT in our foot clinic during 2008-2014 were consecutively included into the study. Fourteen patients who underwent PTA during the same period were included in a control group. The serum levels of pro-angiogenic cytokines such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (b-FGF), platelets-derived factors (PDGF-AA and PDGF-BB), and anti-angiogenic cytokine endostatin were measured by Luminex before revascularization and at 1, 3 and 6 months after the procedure and were correlated with the changes of $TcPO_2$ at the same intervals. The number of injected CD34+ cells was measured by fluorescence-activated cell sorting. Wilcoxon pair test and Spearman correlation coefficient were used for statistical analysis.
Results: We did not observe any significant increase of serum levels of pro-angiogenic cytokines in both groups. By contrast, the anti-angiogenic cytokine endostatin increased significantly at 1 and 3 months after ACT (both $p < 0.001$), however, no significant changes of endostatin after PTA was observed. $TcPO_2$ increased significantly at 1 and 3 months after ACT (from 17.9 ± 10 to 35 ± 15.8 mmHg, respectively 39.4 ± 11.6 mmHg, both $p < 0.001$). A significant correlation of $TcPO_2$ with endostatin at 1 month after the ACT ($r = 0.53$; $p < 0.001$) was observed. There was no significant correlation between endostatin and the number of injected CD34+ cells in ACT group.
Conclusion: Our study showed no relation between serum levels of pro-angiogenic cytokines and clinical effect of ACT measured by $TcPO_2$. On the other hand, the anti-angiogenic cytokine endostatin could be a potential marker of local vasculogenesis after cell therapy in diabetic patients with critical limb ischemia because of its significant increase after this therapy in contrast to PTA.
Supported by: grant AZV no. 16-27262A and MZO 00023001
Disclosure: **A. Němcova:** None.

1014

Are conventional risk factors enough to predict peripheral vascular disease?

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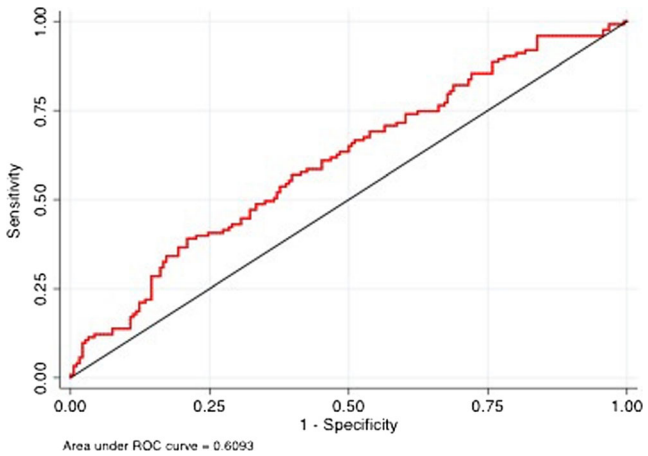
Background and aims: Patients with peripheral arterial disease (PAD) are at a high risk of lower limb amputations and cardiovascular mortality.

Screening for PAD is recommended in individuals with type 2 diabetes if one or more risk factors are present: age≥50 years, duration of diabetes≥10 years, overweight, obesity, hypertension, dyslipidemia and smoking. PAD is asymptomatic in the majority. Asymptomatic individuals have worse clinical outcomes. Screening, diagnosis and adequate treatment of risk factors is necessary even in asymptomatic individuals. In this study we aim to develop a prediction model that can accurately identify asymptomatic individuals at high risk of PAD.

Materials and methods: This was a cross-sectional study of 309 patients with diabetes and one risk factor for PAD: age≥50 years, diabetes duration≥10 years, hypertension, dyslipidemia, BMI≥23 kg/m^2 or smoking, attending the diabetes outpatient from April 2016 to October 2016. Symptomatic patients and those with preexisting PAD were excluded. PAD was diagnosed in individuals with an ankle brachial index (ABI) ≤0.9 and >1.3. Mean (95% CI), frequency (%), p values at 5 % level of significance were calculated using STATA 14. A logistic regression model to predict PAD was developed.

Results: Mean age (years), duration of diabetes (years), HbA1c % and BMI (kg/m^2) was 59.6 (58.7-60.5), 13.0 (12.2-13.8), 8.4(8.2-8.6) and 26.5(26.1-29.9). Sixty-five percent were men. Hypertension, dyslipidemia, smoking was present in 79.6%, 93.8% and 21.6%. Ischemic heart disease (IHD) was present in 11% and 2% had a previous stroke. PAD was diagnosed in 123 (39.8 %) patients. The log odds of developing PAD = -0.5+0.01*age-0.07*sex-0.01*diabetes duration in years + 0.06*hypertension + 0.5*dyslipidemia - 0.05*smoking - 0.1*HbA1c + 0.002*BMI + 0.5*IHD + 1*stroke. The Hosmer-Lemeshow goodness of fit showed good calibration(p=0.5). The AUC was 0.61.

Conclusion: The predictive model, despite good calibration had poor discrimination. Thus traditional risk factors are insufficient to discriminate between asymptomatic patients with and without PAD. It is hence necessary to look beyond conventional risk factor modelling, identify newer biomarkers and develop models to improve the predictive capacity of existing models. Till such time, it is essential to continue screening all type 2 diabetes individuals for PAD as per current recommendations.



Disclosure: **B. Sosale:** None.

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Glucose variability and cardiovascular mortality in type 2 diabetes with lower limb lesions: Italian Leukaemia Association Treviso Project

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Background and aims: Diabetic foot treatment is a good target to reduce non traumatic limb amputations and cardiovascular mortality. Epidemiological studies confirm that vascular mortality prevalence in diabetic foot is similar to lung cancer mortality and its reduction is a public health burden. One possible link between diabetic peripheral and coronary heart disease is common distal limb and coronaric neuroischemia where vasculogenesis by bone marrow derived circulating endothelial progenitor cells and angiogenesis by local distal cells could reflect glucose variability load.

Materials and methods: 74 type two diabetic (T2D) patients with neuropathic foot lesions without (N1=30) or with (N2=44) critical limb ischemia (pO2 <30 mm Hg). N2 were older (60±2 vs 69±1 yrs), without differences in diabetes age, BMI, lipidis and HbA1c (8.2±0.3 vs 7.9±0.2). N2 patients were more complicated relatively to retinopathy, CHD, arterial hypertension and nephropathy. At time 0 we evaluated bone marrow precursor (BMPC)/peripheral blood precursor cells (PePC) CD34+ and bone marrow endothelial progenitor (BMEP)/peripheral blood endothelial progenitor cells (PeEP) CD34+KDR+ by flow citometry FACSCanto. We also considered available retrospective biochemical data (2-12 years) for mathematical indexes of variability relative to HbA1c and glycemia (Stability (SI) and Lability (LI) indexes, Standard Deviation (SD), Coefficient of Variation (CV), CONGA), and prospective 5 years interval time for death observation.

Results: We observed 5 cardiac deaths in N2 group and no deaths in N1 group. BMPC/PePC CD34+ ratio was significant higher in N1 vs N2 (71±18 vs 39±7 p=0.046) with a non significant BMPC CD 34+ increment in N1 vs N2 (4±1% vs 2,6±0,3 p=0.089) PeEP were significant higher in N1 vs N2 (7,8 ±1,1% vs 4,5±0,8 p=0.017). There were good correlations beetween SI for HbA1c and % BMPC (p=0.007 R^2=.364), SI for HbA1c and BMPC/PePC ratio (p=0.003, R^2=.29); correlation beetween SI for HbA1c and BMEP /PeEP ratio was borderline (p=0.0578, R^2=.132). In N2 group we also compared (for omology) alive (AN2) vs dead (DN2) patients and we found for HbA1c: SD: 6.27 [8.71] vs 10.97 [21.41] (median [IQR]), p=0.0364; CV: 10.20 [10.53] vs 16.96 [29.41], p=0.0217; CONGA: 5.59 [7.87] vs 20.75 [33.47], p=0.0043; LI: 8.10 [23.07] vs 93.08 [259.29], p=0.0008.

Conclusion: For the first time, we demonstrated that extracellular/hemathologic glycosylation could directly or indirectly impacts in tissue bone marrow progenitor stem cells capacity and distal tissues repair function. Mathematical indexes demonstrated to have a possible good clinical prognostic value for cardiac death in complicated neuroischemic T2D patients with diabetic foot. We hypothyze that at the beginning of prediabetic history normal bone marrow is an extremely sensible euglicemic setting where homeostasis of endothelial progenitor and tissue cells is prematurely lost with irreversible regeneration capacity.

Supported by: AIL Treviso Section

Disclosure: **M. Sambataro:** None.

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Negative pressure wound therapy inhibits inflammation in diabetic patients with foot ulceration

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Background and aims: Negative pressure wound therapy (NPWT) was one of the most important treatments of diabetic foot, but the underlying mechanisms still remain elusive. This study aimed to evaluate the inflammatory signals involved in the effects of NPWT on diabetic foot ulcers.

Materials and methods: We enrolled 22 patients with diabetic foot ulceration, eleven treated with NPWT and others treated with traditional debridement. All the patients were treated and observed for 1 week. Granulation tissue harvested and analyzed in both groups, and was histologically and immunohistochemical analyzed. Enzyme-Linked Immunosorbent Assay (ELISA), Western blot analysis and real-time PCR were performed to evaluate expression of IL-6, TNF-α, iNOS, Nuclear Factor-κB P65, I_kB-α and activating transcription factor-3 (ATF-3).
Results: After 7 days treatment, NPWT could obviously promote diabetic wound healing because of the mild inflammation and densely cell deposited matrix. Meanwhile, NPWT significantly decreased the expression of TNF-α, IL-6 and iNOS (all $P<0.05$). The result of Western blotting and real-time PCR indicated that NPWT obviously decreased the level of I_kB-α and NF-κB P65 and increased the level of ATF-3 (all $P<0.05$).
Conclusion: NPWT exerts an anti-inflammatory effect possibly through the suppression of pro-inflammatory enzymes and cytokines resulting from IkB-α inhibition and ATF-3 activation, which maybe prevented the activation of NF-κB pathway in human diabetic foot wounds.
Supported by: Natural Science Foundation of China (81270397)
Disclosure: **R. He:** None.

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Negative pressure wound therapy effect in diabetic foot ulcer may be mediated through differential gene expression

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Background and aims: Diabetic foot syndrome (DFS) frequently leads to patient disability due to foot and leg amputations. Negative pressure wound therapy (NPWT) has been successfully used for ulcerations in DFS. However, its mechanisms of action on the molecular level are not fully understood. The aim of this research was to assess the effect of NPWT on the gene expression.
Materials and methods: We have included 21 type 2 diabetes (T2DM) patients with foot ulcer treated with NPWT and 8 T2DM patients treated by conventional debridement. Tissue samples were obtained from the bottom of the ulceration at two time points: before the therapy was started and after 8 days of treatment. Total RNA was extracted and gene expression profiling was performed by means of Illumina human gene expression arrays. Differential expression of mRNAs was performed using the standard R Bioconductor pipeline based on 'limma' package.
Results: The studied groups were similar in terms of age at the examination 69.0 ± 8.3 vs. 67.5 ± 4.3 years (p=0.62), sex: 80,9% vs. 75,0% male (p=0.72), T2DM duration: 14.7 ± 7.1 vs. 14.9 ± 6.0 years (p=0.95), and other basic clinical characteristics. We identified 6 genes with differential expression ($p<0.05$) between the two time points studied (after the Benjamini-Hochberg correction for multiple testing). Expression of only one of them - RRP7A which is involved in rRNA processing - increased over 2-fold after the treatment (logFC = 0.322, p=0.032), while the remaining 5 genes were downregulated. Two of differentially regulated genes - CYP27A1(logFC=-0.57, p=0.02) and CLYBL (logFC=-0.08, p=0.034)- associate with mitochondrial function. Two other genes - SRGAP3 (logFC = -0.14, p=0.013) and TRAPPC6A (logFC = -0.12, p=0.032) are associated with endoplasmic reticulum and Golgi apparatus, respectively. Finally, the KIAA1683 gene (logFC = -0.105, p=0.035) encodes a protein interacting with Calmodulin (CaM) messenger protein, which specific function has yet to be determined.
Conclusion: In summary, we found initial evidence that NPWT effect in diabetic foot ulcer may be mediated through differential gene expression. This finding requires further confirmation.
Supported by: NSC Poland
Disclosure: **S. Borys:** None.

1018

Endoplasmic reticulum stress and autophagy dysfunction: implications in skin repair in a mouse model of diabetes

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Background and aims: Emerging evidence links dysfunction in key cellular organelles with insulin resistance, impaired insulin secretion and diabetic complications. This study was aimed at investigating whether dysfunction in endoplasmic reticulum (ER) and changes in the autophagy response, are associated with impaired wound healing and repair in diabetic mice.
Materials and methods: C57BL/6 male mice (n=8/group) were divided into diabetic (streptozotocin-induced; glycemia above 250 mg/dL) and control (non-diabetic) groups. The skin from the dorsal region was excised (6-mm diameter) to create wounds (day 0) and skin samples used to assess molecular markers at baseline. Ten days after wounding skin biopsies were harvested to assess gene and protein expression of metabolic intermediates involved in the unfolded protein response (UPR) ER stress and autophagy.
Results: The expression of UPR pathway proteins GRP78 and GRP94 was increased in the skin of control mice after wounding (8.5 and 2.2-fold vs. before wounding, $p\leq0.05$) without changes in gene expression. Diabetic mice also presented increased expression of these proteins after wounding (10.5 and 5-fold vs. before wounding, $p\leq0.05$), although the post wounding levels for GRP78 were about 2 fold lower compared to control mice ($p\leq0.05$). Skin wounds from diabetic animals did not show increased ER stress-mediated apoptosis since CHOP levels were similar to those in the control mice 10 days after wounding. Moreover, proteins involved in chaperone-mediated autophagy (Lamp2) and macroautophay (Beclin1, p62) were also significantly increased after wounding ($p\leq0.05$) without changes in gene expression.
Conclusion: Macroautophagy is increased in skin wound healing of control mice while in diabetic mice have impaired expression of proteins involved in this pathway. UPR, macroautophagy and chaperone-mediated autophagy were activated during skin wound healing regardless of the presence or absence of diabetes. Thus, autophagy and the UPR activation crucial for wound healing and tissue repair. Under diabetes conditions the macroautophay response was impaired, in part contributing to the dysfunctional healing/repair capacity observed in diabetes. This suggest that macroautophagy could be a potential therapeutic target for diabetic foot ulcers.
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Disclosure: **E. Carvalho:** None.

1019

Effector T-cell accumulation and reduction in TCR diversity as a promising early DFU biomarker

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Background and aims: Chronic diabetic foot ulcerations (DFUs) are the most debilitating complications of diabetes and are caused by uncontrolled infections of foot wounds, often a consequence neuropathy, impaired angiogenesis and chronic low-grade inflammation. Long-term hyperglycemia promotes NF-kB activation, impairing leukocyte activation and migration and leading to a chronic inflammation, but glucose-lowering treatment does not reduce the risk of DFU, because the effects of long-term hyperglycemia on the immune system continue even after

glycemic stabilization. We have demonstrated that diabetes has a profound impact on the circulating T-cell pool, promoting the accumulation of effector T-cells to a point where T-cell receptor (TCR) diversity may become an issue to the immune response and that these effector T-cells aggravate the inflammatory phenotype thorough the secretion of inflammatory cytokines, such as TNF-α and IFN-γ.

Materials and methods: To ascertain if effector T-cell accumulation and reduction in TCR diversity can be used as an early DFU biomarker, we underwent longitudinal studies on patients with first-time, acute, foot ulcerations (n=17), and evaluated the percentage of peripheral blood effector ($CD3^+CD27^-CD28^-$) and naïve ($CD3^+CD27^+CD28^+CD45RO^-$) T-cells, by flow-cytometry and measured the TCR repertoire diversity by PCR-based studies using the well-established Biomed protocol. We also analyzed the same parameters on familiars of patients with DFU (n=5), to evaluate the influence of genetic predisposition on the reduction in TCR repertoire diversity. We divided the patients with diabetic foot ulcerations into two groups, the ones that healed in less than 3 months (n=9) and the ones that did not heal in the 3 months' period (n=8).

Results: When plotting the ratio between effector and naïve T-cells in the two DFU groups, our results show that patients that have acute ulcers that fail to heal in less than 3 months have a much higher ratio (3,5±1,7) than the ones that heal in less than 3 months (p&lt0,05). The higher effector/naïve T-cell ratio is accompanied by a severe reduction in TCR repertoire diversity (p&lt0,05). We also show that sons of patients with DFU have higher effector T-cell counts, when compared to control individuals, even when they are not diabetic, but these results are still not statistically significant due to the reduced sample size.

Conclusion: Taken together, our results demonstrate the usefulness for the quantification of blood effector T-cells and the analysis of the TCR repertoire diversity as early DFU biomarkers and open new perspectives on DFU treatment trough the negative regulation of effector T-cell function.

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Disclosure: **J. Moura:** Grants; EFSD, SPD.

1020

Long-term changes in the RANK/RANKL/OPG system in diabetes patients with and without a Charcot foot

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Background and aims: Charcot osteoarthropathy (Charcot foot) is a rare but severe complication to diabetes mellitus and peripheral neuropathy. While the precise pathogenetic mechanism is unknown, recent studies have reported changes in biomarkers of bone inflammation and resorption in individuals with Charcot foot, specifically within the RANK/RANK-L/OPG system. It has been suggested that there is an increase in the RANKL/OPG ratio in the acute Charcot foot. The aim of this study was to investigate this system further in individuals with diabetes with and without Charcot foot.

Materials and methods: An 8.5-year follow-up case-control study of 44 individuals with diabetes mellitus, 24 of whom also had acute or chronic Charcot foot at the baseline visit in 2005-2007, who were followed up in 2015. Serum from baseline were stored at -80° C, and all samples analyzed by ELISA at follow-up for fsRANK-L and OPG, as well as sRAGE.

Results: Of the 44 participants at baseline, 22 were able to participate in the follow-up, while 9 had passed away, 3 were out of reach and 10 were unable or unwilling to re-participate. No participants had active foot ulcers, recent foot surgery or Charcot activity at follow-up. At follow-up, the average age of the participants was 69 yrs with a diabetes duration of 27 yrs. There were 18 were males and 4 females, 7 with type 1 diabetes and 17 with type 2 diabetes, and the average HbA1c was 59 mmol/mol. From baseline to follow-up, there was a significant difference in the change in levels of fsRANK-L between the diabetes patients with Charcot compared the diabetes patients without Charcot (-0.11 and -0.02 respectively)(p=0.018). There was no difference in the OPG levels, or change to these between the groups. At baseline, there was a significantly higher fsRANK-L/OPG ratio in the diabetes patients with Charcot than in the patients without Charcot (5.3 versus 1.6)(p=0.016), as well as a significant decrease in the fsRANK-L/OPG ratio from baseline to follow-up in the diabetes patients with Charcot (1.8 to 0.5 respectively)(p=0.006). Regarding sRAGE, there were no difference between the diabetes patients with and without Charcot at baseline and there was no difference in the change between baseline and follow-up between them either (p=0.470).

Conclusion: This study showed that there may be an elevated fsRANK-L/OPG ratio in diabetes patients with an acute Charcot foot, and that this increased level seems to decrease again with time to a level similar to diabetes patients without previous or current Charcot foot.

Clinical Trial Registration Number: NCT02335931
Supported by: Toyota Fdn, Brdr. Hartmann's Fdn

Disclosure: **R.B. Jansen:** None.

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Relationship between choroidal thickness, microalbuminuria, neuropathy and metabolic abnormalities in subjects with different degrees of glucose tolerance

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Background and aims: Hyperglycemia is classically related with the development of microvascular complications associated with diabetes mellitus. However, several studies have demonstrated the development of these complications in subjects with prediabetes and the metabolic syndrome. This study aims to identify earlier findings of microvascular complications in subjects with different degrees of glucose tolerance.

Materials and methods: In this cross sectional study 64 subjects underwent a standard protocol comprising anthropometric evaluation, biochemical assessment of fasting glucose, 2h-glicemia, glycosylated hemoglobin, vitamin B12, HDL-cholesterol, triglycerides, urinary sample albumin and the estimation of glomerular filtration by CKD-EPI. Ophthalmologic evaluation was performed by funduscopy and optical coherence tomography (OCT) on both eyes. Autonomic, peripheral, and small fiber neuropathy were respectively assessed by heart rate variability (HRV), Michigan questionnaire and quantitative sensitive test (QST). We used tuning fork to evaluate vibratory thresholds and Semmes-Weinstein 10g monofilament to assess foot sensitivity. A 24-hour ambulatory blood pressure monitorization (ABPM) was performed to evaluate pressure homeostasis.

Results: Patients were classified according to their glucose tolerance as normal glucose tolerance (NGT=14), prediabetes (PDM=20) and type 2 diabetes (T2DM=30). Groups differed by age, systolic blood pressure (day and night) and triglycerides (table 1). Choroidal thickness decreased with decreasing glucose tolerance in the right [NGT 332 (250.5-421.5) vs. PDM 260.0 (214.2-341.2) vs. T2DM 214.0 (170.0-273.0) μm; p<0.001] and left [NGT 320.0 (288.0-428.0) vs. PDM 271.0 (185.2-343.0) vs. T2DM 221.0 (181.0-274.0) μm; p< 0.01] eyes. One patient in the NGT and 3 patients in T2DM group had diabetic retinopathy (p=0.35). Albuminuria increased with decreased glucose tolerance [NGT 5.0 (3.0-19.2) vs. PDM 7.4 (3.0-27.7) vs. T2DM 18.5 (7.3-51.3) mg/dl; p=0.033]. Likewise, a higher pulse pressure, a cardiovascular risk marker, was found in patients with progressive decrease of glucose tolerance (NGT 42.43±5.52 vs. PDM 56.50±13.82 vs. T2DM 53.54±12.08 mmHg. p=0.003). No differences were found in peripheral neuropathy and most of the cardiac autonomic tests.

Conclusion: This study demonstrates a tendency to early microvascular changes related with worsening glucose tolerance.. It also suggests that earlier abnormalities of the choroidal vasculature may be identified in subjects with PDM being possibly an earlier marker of the development of diabetic retinopathy in a population with impaired glucose metabolism.

Table 1

	NGT (n=14)	*PDM (n=20)*	*T2DM (n=30)*	*Valor p*
Age (years)	45.1 ± 12.5	58.4 ± 12.3	55.8 ± 11.1	0.006
Female sex (n-%)	10 (71.4%)	17 (85.0%)	17 (56.7%)	0.103
BMI (kg/m^2)	30.6 ± 4.7	32.8 ± 7.7	32.1 ± 6.2	0.638
Waist (cm)	101.0 ± 13.3	101.4 ± 13.3	105.1 ± 12.0	0.484
SBP day (mmHg)	121.4 ± 13.7	131.8 ± 14.4	133.3 ± 14.1	0.035
SBP night (mmHg)	109.4 ± 12.5	124.9 ± 18.3	125.6 ± 15.5	0.009
Hypertension (n-%)	6 (46.2%)	15 (75%)	24 (71.4%)	0.072
HbA1c (%)	5.0 ± 0.3	5.4 ± 0.4	7.5 ± 2.3	-
HDL (mg/dl)	54.2 ± 13.9	50.2 ± 12.8	45.3 ± 11.1	0.082
Triglycerides (mg/dl)	103 (71 – 145)	108.5 (71.5 – 143.5)	150 (116 – 202)	0.028

Data described as mean ± standard deviation, absolut number (%) or median (P25 - P75).

Disclosure: **F. Gerchman:** None.

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Prediction models for the risk of retinopathy in people with type 2 diabetes. A systematic review

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Background and aims: A complication of type 2 diabetes (T2D) is retinopathy and early detection and treatment of vision-threatening retinopathy is of importance to prevent blindness. Prediction models for retinopathy enable identifying persons at high retinopathy risk, facilitating tailored monitoring. We aimed to identify all prediction models for retinopathy risk applicable to people with T2D and to assess their quality and accuracy.

Materials and methods: A systematic search was performed in PubMed and Embase in March 2016. Studies were included when: (i) the prediction model was applicable to people with T2D; (ii) the outcome was any stage of retinopathy or blindness and (iii) minimal follow-up of the development study was one year. Data extraction and risk of bias assessment was performed using the CHARMS-checklist. Performance of the models (internal validation) was assessed by discrimination, the ability of the model to identify those at risk (e.g. c-statistic), and calibration, the ability of the model to accurately quantify the absolute risk (e.g. Hosmer-Lemeshow-test). Screening, full-text assessment and data-extraction was performed independently by two reviewer and by a third reviewer in case of disagreement.

Results: From 6481 studies, nine were included in the systematic review. The models predicted the development of retinopathy, macular edema or blindness over a period of two to ten years based on two to nine predictors per model, of which). Age, duration of diabetes, HbA1c, systolic blood pressure and presence of a less severe form of retinopathy, were the most commonly used predictors. Eight studies performed an internal validation. Discrimination was reported in five studies with c-statistics ranging from 0.61 (95% CI:0.52 to 0.71) to 0.79 (95% CI: 0.76 to 0.81). Only two studies reported calibration using the Hosmer-Lemeshow test (p-values:0.2 and 0.13). Four studies reported external validation in an independent population as part of the model development and the discriminative ability ranged from 0.55 (95% CI: 0.47 to 0.62) to 0.84 (95% CI: 0.78 to 0.88).

Conclusion: Nine prediction models for the risk of retinopathy in people with T2D are available with moderate to good performance. However, most models were not validated in an external population. Performance of four models in an independent population ranged from poor to good. Before application in clinical practice, external validity of all these models and their impact on the efficiency of care should be assessed.

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Disclosure: **A.A.W. van der Heijden:** None.

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Diabetic retinopathy in cystic fibrosis related diabetes

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Background and aims: Diabetes mellitus is a major comorbidity associated with cystic fibrosis. The prevalence of cystic fibrosis related diabetes (CFRD) has risen over time contributing up to 40% of adults and 25% of adolescents with cystic fibrosis (CF).The life expectancy of these patients has increased significantly in the recent years and it is more likely that some will develop

microvascular complications.Aim of this study is to determine the prevalence and predictors of diabetic retinopathy (DR) in patients with cystic fibrosis related diabetes (CFRD)

Materials and methods: A retrospective study of patients attending the CFRD clinic in a tertiary care centre was conducted. Data regarding patients with CFRD and CF exacerbations were obtained from CF database at our centre. Retinal images and grades were obtained from the national retinal screening programme. Patients were included if they had at least 2 retinal screening visits at least 1 year apart

Results: 54 patients with average age of 32.5 years (range 19-63) were included. The mean diabetes duration was 8.6 years (range 2-26); 92% of patients were Caucasian; 26(48%) were men. Baseline mean HbA1C was 7.2% (range 5.1-13.8). At baseline DR prevalence was (77.8%R0, 20.4%R1, 1.9%R2, 0%R3, 0%M1). From baseline DR progressed in 7 patients (17%) after a mean follow up of 3.7 Years. 6(14.6%) patients progressed from R0 to R1 and 3(7.3%) from M0 to M1. There was no progression from R0 to R2 or R3 but 1(2%) patient progressed from R1 to R3. After adjusting for age, gender, duration of diabetes, baseline HbA_1C and retinal grading and study end FEV_1, only total cholesterol at baseline predicted progression of retinopathy (OR 3.6; 95% CI 1.02-13.02). BMI, smoking status, number of exacerbations of CF, type of organism grown in sputum culture/sensitivity and serum creatinine did not have any correlation with DR progression

Conclusion: DR was common in CFRD but sight threatening retinopathy was uncommon. A small proportion of patients developed DR after 3.7 years of follow up. Total cholesterol, not Hba1C or duration of diabetes predicted DR progression. There is a need for further research to understand the pathogenesis of DR in CFRD

Disclosure: **M.S. Ahmed:** None.

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Abnormal renal profile predicts proliferative diabetic retinopathy and diabetic macular oedema in patients with type 2 diabetes: an 8-year prospective cohort study

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Background and aims: To investigate the effect of diabetic kidney disease on the development of diabetic retinopathy (DR) and diabetic macular edema (DME) in type 2 diabetes (T2D).

Materials and methods: An 8-year prospective cohort study with 2,135 patients of T2D were conducted in Taiwan. The baseline and mean follow-up renal profile including serum creatinine level, estimated glomerular filtration rate (eGFR) and urinary albumin creatinine ratio (ACR) were measured. Cox regression analysis was used to evaluate the hazard ratios (HRs) of the renal profile for the new onset of DR, proliferative diabetic retinopathy (PDR) and DME.

Results: A higher serum creatinine level (HR = 2.358 for an increase of 1 mg/dL, $p < 0.0001$), an eGFR <60 mL/min/1.73m^2 (HR = 3.037 to 6.436, $p < 0.0001$), and a urinary ACR >30 mg/g (HR = 3.202 to 6.652, $p < 0.0001$) at baseline were all significant predictors for the development of PDR. After adjusting the baseline values, the mean follow-up values including a higher serum creatinine level (HR = 2.369 for an increase of 1 mg/dL, $p < 0.001$), an eGFR <30 mL/min/1.73m^2 (HR = 6.807, $p < 0.0001$), and a urinary ACR >30 mg/g (HR = 2.344 to 4.193, $p = 0.011$ and 0.003) were significant predictors for the development of PDR. A baseline urinary ACR >30 mg/g (HR = 1.563 to 2.717, $p = 0.018$ and < 0.0001) was significantly predictive for the development of DME.

Conclusion: Both baseline and mean follow-up abnormal renal profiles including a high serum creatinine level, a low eGFR and a high urinary ACR were all significant predictors for new-onset PDR in patients with T2D. A high baseline urinary ACR was a significant predictor for the development of DME.

Disclosure: **Y. Hsieh:** None.

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10 year follow-up of background diabetic retinopathy within a national screening programme in Wales

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Background and aims: The aim of this study was to examine the progression of diabetic retinopathy (DR) in persons with non-sight-threatening DR (NSTDR e.g. background DR [BDR]) over a 10 year period.

Materials and methods: Data from persons with diabetes attending Diabetic Eye Screening Wales (DESW) between 2003 and 2013 were evaluated. Survival analysis (Kaplan Meier and Cox regression) were used to explore the progression from NSTDR to sight-threatening DR requiring referral to hospital eye services.

Results: A total of 1,538 people with Type 1 diabetes and 17,407 with Type 2 diabetes with NSTDR were included in the analysis. Of those with type 1 diabetes 712 had minimum BDR and 826 had Moderate BDR and of those with Type 2 diabetes 10,553 had minimal BDR and 6,854 had moderate BDR. In Type 1 diabetes there was a 2 fold increased risk of progression to Sight-threatening DR in those with moderate compared to minimal BDR when adjusted for age, gender and duration of diabetes (Table 1). Similarly there and a 2.9 fold increased risk of progression to referable DR in type 2 diabetes

Conclusion: Extending the screening interval from 1 to 2 years in persons with type 2 diabetes with minimal BDR appears justified (progression rate of 0.1% in the first 12 months). However, for persons with type 2 diabetes with moderate BDR and Type 1 diabetes with any severity of BDR annual screening should be retained.

Table 1: Cox regression analysis for the progression to sight-threatening DR

	multivariate		multivariate
Age	0.99 (0.97, 0.996)	Age	0.98 (0.98, 0.99)
Gender Male Female	NS	Gender Male Female	NS
Duration of DM </=13 14-26 >/=27	 1.00 0.97 (0.75, 1.25) 0.62 (0.42, 0.91)	Duration of DM </=3 4-8 >/=9	 1.00 1.60 (1.43, 1.80) 2.15 (1.91, 2.42)
Baseline DR status Min BDR Mod BDR	 1.00 2.08 (1.67, 2.59)	Baseline DR status Min BDR Mod BDR	 1.00 2.85 (2.61, 3.11)
		Treatment of DM Diet OHA Insulin Other	 1.00 2.10 (1.71, 2.56) 3.59 (2.88, 4.47) 2.84 (2.28, 3.54)

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The role of clinical covariates in informing personalised retinopathy screening intervals

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Background and aims: Diabetic Retinopathy (DR) is a diabetes complication of the eye and the leading cause of blindness in the working age population. Blindness from DR is preventable through early diagnosis and treatment. Scotland, among other countries, introduced a DR screening programme, where all patients with diabetes aged ≥12 are screened annually. As risk of developing referable DR is low on average, but varies between patients, it seems useful to personalise screening intervals to patients' individual risk. Previous literature has focussed on using prior screening outcome to stratify subsequent risks. This study investigates the benefits of additionally including patient demographics and clinical covariates to stratify risk of progressing to referable DR and shows how using this prediction in setting screening intervals affects discrimination between patients and number of screenings.
Materials and methods: This retrospective cohort study used screening data, for patients aged ≥12 with type 1 (T1D) and type 2 (T2D) diabetes, from the Scottish Diabetic Retinopathy Screening Programme and linked clinical data from Scottish Care Information Diabetes for the years 2007-2015. Binary logistic regression with cloglog link function for interval-censored data was used to model absolute risks of transition from the second screening episode to referable DR. Models with various covariates, including previous DR grade, patient demographics and potentially relevant clinical covariates (BMI, HbA_{1c}, blood pressure, blood lipids, eGFR, visual acuity, smoking status, statin and hypertensive drug use, cardiovascular disease status), were fitted using different model selection mechanisms. Models were developed on a training dataset and evaluated on a test dataset. Models were assessed for their goodness of fit (AIC) and classification (AUROC) along with their impact on yield and number of screenings.
Results: Analyses were based on 215501 patients (T1D n=17788) and 999961 screenings. Screening yield of referable DR was 3.96%/1.34% per annum for T1D/T2D. Patients' previous DR grade was a strong predictor of transition to referable DR (AUROC: 0.6862/0.7126 (T1D/T2D)). Additionally, including a full set of clinical covariates increased the AUROC to 0.7165/0.7761 (T1D/T2D) and statistically significantly ($p<0.001$) improved model fit. Restricting the model to the four most important predictors offered a comparable AUROC and model fit. Models including clinical covariates had better predictive performance than those that only used examination results. Individualised screening intervals could be based on the time at which the predicted risk of a referable DR reaches a specified level e.g. 1%; using 1% as the threshold these models could reduce the number of screenings by 11% for T2D, and leave the number of screenings for T1D unchanged.
Conclusion: This study shows that including clinical covariates improves prediction of transition to referable DR and that it is a useful step towards personalising screening intervals, and reducing screenings for T2D.
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Disclosure: **A. Ochs:** None.

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Prevalence of retinopathy among United States adults with both diabetes and chronic kidney disease

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Background and aims: Diabetes is the leading cause of kidney failure in the United States (U.S.). The presence of diabetic retinopathy (DR) is a reliable screening tool for identifying those at highest risk for chronic kidney disease (CKD) progression. No national population-based estimates of the prevalence and severity of DR exist among people with diabetes and kidney disease. The aim of the present study was to estimate the prevalence of DR among U.S. adults with both diabetes and CKD.
Materials and methods: Prevalence of DR was estimated in adults 40 years and older with both diabetes and CKD participating in the National Health and Nutrition Examination Surveys (NHANES) 2005-2008. DR was defined by the presence of +1 retinal microaneurysm or retinal blot hemorrhages with or without more severe lesions on fundus photographs taken with a digital non-mydriatic camera. Vision-threatening DR was defined by the presence of severe non-proliferative DR, proliferative DR, or clinically significant macular edema. CKD was defined by albumin/creatinine ratio above 30 mg/g or estimated glomerular filtration rate below 60 ml/min/1.73m². Diabetes was defined as HbA1c greater than 6.5% or self-report of diagnosis by a doctor or other health professional. Logistic regression was used to examine demographic and clinical determinants of DR.
Results: The study sample included 387 adults with diabetes and CKD, representing 4.9 million U.S. adults. The prevalence of DR was 36.2% (95% CI 30.1-42.7), and of vision threatening DR 8.2% (95% CI 5.4-12.2). Compared with persons without DR, those with DR were on average older, with higher HbA1c, higher blood pressure, longer diabetes duration, and had insulin treatment. In a multivariable adjusted model, the odds of DR were 50% higher per 1% increase in HbA1c (95% CI 1.2-1.9), 40% higher for every additional 5 year diabetes duration (95% CI 1.1-1.7), 3% higher per 10 mmHg increase in systolic blood pressure (95% CI 1.02-1.1), and 13-fold higher with insulin treatment (OR=13.3, 95% CI 2.2-81.7) ($p<0.001$ for each risk factor, with additional age, sex, and race/ethnicity adjustment). Overall, the prevalence of DR was higher than in the earlier NHANES after adjusting for age, sex, race/ethnicity, mean blood pressure, and HbA1c, whereas the prevalence of vision threatening DR remained largely unchanged over time.
Conclusion: Over one third of this nationally representative sample of adults with both diabetes and CKD had DR, representing 1.8 million U.S. adults at high risk for CKD progression. Many of the studied risk factors associated with DR are modifiable.
Disclosure: **M.E. Pavkov:** None.

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Cluster analyses of patients with dysregulated type 2 diabetes based on routine clinical markers reveal cluster-specific prevalence of diabetes complications

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Background and aims: Type 2 diabetes is a heterogeneous disease with genetic and lifestyle factors contributing to its development and progression. In addition, there is a large variation between patients in the development of diabetes related complications making it unlikely that it represents one disease entity. Here we tested the hypothesis that diabetes patients may be clustered based on routine clinical variables in patients with dysregulated type 2 diabetes referred to a specialist center for treatment.
Materials and methods: Diabetes related clinical measures including age of disease onset, diabetes duration, GAD antibody positivity, and BMI, HbA1c, HOMA-B, and HOMA-IR at presentation, were

used for clustering the patients using the K-means clustering algorithm. Odd ratios for diabetes complications including cardiovascular disease, nephropathy, neuropathy and retinopathy, were calculated using logistic regression in R.

Results: Based on our data from patients referred for dysregulated type 2 diabetes (n = 2290) we found that a maximum 5 cluster solution was possible when tested for within-cluster similarity from 1000 bootstrapped similarity estimates. The five clusters were primarily characterized or driven by early insulin resistance (C1; 21%), autoimmunity (C2; 3%; miss-classified type 1 diabetes), age (C3; 32%), insulin deficiency (C4; 22%), and obesity and insulin resistance (C5; 22%), respectively (Table 1). Retinopathy was significantly more common (odds ratio (CI) 2.45 (1.67-3.47), p = 1.14E-05) in the cluster characterized by insulin deficiency (C4), and nephropathy was significantly more common (odds ratio (CI) 1.80 (1.31-2.49), p = 2.99e-04) in the cluster characterized by obesity and insulin resistance (C5), in logistic regression models adjusted for age of disease onset, sex and diabetes duration. In addition, we found a lower incidence of cardiovascular disease in the small cluster likely to represent miss-classified type 1 diabetes (odd ratio = 0.45, p = 0.04). We found no significant cluster specific association for diabetic neuropathy.

Conclusion: Patients referred for dysregulated type 2 diabetes may be clustered into clinically relevant subgroups based on routine clinical markers. The prevalence of diabetes complications seems to be cluster-specific. Our data underlines the need for a tailored strategy for the treatment of type 2 diabetes and prevention of its complications.

Assigned Cluster / Cluster Characteristics	Cluster 1 early Insulin resistance	Cluster 2 Autoimmunity	Cluster 3 Age	Cluster 4 Insulin deficiency	Cluster 5 Obesity and Insulin resistance	All patients
Patients (n)	490	65	727	510	498	2290
Patients (%)	21	3	32	22	22	100
HbA1c (Mean (SD))	54.5 (14.3)	72.7 (18.7)	55.9 (10.7)	78.1 (17.0)	80.5 (17.0)	66.4 (7.9)
HOMA-IR (Mean (SD))	3.2 (2.0)	1.8 (1.6)	3.4 (1.7)	1.9 (1.2)	5.2 (3.0)	3.4 (9.3)
HOMA-beta (Mean (SD))	93.2 (42.8)	39.5 (33.9)	87.1 (47.7)	30.8 (15.3)	59.6 (32.5)	68.5 (18.9)
BMI (Mean (SD))	30.7 (5.7)	28.2 (6.2)	30.0 (5.2)	28.2 (4.7)	38.3 (7.4)	31.5 (2.3)
Age diagnosed (Mean (SD))	55.9 (11.6)	49.2 (10.0)	56.8 (9.7)	43.8 (10.8)	47.3 (10.6)	51.4 (45.0)
Diabetes duration (Mean (SD))	0.9 (0.6)	6.8 (8.8)	9.2 (5.3)	13.2 (7.7)	7.4 (5.6)	7.9 (6.9)
GAD (Mean (SD))	2.43 (9.50)	240.1 (28.3)	2.2 (7.7)	3.3 (10.9)	2.4 (7.2)	9.3 (11.9)
Retinopathy (%)	14	25	26	52	24	28
Nephropathy (%)	20	13	26	32	33	25
Neuropathy (%)	26	23	48	42	37	35
CVD (%)	25	15	38	20	27	27

Disclosure: **A. Ali:** None.

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An effective risk-stratification algorithm to prioritise diabetes retinal screening in previously unscreened, low income, minority communities

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Background and aims: Routine screening for diabetic retinopathy (DR) is a cornerstone of secondary prevention in people with diabetes because treatment of sight-threatening DR (STDR) - if recognized early enough - is effective in preventing visual loss. Yet diabetes remains the single most common cause of blindness in the working-age population in the United States. Low DR screening rates account for this in part, and are especially prevalent in low-income and minority populations. The aim of this study was to develop and validate a risk-stratification algorithm (RSA) to identify patients at high risk for STDR to prioritize DR Screening based on risk. Our overall goal is to improve DR Screening in low income, safety net populations in resource poor environments.

Materials and methods: The algorithm was developed with patient registry data in Clinic 1, and used to predict STDR risk using data from Clinic 2. Both clinics serve primarily Latino and African American patients in low-income areas of a large metropolitan area. In Clinic 1, we used readily available clinical data (age, sex, albumin-creatinine ratio, A1C, insulin use status) to develop an RSA. The RSA was set up without the benefit of results from earlier DR screening as a parameter for the algorithm. This was necessary because in our low-resource health care system many patients did not have prior retinal screening. To our knowledge this is the first RSA developed specifically for a low-resource, safety net population.

Results: Risk factors for DR were similar in the 2 clinics: presence of microalbuminuria (24-28%) and A1C were similarly distributed (p=NS). However in Clinic 1, patients were younger (mean 53 vs. 57 yrs) and fewer used insulin (15 vs 22%) than in Clinic 2 ($p<0.05$). The RSA to predict STDR was developed in Clinic 1 using data from 752 patients with diabetes who had undergone routine DR Screening (%STDR = 13.9). Area under the Receiver Operating Characteristics curve was 79.8% with sensitivity 69.2% and specificity 79.0%. We then applied the RSA to two retrospective validation cohorts in Clinic 2 to determine its effectiveness to identify patients at high risk for STDR. Cohort A included 585 diabetic patients who underwent routine teleretinal DR Screening. STDR was found in 55 patients (9.4%), most of whom were ranked as high risk by the RSA. By applying the RSA, 76.4% of the patients with STDR would have been identified before the program completed screening half of all patients. In Cohort B, there were 105 patients with STDR (8.9% of 1,178). Of these, 88.6% (n=93) would have been similarly identified by the RSA.

Conclusion: These data suggest that using a combination of simple and routinely obtained demographic and laboratory data, it is possible to predict the likelihood of identifying STDR. We conclude that a simple risk-stratification algorithm can be used to prioritize patients for early DR screening in low-income, minority patients. Because the algorithm was successful without the benefit of results from a previous DR screening, this approach may be useful in other low resource communities with a high prevalence of diabetes.

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Relation between the glycaemic exposure indicated by total excessA_{1c} index and retinopathy in type 1 diabetes: a DCCT/EDIC subgroup analysis

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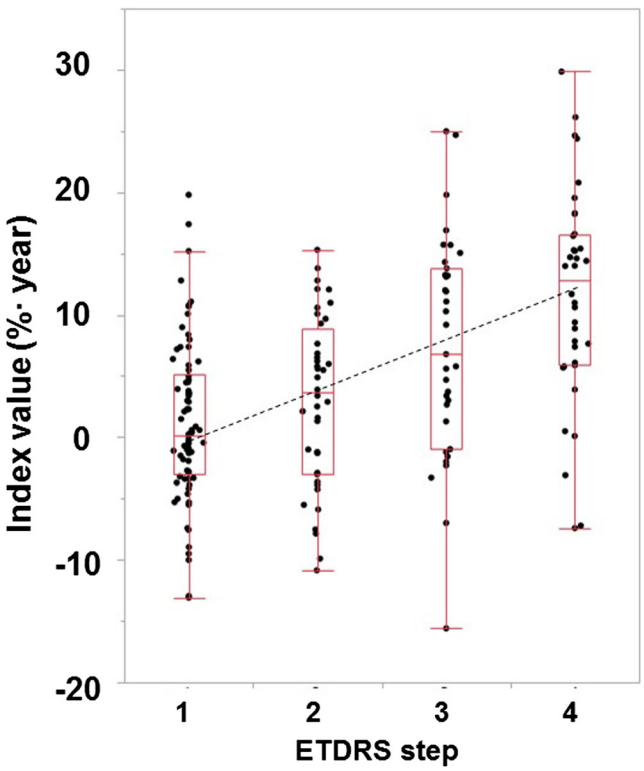
Background and aims: It has not been sufficiently examined that the combination of how high and how long of the glycemic exposure may give effects on retinopathy in patients of type 1 diabetes, though degree of hyperglycemia indicated by HbA1c and duration of diabetes were shown to be the most important factors on retinopathy in DCCT. We have reported that an index, total ΣexcessA1c, integrating both data of HbA1c and duration of diabetes may substantially predict retinopathy, if we use HbA1c data of the total diabetes duration to exclude the disturbing effect of metabolic memory in our type 1 diabetes patients. We examined relation between excess glycemic exposure and early retinopathy in type 1 diabetes using the total excessA1C value in the public data of DCCT/Epidemiology of Diabetes Interventions and Complications (EDIC).

Materials and methods: To examine only selected cases of DCCT/EDIC, those who had HbA1c data during nearly their entire period of hyperglycemia, we included patients of primary prevention with the shortest diabetes duration (≤14 months) at DCCT baseline. We also included only patients who had data of retinopathy in all of the three periods (year 5, year 9 and year13 after diabetes onset). Retinopathy was evaluated by steps in the final Early Treatment Diabetic Retinopathy Study (ETDRS) severity scale for persons. Total excessA1C(7.5%) was calculated by adding all the values of each yearly HbA1c value minus a threshold of 7.5% (National Glycohemoglobin Standardization Program value; 58mmol/mol) from the first year of onset of diabetes to a given year. We calculated it supposing that each case had one-year duration at DCCT baseline. We examined relations between total excessA1C(7.5%) value and retinopathy steps of ETDRS (step 1-4). The Wilcoxon rank sum test was performed using JMP version 13.0.0 (SAS, Cary, NC). Two-sided P value<0.05 was considered significant.
Results: The mean duration of diabetes of the 70 patients who fulfilled the criteria was 12.2 months (range 8-14) at the DCCT baseline, indicating that patients were followed from almost just after onset. In each of 4 steps of retinopathy, there were no significant differences in total excessA1C(7.5%) value between any pairs of groups of year 5, 9 and 13 (P>0.3278 in all of 12 pairs). Then we combined values of year 5, 9 and 13 in each 4 steps (n=70 in each step). P values between step 1-2, step 2-3 and step 3-4 were 0.1463, 0.0254 and 0.0418, respectively. Median values of total excessA1C(7.5%) showed a substantial linear distribution (0.18 in step 1, 3.65 in step 2, 6.80 in step 3 and 12.84 in step 4; Figure). For example, the value of 13 in total excessA1C(7.5%) almost corresponds to the HbA1c value of 10% for 5 years, 9% for 9 years and 8.5% for 13 years, respectively.
Conclusion: Total excessA1C may have a capacity in describing the relation between glycemic exposure and early retinopathy in type 1 diabetes regardless of years of observation.



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PS 088 Treat the risk of retinopathy

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Can (poly)phenols metabolites ameliorate the outcome of diabetic retinopathy

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Background and aims: Diabetes Mellitus is a chronic disease with increasing worldwide prevalence, with several associated complications, including Diabetic Retinopathy (DR). DR is the most frequent microvascular complication affecting diabetic adults. Despite significant efforts, the pathways underlying DR are not completely clarified, compromising the development of efficient treatments. Here we explore one potential therapeutic approach that relies on products resultant from human metabolization of phenolic compounds. These compounds have been studied by Santos and co-workers for their cytoprotective, hypertension-lowering, and anti-inflammatory properties. Retinal inflammation and an imbalance between pro- and anti-angiogenic proteins is observed in DR. Therefore in this work we aim to explore the potential of these metabolites for the treatment of DR, firstly in an *in vitro* setup mimicking hyperglycemia and secondly in an animal model of DR.
Materials and methods: Human RPE cell line, D407, was cultured with increasing concentrations of glucose, under normal and hypoxia conditions, and challenged with Catecol-O-sulfate. Expression of PEDF, VEGF and Glut1 were assessed by Western blot.
Results: Our *in vitro* studies shown that exposure of D407 cells for 24h to Catecol-O-sulfate (5 μM) under hyperglycemic and hypoxia conditions leads to an increase in the expression of anti-angiogenic protein PEDF and reduction of expression of pro-angiogenic VEGF. We have also analyzed the expression of the glucose transporter in the retina (Glut1), whose expression is reduced after exposure to Catecol-O-sulfate, in both hyperglycemic and hypoxic conditions.
Conclusion: Catecol-O-sulfate, a metabolite resultant from dietary (poly)phenols shown to have a beneficial role in decreasing the expression of VEGF and expression of Glut1, and increasing the expression of PEDF under hyperglycemia conditions in D407 RPE cells. Further studies will include testing other metabolites in the same conditions and use an animal model of DR to evaluate their therapeutic potential for DR.
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Disclosure: **D.F. Santos:** None.

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Association of apnoea hypopnea index during rapid eye movement sleep with diabetic retinopathy in patients with type 2 diabetes

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Background and aims: Forty to 80 % of patients with type 2 diabetes (T2DM) have sleep disordered breathing (SDB). SDB may exacerbate DR via poor glycemic control, hypertension, oxidative stress and chronic

inflammation which are known risk factors for diabetic retinopathy (DR). Although a few previous studies with portable sleep test showed independent association between SDB and DR, the overall results were conflicting. On the other hand, it is well known that rapid eye movement (REM) sleep is associated with greater sympathetic activity than in non-REM (NREM) sleep and that SDB events cluster in REM sleep. Furthermore, a few recent studies showed that apnea hypopnea index in REM sleep (REM-AHI) which can be measured only by polysomnography is associated with HbA_{1c} and presence or onset of hypertension whereas NREM-AHI wasn't. We therefore hypothesized that REM-AHI is associated with DR. However, no study evaluated this association and hence we performed the present study.

Materials and methods: We recruited 264 T2DM patients who were followed in our diabetes clinic and who underwent a fully attended polysomnography. Patients with heart failure, active lung disease, currently treated for SDB and REM sleep <30 minutes during polysomnography were excluded. Patients with simple or more severe DR were defined as having DR. In the first analysis, multivariate stepwise logistic regression analysis for DR included categorical REM-AHI (quartile) as the independent variable. The model was adjusted for known risk factors for DR such as diabetes duration, HbA1c, BMI, hypertension and dyslipidemia. The following variables with a p <0.25 in the univariate analysis were also included; age, sex, insulin use, ACE inhibitor/angiotensin receptor blockers use and sleep pills use. In the secondary analysis which included ln_REM-AHI, ln_NREM-AHI value was included for the model.

Results: Characteristics of the patients (n=131) are summarized in the table. In the multivariate logistic regression analysis, quartile of REM-AHI (p=0.011 for trend) and T2DM duration (OR: 6.131; 95 % CI: 2.616-14.369, p <0.001) were independently associated with DR. The OR and 95 % CI for each quartiles of REM-AHI compared with Q1 (reference) are as follows; Q2: 3.944 (0.780-19.938), Q3: 11.928 (2.405-59.149), Q4: 13.372 (2.178-82.099). Similarly in the secondary analysis, ln_REM-AHI (OR 3.196, 95 % CI 1.392-7.337, p=0.006) and T2DM duration (OR 5.976, 95 % CI 2.604-13.719, p <0.001) were independently associated with DR whereas NREM-AHI wasn't.

Conclusion: This is the first study to demonstrate independent association between REM-AHI and DR in patients with T2DM. SDB, especially during REM sleep could be the potential risk factor for diabetic retinopathy.

Table Characteristics of the patients.

	Overall	REM-AHI Q1	Q2	Q3	Q4	*P* value
Age, years	57.5 ± 11.8	57.2 ± 11.7	59.1 ± 11.3	57.8 ± 11.9	55.2 ± 12.9	0.478
Male sex, %	90.1	91.2	88.2	94.4	85.2	0.699
T2DM duration, years	7 (2-14)	8 (4-14)	8 (3-14)	8 (4-14)	5 (1-12)	0.613
DR, %	23.7	8.8	20.6	36.1	42.1	0.047
Hypertension, %	67.2	58.8	64.7	69.4	77.8	0.452
Dyslipidemia, %	61.8	44.1	76.5	52.8	77.8	0.008
BMI, kg/m²	26 (24-29)	24 (23-27)	27 (25-30)	27 (24-29)	27 (25-32)	0.002
HbA1c (NGSP), %	7.6 (7.0-8.8)	7.5 (6.8-8.9)	7.5 (6.8-8.5)	7.9 (7.2-9.0)	7.5 (6.7-9.0)	0.500
Insulin use, %	9.9	2.9	8.8	19.4	7.4	0.233
AHI, events/h	29.8 (20.1-43.1)	17.1 (8.0-29.7)	28.6 (20.1-38.1)	36.0 (24.9-51.1)	40.1 (26.4-65.1)	0.000
REM-AHI, events/h	36.6 (21.1-53.5)	13.9 (7.2-17.7)	28.9 (24.7-33.3)	45.8 (41.8-52.7)	63.2 (57.7-69.7)	0.000

Data are mean ± SD, median (interquartile range) or %. REM, rapid eye movement; AHI, apnea hypopnea index; T2DM, type 2 diabetes; DR, diabetic retinopathy. Analysis performed using the chi-square test for categorical variables, the analysis of variance (ANOVA) for normally distributed variables and the Kruskal-Wallis test for skewed variables.

Disclosure: **A. Nishimura:** None.

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Deregulation of the retinal renin-angiotensin system precedes the onset of diabetic retinopathy

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Background and aims: The renin-angiotensin system (RAS) is a set of complex pathways with a well-defined function in the regulation of blood pressure and body fluid homeostasis. Deregulation of the RAS has been found in different pathologies and this was mainly attributed to the over-activation of renin and the renin receptor. Recently it was found that the RAS encompass a protective axis which includes the MAS receptor. The RAS is present in the eye and its dysfunction is associated with the development of ocular pathologies. Evidences point to the contribution of the RAS in diabetic retinopathy, but at what stage of the disease the deregulation of this system occurs is still unknown. The aim of the present study is to assess the RAS in the retina of a mouse model of diabetes at an early stage of the disease.

Materials and methods: The Ins2Akita is a well described experimental model to study diabetes-associated complications, it has a C57BL/6 background and spontaneously develops type 1 diabetes. Hyperglycemia begins at 2-months of age and this model develops symptoms of diabetic retinopathy around 6-months of age. Retinas of diabetic and non-diabetic (C57BL/6) mice with 4-months of age were collected and assessed for the expression of renin, renin receptor and MAS receptor using Western blot.

Results: It was found that the expression of renin was significantly increased in the retina of the diabetic mice when compared with the non-diabetic. The expression of the renin receptor was unchanged in the retinas of both diabetic and non-diabetic mice at 4-months of age. The expression of the MAS receptor was significantly decreased in the retinas of diabetic mice compared with age-matched controls.

Conclusion: Our data demonstrate that the RAS is deregulated in the retina of a diabetic model, at a stage where retinopathy is not yet installed. We hypothesized that the deregulation of the RAS might contribute to trigger diabetic retinopathy. We are currently exploring further ages to completely characterize the RAS profile in diabetic retinopathy, which will allow to better understand the involvement of this system on the disease course.

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Apolipoprotein A1 and B predicts development of diabetic retinopathy in patients with type 2 diabetes

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Background and aims: We aimed to determine whether, in patients with type 2 diabetes, apolipoprotein A1 and B level have stronger associations with diabetic retinopathy (DR) than do traditional lipids.

Materials and methods: From January 2006 to December 2009, a total of 599 patients with type 2 diabetes without diabetic retinopathy were consecutively enrolled. The serum apolipoprotein A1 (apoA1) and B (apoB) concentrations were measured by rate immunonephelometry assay, and a standardized clinical examination and retinal photographs were checked annually by an ophthalmologist. We used a Cox proportional hazard regression analysis to test the associations between diabetic retinopathy and apoA1 and apoB.

Results: The median follow-up time was 8.8 years. The mean age was 54.6 ± 9.9 years, and the duration of diabetes was 6.2 ± 5.7 years. During the follow-up period, 149 patients (24.9%) developed DR. The patients in the DR group had a higher level of apoB (85.6 ± 21.0 vs 92.5 ± 22.6 mg/dL, P = 0.001) and apoA1/apoB (1.6 ± 0.6 vs. 1.5 ± 0.5, P =0.001), although at the baseline time they had had apoA1 (128.0 ± 21.5 vs. 126.3 ± 20.8 mg/dL, P = 0.405) and LDL cholesterol (106.9 ± 32.6 vs. 103.6 ± 33.3 mg/dL, P = 0.319) levels. Baseline apoB (r = 0.250, P <0.001) and apoA1/B (r = -0.157, P <0.001) levels significantly correlated with LDL cholesterol level. A Cox hazard regression analysis revealed

that the development of DR was significantly associated with the high level of serum apoB (per 10 mg/dL increase; hazard ratio 1.13, 95% CI 1.04-1.21, $P = 0.002$) and apoA1/B level (hazard ratio 0.61; 95% CI 0.42-0.88, $P = 0.008$) after adjusting for sex, age, diabetic duration, mean $HbA1_C$, treatment of insulin, ACE inhibitor/ARB and lipid lowering agents. In contrast, LDL cholesterol level showed insignificant association with DR (per 10 mg/dL increase; hazard ratio 0.97, 95% CI 0.92-1.02, $P = 0.185$).

Conclusion: In conclusion, we found that, in patients with type 2 diabetes, the ApoB and apoA1/B are stronger predictors for the development of DR than the LDL cholesterol level.

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Disclosure: **J. Yun:** None.

1035

Methylglyoxal activates microglia and induces heat shock proteins in the rat retina

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Background and aims: Reactive metabolites such as methylglyoxal are involved in the damage of the neurovascular unit (NVU) of the diabetic retina. Our aim was to analyze methylglyoxal-induced damage of the NVU *in vivo*.

Materials and methods: Methylglyoxal (50 mM) was applied to Wistar rats by drinking water for 4 weeks. Microglial activation was quantified by immunohistochemistry. Retinal gene expression was analyzed by DNA microarray (GeneChip Rat Gene 2.0 ST) and gene set enrichment analysis.

Results: Oral application of methylglyoxal resulted in activation of retinal microglia from 6.5 $CD74^+/mm^2$ in untreated controls to 18.5 $CD74^+/mm^2$ in treated animals ($p<0.01$). Ranking of retinal expression changes revealed the crystallin gene family as the most prominent candidate. In addition, gene set enrichment analysis indicated regulation of several pathways, including carbohydrate metabolism, translation, MAPK signaling pathway, and insulin resistance.

Conclusion: In the NVU of the retina, reactive metabolites are associated with microglial activation, induction of heat shock proteins and pathway changes.

Supported by: DFG SFB 1118

Disclosure: **A. Schlotterer:** Grants; DFG CRC 1118 "Reactive metabolites as cause of diabetic complications".

1036

Behavioural risk of diabetic retinopathy among Bangladeshi subjects with type 2 diabetes: a 15-year follow-up study

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Background and aims: Lifestyle plays an important role in the development of diabetic retinopathy (DR). Previous studies have examined individual dietary and lifestyle factors in relation to type 2 diabetes mellitus (T2DM), but the combined effects of these factors for DR among T2DM are largely unknown. The objectives of this study were to investigate the prevalence of diet, lifestyle, and the risk of diabetic retinopathy and determinants of DR among T2DM.

Materials and methods: We followed 977 T2DM subjects from 1993 to 2008; these subjects were free of diagnosed cardiovascular disease, DR and cancer at base line. Information about their diet and lifestyle was updated periodically. A low-risk group was defined according to a combination of five variables: a body mass index of less than 25; a diet high in cereal fiber and polyunsaturated fat and low in trans fat and glycemic load (which reflects the effect of diet on the blood glucose level); engagement in moderate-to-vigorous physical activity for at least half an hour per day; no current smoking; and regularly eye examine, at least six months within a year.

Results: During 15-years of follow-up, we documented 495 new cases of DR. Co-existing of hypertension was the single most important predictor of DR. Lack of exercise, a poor diet, current smoking, and abstinence from eye check-up were all associated with a significantly increased risk of DR, even after adjustment for the hypertension. A total of 95 percent of the diabetes related DR in this cohort (95% CI, 87 to 99 percent) could be attributed to habits and forms of behavior that did not conform to the low-risk pattern.

Conclusion: In conclusion, our findings suggest that type 2 diabetes related DR could be prevented by weight loss, regular exercise, modification of diet, abstinence from smoking, and the periodical eye examination. Weight control would appear to offer the greatest benefit.

Supported by: BADAS

Disclosure: **K.R. Ahmed:** None.

1037

Development and progression of diabetic retinopathy and associated risk factors in type 1 diabetic patients: a 15-year follow-up study

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Background and aims: Diabetic retinopathy (DR) is an important cause of visual impairment in developed countries, especially in working age adults. The aim of this study was to evaluate the 15-year incidence of development and progression of retinopathy in a sample of type 1 diabetic patients and determine the associated risk factors in these patients.

Materials and methods: 123 type 1 diabetic patients (62 male / 61 female) were enrolled in this study and followed for 15 years. Basic and anthropometric parameters assessed were age, gender, diabetes duration and body mass index (BMI). Glycated hemoglobin (HbA_1c), total cholesterol, HDL and LDL cholesterol, triglycerides (TG), C-reactive protein (CRP), homocysteine (HCY), fibrinogen (FIB), plasma viscosity and serum creatinine were determined using routine laboratory methods. Urinary albumin excretion rate (UAE) was measured from a 24-hr urine sample. Blood pressure was measured with a mercury sphygmomanometer after a 10-min resting period. Ophthalmologic examination included binocular indirect slit lamp fundoscopy, color fundus photography after mydriasis according to the EURODIAB retinal photography methodology and optical coherence tomography of the macula. Possible risk factors for the development and progression of DR were examined in backward stepwise Cox's multiple regression analysis.

Results: At the beginning of the study patients were 24.60 ± 4.45 years old with mean diabetes duration of 9.64 ± 4.42 years. Mean/median values of BMI (23 ± 2.43 kg/m^2), total cholesterol (4.92 ± 1.11 mmol/L), HDL cholesterol (1.57 ± 0.36 mmol/L), LDL cholesterol (2.73 ± 0.77 mmol/L), TG (0.82 ± 0.43 mmol/L), CRP (0.8 (0.1 - 5.1) mg/L), HCY (10.5 (7.5 -14.2) μmol/L), FIB (2.9 (1.9 - 4.1) mg/L), plasma viscosity (1.55 ± 0.10 mPa.s), serum creatinine (81.59 ± 15.05 μmol/L), systolic (113 (95 - 130) mmHg) and diastolic blood pressure (75 (60 - 85) mmHg) were within normal range for diabetic patients, whereas HbA_1c (7.72 ± 1.48 %) and UAE (10.05 (3.18 - 8036.07) mg/24h) were elevated. At baseline, 87 (71%) patients had no retinopathy and

36 (29%) had nonproliferative diabetic retinopathy (NPDR). After 15 years, 54 patients (43.9%; 29.3/1000 person-years) developed NPDR or progressed to proliferative diabetic retinopathy (PDR). None of the patients had diabetic macular edema (DME) at baseline, nor has it developed after 15 years. From the 87 patients with no retinopathy at baseline 24 (27.6%; 18.4/1000 person-years) developed NPDR, while from the 36 patients with NPDR at baseline 30 (83.3%; 55.5/1000 person-years) progressed to PDR. Higher HbA_1c (HR=2.276, P=0.006), lower HDL cholesterol (HR=0.161, P=0.025) and higher UAE (HR=0.388, p=0.045) were significant risk factors for development and progression of retinopathy, whereas the presence of DR at baseline (HR=2.319, p=0.023) was significant factor for its progression to PDR. Diabetes duration, BMI, inflammatory and hemostatic disturbance markers showed no significant values in the statistical analysis.

Conclusion: The results of this study suggest that 15-year incidence of development and especially progression of retinopathy in type 1 diabetic patients is still very high. This points to the need for close monitoring of type 1 diabetic patients aimed at early detection, prevention or limitation the progression of retinopathy, especially those with higher HbA_1c, lower HDL cholesterol, higher UAE and the initial presence of DR.

Disclosure: **M. Tomić:** None.

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Impact of puberty and long term glycaemic control from diabetes onset on incidence of simplex and proliferative retinopathy in type 1 diabetes: the VISS-study

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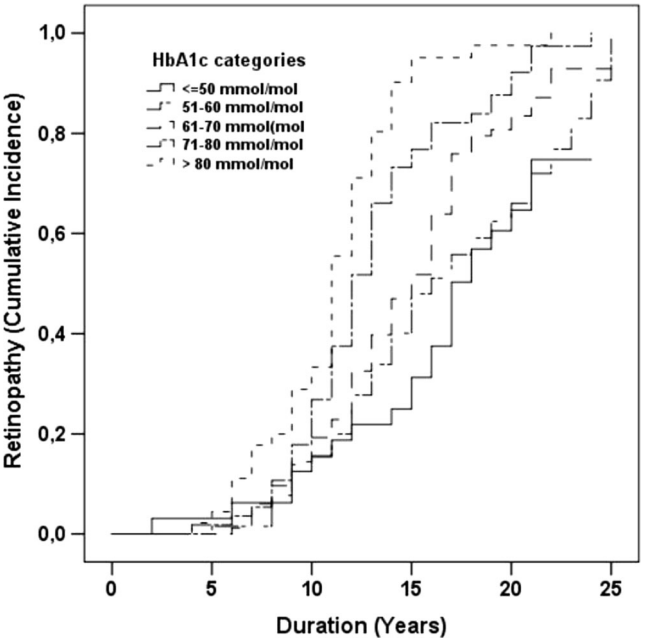
Background and aims: Glycaemic control and diabetes duration are strong risk factors for diabetic retinopathy but there is evidence that the effect of duration is not uniform, being modified by puberty. Our aim was to evaluate sex, onset before, during and after puberty and HbA1c followed from diagnosis as risk factors for developing diabetic simplex and proliferative retinopathy.

Materials and methods: In a population based prospective observational study, 451 patients diagnosed with type 1 diabetes before the age of 35 years during 1983-1987 in the region of South East Sweden were followed. Long term weighted mean HbA1c (wHbA1c) from diagnosis and during the whole follow up of 20 - 25 years was calculated and categorized into 5 levels, ≤50, 51-60, 61-70, 71-80 and >80 mmol/mol. Retinopathy was evaluated by fundus photography.

Results: The time to first appearance of any retinopathy, studied in a subgroup of patients (n=281), who had not moved out from our region, decreased with increasing HbA1c, the difference being most obvious after about 15 years duration (See Figure!). With time most patients developed background retinopathy and the difference in background retinopathy between the HbA1c categories decreased or disappeared. At the end of the follow up 10 of 32 with wHbA1c ≤50 mmol/mol were without any retinopathy while only 1 of 44 in the group >80 mmol/mol had not retinopathy. 58 patients developed proliferative retinopathy. In Cox hazard regression analysis wHbA1c categories, puberty but not sex were associated with development of background retinopathy whereas only wHbA1c categories were associated with proliferative retinopathy. Onset before puberty was associated with prolonged time to appearance of background retinopathy.

Conclusion: There is a strong positive association between long term mean wHbA1c measured from diagnosis and rate of appearance of background retinopathy but with time background retinopathy occur in the majority of patients in spite of excellent glycaemic control. Diabetes onset before puberty requires longer duration to develop background but not proliferative retinopathy.



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Disclosure: **H.J. Arnqvist:** None.

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Prevalence and progression of diabetic retinopathy in 499 type 1 diabetic pregnancies

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Background and aims: 1) to study the prevalence of diabetic retinopathy during pregnancy in women with type 1 diabetes 2) to document the progression during pregnancy and factors associated with this progression.

Materials and methods: We studied a cohort of 499 type 1 diabetic pregnancies followed in the same centre from 1997 to 2015. Management followed the French guidelines. Retinal examination was performed by one ophthalmologist: each trimester in the absence of retinopathy, each month in case of retinopathy at first examination. Diabetic retinopathy was classified according to the ETDRS. Progression was defined as at least one stage of deterioration (apparition or aggravation of the retinopathy).

Results: The mean age was 29.7±4.8 years with duration of diabetes of 13.6±8.1 years. The metabolic control was improved during pregnancy. At inclusion 69.7% of women had normal fundus photography, 23.8% a nonproliferative diabetic retinopathy (NPDR) and 6.4% a proliferative diabetic retinopathy (PDR). The progression of retinopathy occurred in 21.8% women (apparition in 24.4% and aggravation in 15.9%). The regression rate at 1 years' post-partum was 9.3%. Women who

demonstrated progression had a higher preconceptional, 1st and 2nd trimester HbA1c ($p<0.05$, $p< 0.01$ and $p<0.01$) compared to the women without progression. Additionally, the drop in HbA1c was greater between preconception and first trimester ($p<0.01$), between first and third trimester ($p<0.001$), and between preconception and the lower HbA1c during pregnancy ($p<0.001$) among the women who had progression. After multivariate analysis, risk factors for retinopathy progression were duration of diabetes > 10 years ($p<0.0001$), nulliparity ($p<0.05$), and absence of retinopathy before pregnancy ($p<0.001$).
Conclusion: This study highlights the ongoing risk of retinopathy progression during pregnancy among women with type 1 diabetes. Our study reinforces other published works that found a progression of retinopathy associated with a more important fall in HbA1c level during pregnancy and validates the need for close follow up, especially in women with risk factors.
Disclosure: **A. Vambergue:** None.

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Quantitative measurement of retinal thickness in diabetic retinopathy and qualitative correlation between the retinal thickness and visual acuity

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Background and aims: The conventional practice of assessing the macula for its thickness, involving slit-lamp biomicroscopy, fundus photography, and fluorescein angiography, is less sensitive to subtle changes and is limited to provide qualitative findings. Optical Coherence Tomography (OCT) provides a rapid and non-invasive method to assess retinal structures at a microscopic level. Macular edema remains the most common reason for intervention by ophthalmologists in patients with diabetes confronting visual loss and the degree of macular thickening is significantly correlated with visual acuity. Measuring macular edema is therefore of major importance in imaging patients with Diabetic Macular Edema (DME)
Materials and methods: We conducted a prospective evaluation across 100 patients with Type 2 Diabetes (T2DM) with DR (184 eyes) to assess the grade of DME by a comprehensive ophthalmologic examination to analyze retinal thickness, morphology and presence of vitreo-macular traction using OCT and correlate the macular thickness to Best Corrected Visual Acuity (BCVA) by utilizing the dilated fundus photography, fluorescein angiography and OCT. Statistical analyses were performed using independent t-test and Chi-Square test
Results: OCT precisely differentiated 4 types of DME, namely Type 1, 2, 3 and 4 which were simple (n=11), diffuse without cyst (n=33), cystoid (n=48), and vitreomacular traction (n=8) respectively. There was a significant correlation between OCT and Fundus Fluorescein angiography (FFA) ($p< 0.00001$) and Best Corrected Visual Acuity (BCVA) across macular thickness range <200 to > 500 μm. ($p< 0.00001$). 50 patients were detected with the retinal thickness > 400 μm with 16 patients with retinal thickness <200 μm. The worst BCVA (> 20/62.5) was observed in 6 patients with retinal thickness > 500 μm with the best reported BCVA (20/20-20/30) was in 17 patients with retinal thickness <200 μm
Conclusion: Our study with a triple correlates (OCT, FFA and BCVA) provides insights to the early prediction of diabetic maculopathy defined according to the Early Treatment Diabetic Retinopathy Study classification. The association between these parameters would prove useful in monitoring disease progression and identifying parameters that affect visual function. The increased macular thickness may be an earliest predictor to detect the onset of diabetic retinopathy in association with other correlates. This could help increase the reliability and precision to accurately predict the prognosis in patients with DME and help determine an appropriate therapeutic approach especially in early stages when structural changes are not yet evident with the conventional techniques
Disclosure: **K. Shah:** None.

PS 089 Novel treatments of retinopathy

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Calcium dobesilate prevents the oxidative stress and inflammation induced by diabetes in the retina of db/db mice

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Background and aims: Calcium Dobesilate (CaD) is beneficial in early stages of diabetic retinopathy (DR), but its mechanisms of action remains to be elucidated. The aim was to investigate the effect of CaD on proinflammatory cytokines and oxidative stress.
Materials and methods: db/db mice were randomly assigned to daily oral treatment with CaD (200 mg/Kg/day) or vehicle for 15 days. Biomarkers of oxidative stress (dihydroethidium, malondialdehyde), NF-κB, and proinflammatory cytokines (IL-1β, IL-6, IL-8, TNF-α, MCP-1) were examined in the retina by immunohistochemical analysis. Mitochondrial manganese superoxide-dismutase (MnSOD) was measured by Western blot. Cultures of human retinal endothelial cells (HRECs) were used for complementary experiments.
Results: CaD significantly reduced the biomarkers of oxidative stress and ameliorated the downregulation of MnSOD in the retina of db/db mice. In addition, CaD prevented the increase of NF-κB, IL-6, IL-8, TNF-α and MCP-1 induced by diabetes. These effects were associated with a significant reduction of vascular leakage. CaD inhibited the activation of NF-kβ induced by IL-1β by preventing IKKB-α phosphorylation in HRECs and reduced the upregulation of IL-6 and IL-18 induced by TNF-α in a dose-dependent manner.
Conclusion: Our results suggest that antioxidant and antinflammatory effects are crucial in accounting for the effectiveness of CaD for treating DR.
Disclosure: **P. Bogdanov:** None.

1042

Increased plasma concentrations of vascular endothelial growth factor 165 and vascular endothelial growth factor 165b in diabetes patients with diabetic retinopathy

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Background and aims: Diabetic retinopathy (DR) is the most frequent cause of blindness among younger adults in the western world. No blood biomarkers exist to detect DR. We therefore speculated if the plasma concentration of vascular endothelial growth factor $(VEGF)_{165b}$ and $VEGF_{165}$ could be early markers of retinal damage. The aim of the study was to measure the plasma concentration of $VEGF_{165}$ and $VEGF_{165b}$ and to investigate whether the plasma concentration of $VEGF_{165b}$ and $VEGF_{165}$ in diabetes patients with DR differed from the plasma concentration of $VEGF_{165b}$ and $VEGF_{165}$ in control persons and in diabetes patients without DR.
Materials and methods: A digital ELISA assay ($VEGF_{165b}$) using a Simoa HD-1 Analyzer (Quanterix©, Lexington, MA 02421, USA) and a conventional ELISA assay ($VEGF_{165}$) (QuantiGlo ELISA, RnD Systems, Abingdon, OX14 3NB, UK) were applied on a cohort of type 1 and type 2 diabetes patients characterised with DR (n=466) and without DR (n=144) as well as on a sex and aged matched control group (n=169). The diabetes patients without DR consisted of a group of diabetes patients with HbA1c ≤ 5.5% (n=77) and a group of patients with HbA1c ≥ 9.7%

(n=67). The patients with DR were divided in a group of patients with DR at time of inclusion into the cohort (n=270) and in a group of patients who developed DR during follow-up (n=196).
Results: $VEGF_{165}$ was statistical significantly increased in patients with DR, both in the group of patients with DR at time of inclusion into the cohort (40 ng/L, $p < 0.001$) and in the group of patients who developed DR during follow-up (35 ng/L, $p = 0.02$) compared to the control group (31 ng/L). Further, $VEGF_{165}$ was increased in the group of diabetes patients with HbA1c ≥ 9.7% (40 ng/L, $p < 0.01$). $VEGF_{165b}$ was increased in diabetes patients with DR at time of inclusion into the cohort (19 pg/L, $p < 0.001$) and in diabetes patients developing DR during follow-up (16 pg/L, $p = 0.06$) compared to the control group (13 pg/L).
Conclusion: We determined the plasma concentration of $VEGF_{165}$ and $VEGF_{165b}$ by ELISA and a digital ELISA method, respectively. Plasma $VEGF_{165}$ and $VEGF_{165b}$ were increased in diabetes patients with DR, and $VEGF_{165}$ was also increased in diabetes patients with high HbA1c without DR. These findings support the existing knowledge on the mechanism of $VEGF_{165}$ and $VEGF_{165b}$ in DR but the difference between the groups was not large enough to identify patients at risk.
Supported by: The Lions Club International Denmark
Disclosure: **E.R.B. Petersen:** Grants; The Danish Council for Independent Research/Medical Sciences, The Research Council of Vejle Hospital, The Danish Research Fund, The Lions Club International Denmark.

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Plasma glutamine and glutamic acid are potential novel biomarkers for predicting long-term clinical outcomes of diabetic retinopathy

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Background and aims: Some diabetic patients experience few or no complications despite a long disease duration. We identified differences in plasma metabolites in type 2 diabetes patients with and without diabetic retinopathy (DR) and a disease duration of 15 years or more.
Materials and methods: From September 2014 to July 2015, a cohort of 183 elderly type 2 diabetes patients was established. Their clinical data and biospecimens were collected in accordance with the guidelines of the Korean Diabetes Association and the National Biobank of Korea, and DR phenotypes were identified by ophthalmologic specialists. Plasma metabolites were profiled with a comprehensive metabolomic approach using gas chromatography time-of-flight mass spectrometry and ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry with multivariate statistical analyses.
Results: For metabolite analysis, 32 case-control sets with adjusted clinical variables were selected. The mean age of the subjects was 66.4 years and mean diabetes duration was 22.2 years. Metabolomic analysis revealed various amino acids, organic compounds, and carbohydrates that differed between age- and sex-matched non-diabetic controls and diabetic subjects. Among these metabolites, glutamine and glutamic acid were identified as the most accurate biomarkers for the presence of DR in subjects. ROC analysis showed a high diagnostic value of glutamine (AUC = 0.671), glutamic acid (AUC = 0.656), and the ratio between the two (AUC = 0.742) for DR. The results were consistent in validation analyses.
Conclusion: Our study suggests that glutamine, glutamic acid, and their ratio may be useful as new biomarkers for predicting the prognosis of DR in elderly type 2 diabetes patients.
Clinical Trial Registration Number: KCT0001269
Supported by: HD14B1246, and HC15C3364
Disclosure: **S. Rhee:** None.

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Circulating extracellular vesicles from diabetic and healthy subjects show different miRNA patterns

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Background and aims: We previously demonstrated that extracellular vesicles (EV) derived from mesenchymal stem cells (MSC) cultured in high glucose and/or hypoxia are able to enter retinal pericytes, causing their detachment from substrate and migration, and stimulating angiogenesis in vitro. Our present hypothesis is that circulating EV in diabetes may influence small vessel homeostasis, and that molecular differences can be identified in EV from healthy controls and diabetic subjects, with/without microvascular complications. The aim of this study was the molecular characterization and comparative analysis of EV derived from healthy and diabetic subjects with and without microvascular complications, and their influence on pericyte detachment and angiogenesis.
Materials and methods: EV were extracted from plasma of 4 type 1 diabetic patients with microvascular complications (nephropathy and retinopathy) (CDM, sex: 1F/3M, age: 48.0±16.3, disease duration: 22.5±10.2), 4 without complications (DM, sex: 1F/3M, age: 44.5±8.1, disease duration: 21.0±9.6) and 4 healthy controls (noDM, sex: 1F/3M, age 39.0±9.2). EV expression of surface molecules was measured by FACS. microRNA (miRNA) content was evaluated by Taqman Human MicroRNA Arrays - cards A and B, which allow detection of 754 different miRNAs. Human retinal pericyte (HRP) detachment was evaluated by cell counting after 24 hr exposure to EV. Proliferation and apoptosis were measured in HRP still attached to culture wells. In vitro angiogenesis in HRP-endothelial cell co-cultures was analyses by tube formation in Matrigel after 48 hr EV exposure.
Results: FACS analysis of surface molecules showed no significant changes between groups. Only miR-106a was increased in the DM compared with the noDM group ($p<0.05$), while 10 miRNAs differed between the noDM and CDM groups. Five of them, with anti-angiogenic properties (miR-150, miR-155, miR-342-3p, let-7-g, miR-1243), were decreased, and 5, with pro-angiogenic properties (miR-17, miR-106a, miR-484, miR-580, miR-21*), were increased ($p<0.05$, all). As regards CDM vs DM subjects, 7 miRNAs were significantly modulated: 2 decreased (miR-30b, miR-21*) and 5 increased (miR-139-5p; miR-342-3p; miR-150; miR-24; miR-1243) ($p<0.05$, all). EV from CDM subjects increased HRP detachment (+21.7%, $p<0.05$ vs noDM), while EV from DM subjects had no effect. HRP which remained attached to wells showed no signs of apoptosis. Moreover, EV from CDM subjects increased the formation of vessel-like structures in vitro in comparison with EV from healthy controls ($p< 0.001$).
Conclusion: These observations suggest that EV patterns may be different in diabetic patients, with or without complications, compared to healthy subjects. In particular, an imbalance between miRNAs with pro- and anti-angiogenic functions could lead to abnormal microvascular proliferation, and contribute to proliferative diabetic retinopathy. Further studies could provide predictive options for diagnostic purposes and tools for the treatment of vessel abnormalities in diabetes.
Supported by: EFSD/Lilly
Disclosure: **A. Mazzeo:** Grants; EFSD - Lilly Fellowship 2016.

1045

Lixisenatide protects the neurovascular unit in diabetic retinopathy

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Background and aims: Diabetic retinopathy is a disease of the entire neurovascular unit (NVU), with the pathogenic hallmarks of vasoregression, neuronal dysfunction and glial activation. GLP-1 receptor agonists are beneficial, not only in the treatment of diabetes and its complications, but also in several neurodegenerative and neuroinflammatory diseases. In this study we analyse the effects of the GLP-1 receptor agonist lixisenatide on the neurovascular unit in a rat model of diabetic retinopathy.
Materials and methods: Streptozotocin-induced diabetic Wistar rats were treated with Lixisenatide (150 μg/kg body weight) for 12 weeks (DC+Lixi). Untreated animals served as controls (DC). Vasoregression was quantified using retinamorphometry (QRM) for pericytes (PC) and acellular capillaries (AC). Neuroretinal function was assessed with multifocal-electroretinography (mfERG). Glial activation was analysed with western blots for GFAP (Müller glia) and Cd74/Iba1 whole mount immunofluorescence staining (microglia). Methylglyoxal was quantified using HPLC-MSMS.
Results: Lixisenatide prevented pericyte dropout (1885±42.7 vs 2120±35.9 PC/mm²; DC vs DC+Lixi; p<0.01) as well as formation of acellular capillaries (64.7±4.8 vs 88.5±4.8 AC/mm²; DC vs DC+Lixi; p<0.01). Neuronal function of the inner retina improved upon treatment with Lixisenatide (b-wave amplitude: 0.87±0.15 vs 1.46 ±0.06 μV/segment; DC vs DC+Lixi; p<0.01). GFAP was significantly decreased in the Lixisenatide groups (0.28±0.04 vs 0.14 ±0.04 mean AU; DC vs DC+Lixi; p<0.05) indicating reduced Müller glia activation. Microglial activation was also markedly reduced in treated animals (20.1±1.5 vs 7.5±1.6 $Cd74^+$ microglia/mm²; DC vs DC+Lixi; p<0.01). Lixisenatide treatment showed no effects on retinal methylglyoxal levels (12.0 vs 10.2 pmol/mg; DC vs DC+Lixi; n.s.), bodyweight (372±26 vs 367±23 g; DC vs DC+Lixi; n.s.) or HbA1c (12.5±1.1 vs 12.0±1.3 %; DC vs DC+Lixi; n.s.).
Conclusion: In this study we demonstrate the beneficial effects of Lixisenatide on the neurovascular unit in the diabetic retina. Lixisenatide protects from vasoregression, neuronal dysfunction and glial activation independently of metabolic effects proving its efficiency in the treatment of diabetic microvascular complications.
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Disclosure: **K. Acunman:** Grants; Sanofi-Aventis Deutschland GmbH.

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MicroRNA 124a inhibits microglial migration and activation

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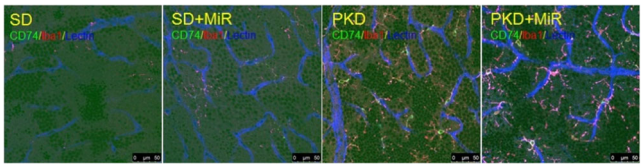
Background and aims: MicroRNAs (MiR) are a family of regulatory molecules involved in many physiological processes, including differentiation and activation of cells of the immune system. MiR-124 is a small non-coding RNA molecule with ~21 nucleotides. It is the most abundant microRNA expressed in neuronal cells. However, it is unknown if miR124 plays an important role in regulating microglial migration and activation in retinas with neurodegenerative disease. To investigate the effect of miR124 on microglia, we intravitreally administered miR124 to polycystin-2 transgenic rats, a model of polycystic kidney disease (PKD) assiociated with retinal neurodegeneration, and characterized the Impact on microglial activation.
Materials and methods: In vitro experiment: BV2 cells were transfected with control microRNA, MiR-124a and its inhibitor for 24 hours. Transwell migration assay was performed by using a culture insert of polyethylene terephthalate (PET) with a membrane of 8μm pore size. Cells from 10x random fields were quantified under microscope. In vivo experiment: Male PKD transgenic rats at 8weeks and 10 weeks old were twice intravitreally injected with microRNA 124 and its inhibitor. Eyes were enucleated at 12 weeks. Microglial and retinal vessels were labelled for CD74 and Lectin. Leica SP8 confocal microscope and Leica Application Suite X software were used for taking images and quantification.
Results: MicroRNA 124 not only inhibits microglial migration in vitro, but also suppressed microglial activation in vivo. The number of CD74 positive microglial cells increased in the deep layer of retina and significantly reduced after microRNA 124 treatment compared with the control group.
Conclusion: MicroRNA 124 inhibits microglial migration and activation in vivo and in vitro.



Supported by: EYEnovative
Disclosure: **J. Lin:** None.

1047

Effects of bariatric surgery on retinal microvascular architecture

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Background and aims: Retinal microvasculature changes reflect damage from obesity, hypertension, diabetes and other chronic disease processes. The impact of bariatric surgery induced weight loss on preclinical changes in the microvasculature are relatively unknown. We hypothesised that weight loss following bariatric surgery would be associated with improved structural changes in the retinal microvasculature, specifically with a reduction in retinal arteriolar narrowing and venular widening.
Materials and methods: The study included 22 obese subjects scheduled for bariatric surgery (laparoscopic Roux-en-Y gastric bypass or a sleeve gastrectomy) and 15 lean, age-matched controls. Ophthalmic examination, including fundus photography, was performed at baseline and 6 months. The retinal microvasculature was analysed quantitatively using a semi-automated computer program.
Results: Mean weight loss at 6 months was 26 kg ± 8 kg, (p<0.001) in the bariatric surgery group. The central retinal artery equivalent arteriolar (CRAE) increased (Mean and SE: 136 ± 1.4 μm to 141 ± 1.4 μm, p <0.012) and the central retinal vein equivalent (CRVE) decreased (from 203 ± 1.9 μm to 197 ± 1.9 μm, p <0.046) in the bariatric surgery group by 6 months, with no change in CRAE (from 137 ±1.5 to 135 ± 1.2, p=0.222) or CRVE (195 ± 2.1 to 193 ± 2.2, p=0.550) in the control group. The arteriolar to venular ratio increased in the bariatric surgery group (from 0.67 ± 0.01 to 0.72 ± 0.01, p=0.002), with no change in the control group (from 0.71 ± 0.01 to 0.70 ± 0.01, p=0.550) at 6 months. No change was observed for tortuosity, branching angles or fractal dimension.
Conclusion: The findings suggest obesity-related impairments in retinal arteriolar narrowing and venular widening are reversible after bariatric

surgery inducing weight loss. The capacity for the retinal microvasculature to improve following bariatric surgery, furthers our understanding of the plasticity of the retinal microvasculature, early in the disease course.
Clinical Trial Registration Number: NCT01373892
Supported by: AF, FCF, VSRF, DRF, FMF, JRAF, TSY
Disclosure: **R.J. Tapp:** None.

1048

Thiamine transporters and hyperglycaemia-induced damage in human retinal cells

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Background and aims: Thiamine, a co-factor for transketolase and other glycolytic enzymes, counteracts high glucose-induced damage in microvascular cells *in vitro* and prevents progression of retinopathy and nephropathy in diabetic animals. Impaired thiamine availability may facilitate metabolic damage, and diabetes might be considered a thiamine-deficient state, if not in absolute terms, at least relative to the increased requirements deriving from accelerated glucose metabolism in non-insulin dependent tissues prone to complications. Renal loss via proximal tubules, resulting in reduced thiamine/transketolase activity, has been described in diabetic patients. We previously described 2 single nucleotide polymorphisms (SNPs), rs12694743 and rs6713116, located in the SLC19A3 gene encoding for the thiamine transporter THTR2, and associated with resistance to development of proliferative diabetic retinopathy and end-stage renal disease in type 1 diabetic subjects. The protective effects of these SNPs may work either through loss of function, decreasing the discharge of thiamine, or gain of function, by increasing its uptake in target tissues. The objective of this work was to investigate if, in human retinal cells, a diabetic-like microenvironment is able to modulate the expression of the two thiamine transporters THTR1 and THTR2 and their transcription factor Sp1.
Materials and methods: Human retinal pericytes (HRP), human microvascular endothelial cells (HMEC) and human Műeller cells (MIO-M1) were cultivated for 8 days in physiological glucose (NG), stable high glucose (HG) or intermittent physiological/high glucose conditions (intHG) (n=6). To investigate substrate influence on the expression of transporters, cells were also cultured in thiamine-deficient medium (noT) or in high thiamine conditions (50μmol/l, HT) (n=6). THTR1, THTR2 and Sp1 mRNA expression was checked by relative quantitative RT-PCR and protein expression by Western blotting.
Results: Our results show that both transporters and Sp1 are expressed in HRP, HMEC and MIO-M1, THTR1 being more expressed than THTR2 in all cases. THTR2 and Sp1 mRNA expression decreased in HRP cultured in HG and intHG (THTR2: -20.8 and -36.1% respectively, $p<0.05$ vs NG; Sp1: -17.1 and -19.9%, $p<0.05$), while THTR1 expression was unchanged. On the contrary, THTR2 mRNA expression increased in HMEC (+29.7%, $p<0.05$) and MIO-M1 cells cultured in intHG (+36.4%, $p<0.05$). Protein expression checked by Western blotting confirmed these results. Different thiamine concentrations did not influence THTR1, THTR2 or Sp1 expression.
Conclusion: Diabetic-like conditions are able to modulate the expression of thiamine transporters in retinal cells. However, THTR2, which appears to be the most affected, is regulated in opposite directions in pericytes on one side, and endothelial and Műller cells on the other. Pericytes are the first cells to be affected by early diabetic retinopathy; therefore, decreased expression of THTR2 in these cells may lead to reduced intracellular availability of thiamine, with consequent metabolic damage due to accumulation of toxic metabolites. On the contrary, increased expression of THTR2 in the surrounding cells may be interpreted as an attempt to counteract glucose-induced damage, by stimulating thiamine uptake.
Supported by: EFSD/Boehringer Ingelheim
Disclosure: **E. Beltramo:** None.

PS 090 Understanding and treating nephropathy

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The 'fatty kidney': crosstalk of renal sinus fat with glomerular cells under the influence of the hepatokine fetuin-A

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Background and aims: We previously identified the fatty liver-derived hepatokine fetuin-A to induce pro-inflammatory signalling in perivascular fat cells (PVFC). Renal sinus fat (RSF) is a perivascular fat compartment located around the renal vessels potentially adversely impacting on renal function. In this study, we hypothesized that fetuin-A may impair renal function in NAFLD by proinflammatory signalling in RSF cells (RSFC).
Materials and methods: RSF was quantified by MRI and liver fat content by ^{1}H-MR spectroscopy in 449 individuals at risk for type-2 diabetes. Impaired renal function was assessed by the determination of uACR. To study the crosstalk of fetuin-A with RSF, human RSFC, endothelial cells (EC) from specimens of kidney transplantation donors and a human podocyte cell line (PO) were used. RSFC, EC and/or PO were cocultured in transwell systems for 6-72 h ±600 μg/ml human fetuin-A. Protein secretion was quantitated by Luminex analysis and mRNA expression by realtime PCR. Macrophages and microvessels in renal fat resections were stained by standard immunohistochemistry methods.
Results: While RSF and fetuin-A did not associate with uACR in subjects without NAFLD (n=212; beta=0.04, p=0.94 and n=88, beta=-0.09, p=0.41) positive correlations were found between RSF and fetuin-A with uACR in subjects with NAFLD (n=105; beta=0.40, p=0.0005 and n=41; beta=0.3, p=0.05). In cocultures without fetuin-A, RSFC downregulated proinflammatory and upregulated regenerative factors in EC/PO. Proinflammatory expression levels in RSFC were higher than in PVFC. Coculture of RSFC with EC/PO downregulated IL-6/-8 and MCP-1 expression, induced HGF in EC and decreased TGF-β in PO. Fet-A/palmitate increased IL-6/-8 and MCP-1 in RSFC potentially. HGF was even inhibited. Downstream of Fet-A/palmitate-induced TLR4 activation, NFκB- and JNK-mediated signalling was involved. Fet-A-treated RSFC inverted the benign effects on EC and PO from an anti- to a proinflammatory status. HGF, IL-8, MCP-1, ICAM-1 and ALCAM expressions were stimulated in EC, IL-6/-8 in PO. TGF-β was slightly reduced. In RSF resections, M1/M2 macrophages and many microvessels could be detected.
Conclusion: In vitro, treatment of RSFC with elevated fetuin-A doses inverted the benign effects of RSFC on glomerular cells from a reduced into an induced unhealthy proinflammatory status indicating a Janus-faced characteristic of RSFC. Analysis of our cohort indicates that RSF, particularly in conjunction with fatty liver, may influence renal function in apparently healthy individuals. Taken together, the crosstalk of the fatty liver and the kidney via RSF may adversely affect renal (patho)physiology.
Supported by: German Center for Diabetes Research
Disclosure: **D.I. Siegel-Axel:** None.

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Lifetime dietary macronutrient intake ratios alter kidney function and structure into old age

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Background and aims: Chronic kidney disease is prevalent in diabetes (DKD) affecting ~30% of patients, increasing their risk for stroke and cardiovascular disease. DKD is a progressive disorder where changes in glomerular filtration rate (GFR) and structural abnormalities such as tubulointerstitial fibrosis (TIF) and glomerulosclerosis (GS) can predict progression. Despite dietary protein restriction appearing in guidelines for advanced DKD management, the influence of macronutrients on kidney function and structure over the course of ageing has not been investigated. Hence, in this study we use a novel geometric framework to assess the long term effects of macronutrient combinations on kidney function and structure in ageing.
Materials and methods: Male and female C57 mice (N=4/group) were fed *ad-libitum,* 1 of 25 experimental diets that varied across a spectrum of protein (5-60%), carbohydrate (20-75%) and fat (20-75%), at low, medium or high energy contents. At 15 months of age (late middle age), organs and blood were collected. TIF and GS were assessed histologically, whilst serum cystatin C was used as a surrogate for GFR. Blood urea and ions were analysed by COBAS. Risk factors for DKD (body composition, glucose tolerance, systolic (SBP) and diastolic (DBP) blood pressure, and lipids) were assessed just prior to death by gold standard techniques. Using a geometric framework, we produced 4-dimensional models (general additive models) to visualise and quantify the impacts of macronutrient consumption, as both main effects and interactions on each of the DKD factors of interest. Statistical analysis was performed using the mgcv package for R.
Results: Serum cystatin C ranged from 126 to 1006 ng/ml, and was negatively correlated with dietary protein intake (P<.0001), whereby GFR was decreased with lower protein intake. GFR was not influenced by dietary consumption of fat or carbohydrate. A spectrum of TIF was observed in kidney sections (0.5 to 9%). Lower protein consumption in the context of either a high fat or high carbohydrate intake predicted greater TIF (P=0.0005, P= 0.016, respectively). GSI tended to be greater with higher fat intake in the context of lower protein or carbohydrate, although this did not reach significance (p=.083). When lifetime dietary intake of protein was lower, coupled with high fat or increased caloric intake, GFRs were lower and kidney fibrosis was increased in conjunction with increased adiposity (P=.16 and P=.01). Surprisingly, long term consumption of macronutrient combinations which increased SBP or DBP, elevated plasma LDL cholesterol or triglycerides or resulted in glucose intolerance, did not associate with decreased GFR or kidney structural damage.
Conclusion: This framework shows that both individual macronutrients in isolation and their relative combinations affect risk factors for DKD over a lifespan. Overall lower protein intake in conjunction with higher consumption of fat, or calories, results in a phenotype with the lowest GFR and the most kidney structural damage as assessed by TIF and GSI.
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Disclosure: **A.K. Fotheringham:** None.

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Relation between renal vitamin D-endocrine system and RANK/RANKL-signalling pathway in diabetes nephropathy: effect of vitamin D_3 treatment

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Background and aims: Substantial deficiency of vitamin D_3 (D_3) and disruption in the functioning of D_3-endocrine system may play a crucial role in the development of diabetic nephropathy, but the precise mechanism is not fully explored. Growing evidence suggests that receptor activator of nuclear factor κB (RANK)/RANK ligand-signaling pathway can be involved in the development of various renal diseases and close association may exist with D_3-associated regulatory processes. We, therefore, investigated the relationship between the changes in renal vitamin D-endocrine system and RANK/RANKL-singaling pathway in diabetes nephropathy and estimated the effect of vitamin D_3 treatment.
Materials and methods: Type 1 diabetes was induced in male C57BL/J6 mice (23.0 ± 1.6 g) by i.p. injection of high dose of STZ (150 mg/kg b.w.). After 2 weeks of STZ-induced diabetes mice were treated with or without vitamin D_3 (15 IU/mouse per os, for 8 weeks). Serum $25OHD_3$ was assessed by ELISA. The mRNA expression of D_3-endocrine system components and pro-inflammatory cytokines was measured by RT-qPCR. Levels of VDR, RANK and RANKL were detected by immunoblotting. VDR and RANK localization in kidney tissue was visualized using immunohistochemistry. Histological features were assessed by H&E staining.
Results: Serum level of $25OHD_3$ was found to be reduced to 24.6 ± 2.1 in diabetes vs. 40.3±2.8 nmol/L in control, indicative of diabetes-induced D_3 deficiency ($p< 0.05$). D_3 deficiency correlated with abnormalities in D-endocrine system as are evident from decreased levels of cubulin, megalin and CYP24A1 mRNAs and increased expression of CYP27B1 and DBP mRNAs. It was also shown that diabetes induced more than 2-fold decrease in VDR protein level in kidney tissue of diabetic mice vs. control ($p<0.05$). These changes were accompanied by upregulation of the expression of key pro-inflammatory markers in kidney tissue: nuclear factor κB (NF-κB)/p65, IL-6, IL-1β, and TNF-α. Diabetes-related inflammation and failures in D_3-endocrine system were shown to be associated with overexpression of RANKL and RANK proteins (1.6- and 1.4-fold respectively vs. control, $p<0.05$). Given that RANKL is referred to as an upstream effector of the NF-κB activation, it can be implicated in the mechanism of impaired genomic regulation of cytokines expression as an underlying effector of diabetic nephropathy. Full restoration of $25OHD_3$ content and partial normalization of kidney tissue structure were achieved by D_3 treatment. Vitamin D_3 administration caused a partial normalization of D-endocrine system that led to VDR-mediated decrease in mRNA expression of pro-inflammatory cytokines as well as protein levels of RANK and RANKL in renal tissue of diabetic animals.
Conclusion: The present study confirmed that to a large extent diabetes-induced kidney abnormalities can be associated with the upregulation of RANK/RANKL signaling pathway and NF-κB-mediated expression of pro-inflammatory cytokines that correlated with insufficient vitamin D_3 availability and VDR downregulation. Our results can explain protective VDR-mediated effects of vitamin D_3 against diabetes-induced kidney injury through RANK/RANKL signaling pathway.
Disclosure: **D. Labudzynskyi:** None.

1052

Baseline characteristics in PRIORITY study: proteomics and mineralocorticoid receptor antagonism for prevention of diabetic nephropathy in type 2 diabetes

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Background and aims: The urinary proteomics based classifier CKD273 has been shown to retrospectively identify normoalbuminuric

diabetic patients who progress to overt kidney disease. In the PRIORITY (Proteomic prediction and Renin angiotensin aldosterone system Inhibition prevention Of early diabetic nephRopathy In TYpe 2 diabetic patients with normoalbuminuria) trial, the aim is to confirm that CKD273 can predict microalbuminuria prospectively, and to test whether mineralocorticoid receptor antagonism (MRA) delays progression to microalbuminuria. Here we report the association between CKD273 and traditional risk factors for diabetic nephropathy at baseline.

Materials and methods: PRIORITY is an investigator-initiated, prospective, randomized, double blind, placebo-controlled multicentre clinical trial and observational study in normoalbuminuric type 2 diabetic patients. Patients are stratified into high-or low risk groups based on CKD273. Patients in the high risk group are assigned to spironolactone 25 mg once daily or placebo, whereas the low-risk group is followed on standard care. The trial continues until September 2018, following patients for up to 4.5 years. The primary endpoint is development of microalbuminuria.

Results: In total 2277 type 2 diabetic patients have been screened over a time period of 2.5 years and 1811 are included from 15 sites. Table 1 shows the baseline characteristics. 224 (12.4%) have the high-risk CKD273 pattern. The high- and low-risk populations differ statistically in terms of gender, age, diabetes duration, UACR and eGFR however the differences are numerically small. Univariate regression analyses of CKD273 vs each baseline variable demonstrated weak associations (R^2 of 0.03, p <0.0001) for the strongest correlations with UACR and eGFR respectively. In a logistic regression model predicting CKD273 risk strata, including all baseline variables, eGFR and UACR remain statistically significant (p < 0.0003) with an AUC of 0.70 (95 % CI: 0.65, 0.74).

Conclusion: Classical risk factors for diabetic kidney disease differ only slightly between high and low risk patients based on the urinary proteomics based risk classifier CKD273 in type 2 diabetes, suggesting it provides additional information to the measures already available in the clinic. The potential added value will be tested in this prospective study.

Variable \	Included N=1811	Low-risk N=1587	High-risk N=224	P-value (high vs. low)
Gender, male, *n* (%)	1132 (62)	976 (61)	156 (69)	⊐0.03
Age, years	62 (8)	61 (8)	63 (7)	0.0014
Diabetes duration, years	11 (8)	11 (8)	13 (8)	0.0010
Systolic blood pressure, mmHg	134 (14)	133 (12)	134 (13)	0.07
Diastolic blood pressure, mmHg	79 (9)	78 (9)	78 (9)	0.77
eGFR, ml/min/1.73 m^2	87 (15)	88 (15)	81 (17)	<0.0001
UACR, Median (IQR), mg/g	7 (3 – 9)	5 (3 – 8)	7 (4 – 12)	<0.0001
HbA1c, mmol/mol	58 (12)	57 (17)	59 (13)	0.22
LDL cholesterol, mmol/L	NA	2.4 (0.9)	2.4 (1.0)	0.78

Table 1 Baseline characteristics of the overall study cohort and by subgroup, Mean (SD)

Clinical Trial Registration Number: NCT02040441
Supported by: EU FP7 grant agreement no. 279277
Disclosure: **N. Tofte:** None.

1053

Adiponectin receptor agonist, AdipoRon, protects cultured podocytes against lipopolysaccharide-induced inflammation and apoptosis

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Background and aims: Chronic inflammation has emerged to play a significant role in the pathogenesis of diabetic kidney disease. Several diabetes-associated factors can induce chronic inflammation, such as bacterial lipopolysaccharides (LPS) in the serum. On the other hand, type 2 diabetes is often accompanied with lowered serum levels of adiponectin hormone. As adiponectin has anti-inflammatory effects, its deficiency or downregulation of its receptor may support chronic inflammation and predispose podocytes to cellular injury and development of albuminuria. The aim of this study is to investigate whether the first small-molecule agonist of the adiponectin receptor, AdipoRon, protects cultured podocytes from LPS-induced inflammation and cell death.

Materials and methods: Human kidney samples were obtained from surgical nephrectomies and were taken from the nonmalignant part of the kidney. Paraffin sections were stained with AdipoR1 antibody and positive staining area (%) within 10 glomerular sections per patient was quantified by HistoQuant software. Differentiated human podocytes (AB8/13) were pre-treated with AdipoRon or vehicle for 2 hours and exposed to LPS for additional 1 or 24 hours. Culture media was collected after 24 hours and secreted cytokines were measured by ELISA. Protein levels of cleaved caspase-3, p-JNK and p-IκBα were measured from cell lysates by quantitative immunoblotting.

Results: We found that Adiponectin receptor 1 (AdipoR1) is strongly expressed in human glomeruli and especially in podocytes. In females, patients with type 2 diabetes (n=11) had 14% less AdipoR1-positive area in comparison to non-diabetic controls (n=16) (p<0.05). In men, no difference was observed between type 2 diabetes patients (n=23) and non-diabetic controls (n=24). The average age did not differ between the groups. Downregulation of renal AdipoR1 expression has been previously observed in albuminuric rodent models of type 2 diabetes, indicating that it may play a role in the development of diabetic kidney complication.

In the in vitro studies, we found that 24-hour LPS stimulation caused 2-fold increase in the expression of cleaved caspase-3 (p<0.001) in cultured podocytes, and was restored back to the level of the control by AdipoRon (p<0.001). This indicates that AdipoRon prevents LPS-induced apoptotic cell death. Simultaneously, LPS stimulation caused 3-fold upregulation (p<0.01) of the secretion of tumor necrosis factor-α (TNF-α), which was reduced by 41% by AdipoRon (p<0.01). As TNF-α has pro-apoptotic and pro-inflammatory effects, the anti-apoptotic effects of AdipoRon are likely mediated via lowering inflammation. In line with this, we found that 1-hour LPS stimulation upregulated phosphorylation of JNK (Thr183/Tyr185) and IκBα (Ser32/36), which was prevented by AdipoRon. The JNK and NFκB pathways, which are activated by phosphorylation of JNK and IκBα, respectively, are known to mediate inflammatory responses and cell survival.

Conclusion: Our results suggest that activating the adiponectin receptor by AdipoRon is a potent way to alleviate inflammation and apoptosis of podocytes. Further studies are needed to investigate whether AdipoRon could prevent inflammation-related glomerular injury in vivo.

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Disclosure: **S. Lindfors:** Grants; Finnish Kidney Foundation; Finnish-Norwegian Medical Research Foundation; Päivikki and Sakari Sohlberg Foundation; Finnish Diabetes Research Foundation; Sigrid Juselius Foundation.

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The phosphodiesterase inhibitor cilostazol reduced the risk of chronic kidney disease in type 2 diabetes without diabetic kidney disease

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Background and aims: The purpose of this study was to investigate a possible association between the use of cilostazol (a phosphodiesterase type 3 inhibitor) and the development of chronic kidney disease (CKD) through a post-hoc analysis of a cohort of patients with type 2 diabetes
Materials and methods: From January 2000 to December 2006, 620 patients with type 2 diabetes without diabetic kidney disease (estimated glomerular filtration rate [eGFR] > 90ml/min/1.73m2 and normoalbuminuria [24-hour urine albumin excretion < 30mg/day by consecutive two or more measurements]) were enrolled. The indications for cilostazol use were patients who were over 40 years of age or who had additional cardiovascular risk factors (a family history of cardiovascular disease, hypertension, smoking, dyslipidemia) or had a symptom with intermittent claudication and peripheral artery disease in one or both limbs. The eGFR was measured more than once every year to check the patients' renal function. New-onset CKD was defined as a decreased eGFR of < 60 ml/min/1.73m^2 using a Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. A logistic regression model was used to estimate the adjusted odds ratio with 95% confidence intervals of incident CKD associated with cilostazol use both overall and by cumulative duration of use.
Results: The mean age and duration of diabetes were 54.5 ± 8.3 years and 6.9 ± 5.6 years, respectively. The baseline eGFR and albumin excretion rate were 101.9 ± 8.1 and 10.1 ± 6.7 mg/day. The median follow-up time was 12.1 years. During the study, 86 patients (13.9%) progressed to CKD. After adjusting for multiple confounding factors, the use of cilostazol was significantly associated with a reduced risk of incident CKD (adjusted OR 0.49, 95% CI 0.26-0.93; P = 0.028). A duration-response relation was also observed. The use of cilostazol for more than 10 years associated with a reduced risk of CKD (adjusted OR 0.50, 95% CI 0.25-0.98; P = 0.044). When the duration-response was considered as a continuous variable, the significant association between cilostazol and the development of CKD remained (adjusted OR 0.94 per one year cilostazol use, 95% CI 0.89-0.99; P = 0.017).
Conclusion: The results of this study showed that the use of cilostazol, a phosphodiesterase inhibitor, was associated with a reduced risk of new-onset CKD. In fact, a longer duration of cilostazol use tended to decrease the risk of new-onset CKD.
Disclosure: **Y. Ahn:** None.

1055

Resistant starch ameliorates advanced glycation endproduct-induced albuminuria in a mouse model of type 2 diabetes

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Background and aims: Long-term excess intake of dietary advanced glycation endproducts (AGEs) contributes to chronic renal injury. Recent research implicates gut dysbiosis in the progression of diabetic nephropathy, however, the role of dietary AGEs in gut dysbiosis and renal injury in the context of diabetes has not yet been explored. The aim of this study was to investigate whether excess consumption of dietary AGEs cause gut dysbiosis, exacerbating renal injury in a mouse model of type 2 diabetes. A secondary aim was to elucidate whether a high fibre diet (resistant starch), may be protective against diabetic nephropathy via altering gut homeostasis.
Materials and methods: Six week old diabetic mice (db/db) (BKS.Cg-Dock7m+/+Leprdb/J) on a C57BL/KsJ background and age-matched non-diabetic control mice (db/m) were randomised (n=12/group) to receive a low AGE (LAGE, unbaked rodent chow) or a high AGE diet (HAGE, baked at 160°C for 1 hour), with or without 25% resistant starch (RS) for 10 weeks. All diets were isocaloric. 24-hour urine was collected for the assessment of albuminuria. At 15 weeks of age, mice were fasted and an oral glucose tolerance test (2g/kg lean body mass) was performed. At 16 weeks of age, intestinal permeability was assessed in vivo by the clearance of FITC-labelled dextran (500mg/kg body weight). Glycated haemoglobin was assessed using a Roche cobas b101 analyser.
Results: The high AGE diet exacerbated albuminuria in db/db mice (874.4±154.8 vs 536.2±96.5μg/24h, P<0.05, db/db HAGE vs db/db LAGE), and this AGE-induced increase in albuminuria was attenuated by RS (874.4±154.8 vs 515.5±71.9μg/24h, P<0.05, db/db HAGE vs db/db HAGE + RS). Db/db mice had increased gut permeability compared to db/m mice (2.38±0.32 vs 1.05±0.11μg/ml, P<0.01, db/db LAGE vs db/m LAGE). Furthermore, the high AGE diet increased gut permeability of db/db mice (3.43±0.43 vs 2.38±0.32μg/ml, P=0.06, db/db HAGE vs db/db LAGE), an effect not observed in RS-fed db/db mice (2.38±0.32 vs 2.86±0.35μg/ml, P>0.05, db/db LAGE vs db/db LAGE+RS). Following OGTT, db/db mice had a higher glucose AUC (74.08±5.98 vs 17.54±2.32 mmol/L x min, P<0.05, db/db LAGE vs db/m LAGE) and glycated haemoglobin (10.47±1.96 vs 4.06±0.12, P<0.05, db/db LAGE vs db/m LAGE) compared to db/m mice. Neither the high AGE diet nor the resistant starch supplemented diets influenced glycaemic control in db/db or db/m mice, as reflected by OGTT AUC (74.08±5.98 vs 75.29±6.45 vs 75.29±6.45 vs 72.64±7.16 mmol/L x min, P>0.05, db/db LAGE vs db/db LAGE+RS vs db/db HAGE vs db/db HAGE+RS), or glycated haemoglobin (10.61±0.46 vs 9.65±0.55 vs 10.98±0.67 vs 10.58±0.67 % glycated haemoglobin, P>0.05, db/db LAGE vs db/db LAGE+RS vs db/db HAGE vs db/db HAGE+RS).
Conclusion: A high AGE diet led to increased intestinal permeability, which was associated with worsening albuminuria in db/db mice. Resistant starch was protective against high AGE induced albuminuria in db/db mice, effects which are not dependant on changes in glucose homeostasis. These preliminary studies support the notion that dietary AGEs contribute to renal disease via alterations in gut homeostasis and suggest a potential role for resistant starch as a renoprotective agent.
Disclosure: **M. Snelson:** None.

1056

One-year eGFR decline rate is a good predictor for prognosis of renal failure in patients with type 2 diabetes

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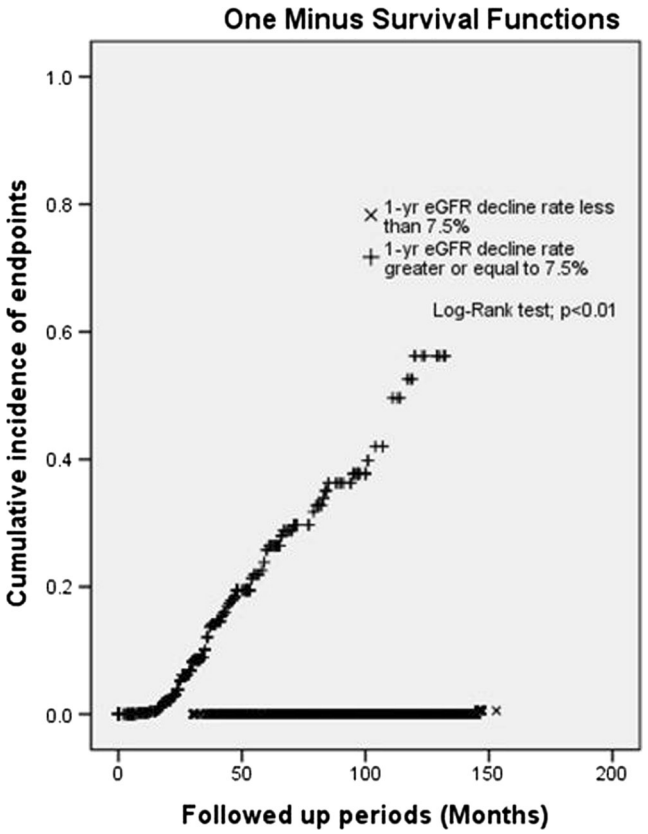
Background and aims: Patients who lose renal function faster than the average age-related decline in eGFR tend to progress to ESRD. Progressive renal decline was defined as an eGFR loss of ≥ 3.3% per year, but the calculation of this annual average renal decline rate requires lifelong observation. In this study, we used a data smoothing technique to analyze eGFR trajectories and successfully identified starting points of meaningful eGFR decline.
Materials and methods: All type 2 diabetes patients seen between June 2001 and October 2014 were candidates for this study. Among these patients, subjects whose eGFR was measured more than twice in a half-year for more than three years were included. Patients with no eGFR examination more than a year during the study period, or a mean eGFR < 60 mL/min/1.73 m^2 at baseline were excluded. We performed a smoothing technique called locally weighted regression method to reduce the fluctuation in eGFR trajectory. Every eGFR value was smoothed ($eGFR_{monthly\ smoothing\ data}$) and average $eGFR_{monthly\ smoothing\ data}$ in every half-year was calculated for each patient ($eGFR_{half\ year}$). We calculated each 1-year eGFR decline rate from the difference between each $eGFR_{half\ year}$ value and that of the previous year. We also used the maximum value of $eGFR_{half\ year}$ for ROC analysis. The endpoint was defined as a decline of $eGFR_{half\ year}$ to less than half of eGFR at baseline.

Results: A total of 2533 patients with type 2 diabetes were included in this study. The mean eGFR at baseline was 77.1 ± 13 mL/min per 1.73 m^2. The mean follow-up period was 9.1 ± 3.0 years. During the follow-up period, 85 (3.4%) patients reached the endpoint. When we performed ROC analysis for the endpoint, AUC of 1-year eGFR decline rate was 0.949. We examined the predictive value of the cut-off value of 7.5%, and found a sensitivity of 98.8% and specificity of 82.3%. The AUC of albuminuria at baseline was 0.684 and that of mean eGFR at baseline was 0.576. When we analyzed the cumulative renal endpoint incidence in subjects with a 1-year eGFR decline rate ≥ 7.5% with the Kaplan-Meier method (Figure), the average survival period was 98 months, and only 1 (0.05%) patient whose 1-year eGFR decline rate was always < 7.5% reached an endpoint.

Conclusion: The predictive accuracy of the smoothed 1-year eGFR decline rate for renal prognosis is high compared with other indicators. In cases where the 1-year eGFR decline rate is ever greater than 7.5%, the prognosis may be poor.



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Disclosure: **S. Meguro:** Grants; Asahi Kasei. Lecture/other fees; Astrazeneka, Takeda, Boehringer-ingelheim.

1057

ADIPOQ gene polymorphisms and changes in renal function in a cohort from the community, the prospective D.E.S.I.R. study

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Background and aims: High adiponectin levels are associated with diabetic nephropathy. Polymorphisms of the adiponectin gene (*ADIPOQ*) have been associated with nephropathy, both in type 1 and type 2 diabetes. Nevertheless, it is not known whether *ADIPOQ* polymorphisms are associated with renal function in the general population. We evaluated whether *ADIPOQ* polymorphisms were associated with changes in renal function in a community cohort, the D.E.S.I.R. (Data from Epidemiological Study on Insulin Resistance Syndrome) study.

Materials and methods: The D.E.S.I.R. study, a prospective cohort from the general population, recruited 5,212 men and women from western France, in the age range 30-65 years. Among those with a 9-year follow-up, 4220 born in mainland France were genotyped for 4 *ADIPOQ* polymorphisms (rs2241766, rs266729, rs17300539 and rs1501299). Adiponectin levels were measured by radioimmunoassay in a sample of 250 subjects. Estimated glomerular filtration rate (eGFR) was calculated according to the "Modification of Diet in Renal Disease" formula (MDRD). Progression towards chronic kidney disease (CKD) during follow-up was defined by an eGFR below 60 ml/min/1.73 m^2 on at least one follow-up visit. We have also considered the criterion "Certain Drop in eGFR" as proposed by the Kidney Disease Improving Global Outcomes (KDIGO) group.

Results: Progression towards CKD (defined as eGFR<60 ml/ min/1.73 m^2) during follow-up was observed in 225 participants. The KDIGO criterion "Certain Drop in eGFR" of CKD progression was fulfilled by 251 participants during follow-up. We observed an association between the *ADIPOQ* rs2241766 SNP (+45T>G) and CKD progression defined by the KDIGO criterion. The +45G carriers had a lower risk of progression with an odds-ratio of 0.61 (95% CI 0.43-0.86, p=0.006, by logistic regression, adjusted for sex, age, and body mass index). Change in eGFR during the follow-up was also associated with +45T>G polymorphism. The +45G allele was associated with a lower decrease in eGFR after 9 years (-6.76, -5.38, -4.35 ml/min/1.73 m^2 in TT, TG and GG genotypes, respectively, p=0.014, by linear regression adjusted for sex, age, BMI). These results remained significant after exclusion of people with diabetes, at baseline or at follow-up. The +45G allele was associated with higher adiponectin levels (p=0.003, ANCOVA adjusted for sex, age, BMI, glycemic status). No association was found between renal parameters and the other *ADIPOQ* polymorphisms.

Conclusion: The *ADIPOQ* +45G allele associated with higher adiponectin levels was negatively associated with progression towards CKD in this community-based cohort. The same allele has been positively associated with nephropathy in our previous studies in people with type 1 and type 2 diabetes. This supports the hypothesis of opposite roles of adiponectin in renal function according to the clinical and metabolic background. High adiponectin levels may be beneficial in healthy subjects.

Disclosure: **F. Fumeron:** None.

PS 091 Insights into diabetic nephropathy

1058

Correlation of diabetic nephropathy with platelet hyperactivity, von Willebrand factor, protein S and protein C activity in patients with type 1 diabetes

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Background and aims: Diabetes is associated with accelerated atherosclerosis and an increased risk of clinical cardiovascular disease. However, little is known about platelet activation as a risk factor for cardiovascular disease in DM1 patients with nephropathy. The aim of this study was to assess the impact of level of fasting plasma glucose, HbA_1c, glomerular filtration rate (GFR), albumin excretion rate (AER) on the platelet activity, endothelial dysfunction and the physiological anticoagulant activity in patients with DM1.
Materials and methods: Study has been reviewed by the Local Ethics Committee it has been performed in accordance with the ethical standards laid down in the Helsinki Declaration. We examined 60 patients with DM1 (age 28,1±6,5, HbA_1c 9,1±1,8%). With normoalbuminuria AER in the morning urine <20 mg/L (n=38); microalbuminuria AER <199 mg/L(n=17); macroalbuminuria AER ≥200 mg/L (n=5). On the background euglycemia (fasting plasma glucose ≤6,5 (5,7±1,06) mmol/l), and hyperglycemia (fpg ≥12 (13,2±2,35) mmol/l) were measured induced platelet aggregation (IPA) in whole blood using ADF, arachidonic acid, ristocetin, thrombin (tRaP-6), collagen by multiple electrode platelet aggregometry (Multiplate); physiological anticoagulants (protein S, protein C, AT-III), and von Willebrand factor was determined by ELISA; GFR by standard EPI formula. Statistical analysis was performed with SPSS 22,0 for Windows, $p<0.05$.
Results: Platelet aggregation using ADF, arachidonic acid, ristocetin was significantly increased on the background of hyperglycemia compared with euglycemia (p=0,01, p=0,02, p=0,016 respectively) (W-test). Platelet aggregation using ADF was higher in patients with macroalbuminuria 82,0 [61,0;86,0] compared with microalbuminuria patients 47,5 [38,7; 68,2], p=0,014 (U-test). von Willebrand factor was higher in patients with microalbuminuria 0,76 [0,54;1,04] and with macroalbuminuria 0,77 [0,58; 0,85] compared with normoalbuminuria patients 0,47 [0,28; 0,59],p=0,01 (U-test). Protein S activity was significantly increased in group with GFR 45-59 ml/min/1.73 m^2 80,3[68,1; 97,2] compared with group with GFR ≥60 ml/min/1.73 m^2 103,8[87,4; 109,9], p=0,021 (U-test). Protein C activity was significantly increased in group with GFR 45-59 ml/min/1.73 m^2 114,5 [95,3; 122,1] compared with group with GFR ≥60 ml/min/1.73 m^2 138,9 [124,2; 158,5], p=0,007 (U-test). Platelet aggregation using ADF was correlated negatively with GFR (r=-0,253;p=0,03). Level of HbA_1c was correlated positively with increased platelet aggregation using arachidonic acid (r=0,299;p=0,03) and protein C activity (r=0,375;p=0,02).
Conclusion: Hyperglycemia, increased level of HbA_1c, diabetic nephropathy are associated with platelet hyperactivity and endothelial dysfunction which may increase the risk of adverse cardiovascular and cerebrovascular events. An increase in the activity of physiological anticoagulants in later stages of nephropathy may be a compensatory reaction of the organism for the hypercoagulable condition in patients with DM1.
Disclosure: **K. Sarkisova:** None.

1059

The assessment of relationship of osteopontin with vascular complications progression and the risk of adverse outcomes in patients with long-term type 1 diabetes

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Background and aims: To investigate the association between osteopontin (OPN) and vascular complications of diabetes, and the risk of adverse events developments in patients with long-term type 1 diabetes mellitus (DM1)
Materials and methods: The study included 156 patients with long-term DM1 (more than 20 years). We have estimated the renal status, severity of cardiovascular pathology, and frequency of adverse outcomes such as: doubling the level of creatinine, reaching the terminal stage of chronic kidney disease (CKD), developing nonfatal myocardial infarction, stroke, amputation, urgent hospitalization, death within 5 years of observation.
Results: Serum OPN was higher in persons with terminal stage of CKD who are on dialysis therapy and who underwent renal transplantation (TP), and lower was - in patients without CKD. The differences were statistically significant: between a group of people without CKD and patients with CKD 1-4, dialysis patients and those, who underwent TP (p=0.0027; p=0.0246; p=0.0249, respectively). In patients with albuminuria, the level of OPN was also significantly higher than in individuals with normoalbuminuria (49.9 ng/ml [24.3, 67.85] vs 28.1 ng/ml [12.4, 46.7]; p = 0.005). Elevated serum levels of OPN were associated with young patients (r=-0.249; p=0.048), low body mass index (BMI) (r=-0.267; p=0.020), increased albuminuria (r=0.42; p=0.0104), serum level of C-reactive protein (r=0.426;p=0.0378), left ventricular hypertrophy - LVMI (r=0,257; p=0,0279), vitamin D deficiency (r=-0.224; p=0.0280). The relationship of OPN with the ADMA marker was found (r=0.290; p=0.0103). According to the OPN serum concentration level, patients were divided into 3 groups: the 1st group of OPN 5-17 ng/ml, the 2d group 17.1-59.0 ng/ml, the 3d group 59.1 -145, 6 ng/ml. Groups 1 and 2 were differed in the degree of calcification of the coronary arteries, such was identificated with using of a multispiral computed tomography of heart with Agatston index definition (p=0.048), groups 1 and 3 were differed with respect to BMI (p=0.003), the level of albuminuria (p=0.010), vitamin D (p=0.047) and ADMA (p=0.013), and groups 2 and 3 - by BMI (p=0,012), albuminuria (p=0,018) and ADMA (p=0,021). The development of adverse events during the 5-year follow-up period (28.2%) was associated with a higher value of the OPN (50.6 ng/ml [23.8, 62.2] vs 38.9 ng/ml [16, 51.2]; p=0.010).
Conclusion: The study demonstrates the diagnostic value of serum OPN not only in verification of specific for diabetes cardiovascular and renal pathology, which has already got formed, but also in predicting the risks of progression of late diabetic complications and mortality of patients with long-term CD1 flow.
Disclosure: **M. Arutyunova:** None.

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The detoxifying capacity of carnosine in vitro and in renal cells

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Background and aims: Methylglyoxal (MG) is a reactive carbonyl species that accumulates under diabetic conditions and essentially contributes to diabetic complications. Carnosine has previously been described as a quencher of reactive metabolites and to mitigate long term diabetic

complications such diabetic nephropathy. We therefore analyzed the mode and detoxification capacity of carnosine against MG in cell free environment and cultured renal cells.
Materials and methods: Interactions of carnosine and MG were evaluated spectrophotometrically and by NMR analysis of the formed adducts. Carnosine dependent reduction of MG induced AGE and CEL formation of from human serum albumin was measured by ELISA and Western Blot. Renal cells were incubated with increasing carnosine to MG ratios and incubation times; cell viability was measured by MTT assay. Putative carnosine transporters were quantitated by qPCR, intracellular carnosine uptake by radiolabeled carnosine-uptake assay.
Results: NMR studies revealed the formation of oligo/polymeric products of MG, catalyzed by carnosine. The addition of carnosine (≥100:1 to MG) and anserine (≥200:1 to MG) reduced the formation of AGEs ($p \leq 0.05$) and CEL ($p \leq 0.05$). In renal cells, carnosine (0 to 5 mM) did not restore MG impaired cell viability (0 to 1 mM) in short- and long-term incubation experiments. Neither cellular SOD and GPx expression, nor SOD activity or GSH levels were altered by carnosine addition. Putative carnosine transporters were only expressed in tubular cells and uptake of radiolabeled carnosine was very low (0.04±0.02% in tubular cells and 0.05±0.02% in mesangial cells after 24h).
Conclusion: Carnosine interacts with MG in vitro by catalyzing the formation of MG oligo- and polymers .This action, however, in cultured renal cells does not result in protection from MG induced toxicity, i.e. does not increase cell viability or elements of the cellular stress protection system. Intracellular carnosine uptake is low, carnosine to MG ratios required to induce protective effects are not reached. Intracellular carnosine metabolism and compartmentalization should now be analyzed. These results suggest high importance of internal carnosine metabolism.
Supported by: SFB 1118 (DDG)
Disclosure: **V. Peters:** None.

1061

The new, volatile biomarkers of chronic kidney disease and coexisting type 2 diabetes

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Background and aims: Chromatographic studies on breath composition are aimed at finding volatile markers useful for medical diagnostics or in screening investigations. Studies leading to the development of screening breath tests are especially important for the diagnostics of chronic kidney disease (CKD) and type 2 diabetes mellitus (T2DM). The aim of the presented study was to confirm diagnostic usefulness of chosen volatile compounds detected in breath, which are suggested as potential biomarkers of renal dysfunction and diabetes.
Materials and methods: Breath samples were collected from 20 patients with CKD. Among these 20 patients, all with CKD, 10 patients had also T2DM or glucose intolerance. The control group, consisting of 6 women and 4 men. All exhaled air samples were analyzed using gas chromatograph (Agilent 6890GC) coupled with mass spectrometer (5975MSD). Thermal desorption was applied as the enrichment method.
Results: Patients were characterized by increased creatinine, urea, homocysteine (Hcy) and glutathione (GSH) levels. Liver enzymes values, lipid levels and inflammation markers remained within normal limits. The group of patients with CKD and diabetic nephropathy had significantly different kidney function parameters ($p<0.05$). Diagnostically promising results were obtained for trimethylamine (TMA) and sulphur compounds. TMA was detected only in CKD patients. Higher breath concentrations of methanethiol (MeSH) were observed in CKD patients with coexisting diabetes than in patients with renal dysfunction only or in the healthy group. There was a tendency of increasing MeSH concentration in breath with increasing total glutathione in plasma ($r=0.53$, $p=0.0026$). Also, a trend of increasing dimethylsulfide (DMS) levels detected in breath was noticed with an increase of hydrogen sulfide concentration in plasma ($r=0.74$; $p=0.00001$) as well as with aspartate aminotransferase (AST), ($r=0.61$; $p=0.001$).
Conclusion: The presented results suggest the possibility of applying TMA, MeSH, and DMS detection in breath as diagnostic methods.
Disclosure: **P. Miarka:** None.

1062

Mitochondrial dysfunction defines a population of young people with type 1 diabetes at risk of kidney disease

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Background and aims: Recent evidence suggests that kidney disease in Type 1 diabetes (T1D) develops much earlier than previously appreciated. In adolescents with type 1 diabetes (T1D), the highest tertile of urinary albumin-to-creatinine ratio (uACR), a marker of endothelial dysfunction, predicts renal and cardiovascular disease risk. Another factor implicated in the development of diabetic kidney disease (DKD) is mitochondrial dysfunction. The objective of this study was to examine the relationships between mitochondrial function, uACR and estimated glomerular filtration rate (eGFR) in young people with T1D.
Materials and methods: In this cross-sectional study, adolescents and young adults with T1D were recruited [n=100; 20.0±2.8 yrs; M:F-54:46, HbA_{1c}-66.1(12.3) mmol/mol; diabetes duration-10.7 ±5.2 yrs; BMI-24.5(5.3) kg/m^2]. Mean uACR was determined from 3 morning urine samples collected from each study participant. The study population was then divided into uACR tertiles. Lower (uACR ≤0.66 mg/mmol; n=33) and middle (uACR 0.67-1.16; n=33) tertiles were defined as having low-moderate risk of future DKD and those in the upper tertile (uACR ≥1.17; n=34) as having the highest risk. SeaHorse XF analyser was used for mitochondrial functional assays in circulating leukocytes. Urinary metabolomics by LC MSMS, GCMS and HPLC measured central carbon metabolites, nucleotides, fatty and amino acids.
Results: A majority (99.7%) of participants had hyperfiltration [CKD_{EPI} eGFR, 135.0(13.8) ml/min/m^2) and individuals in the upper tertile of uACR had the highest median eGFR (P <0.031 vs low risk tertile; age, gender and diabetes duration adjusted). In a generalized linear model which included HbA_{1c}, BMI, diabetes duration, sex and age, a significant inverse relationship was identified between eGFR-CKD_{EPI} and logACR in the upper tertile, as compared with both lower risk tertiles (vs middle, $P=9.8\times10^{-5}$; vs lower, $P=2\times10^{-16}$). Mitochondrial function (ATP dependent respiration) in circulating leukocytes, was decreased in individuals in the higher uACR tertile, compared with the two lower uACR tertiles ($P=0.008$). Multivariate modelling of urinary metabolites identified a signature which separated individuals in the upper uACR tertile from the lower risk tertiles.
Conclusion: Young individuals with type 1 diabetes and higher risk of DKD have mitochondrial dysfunction and an inverse relationship

between GFR and uACR. These features are similar to those in individuals with progressive kidney disease, warranting further investigation.
Supported by: DIACOMP-NIH/NIDDK; KHA; NHMRC
Disclosure: **J.M. Forbes:** Grants; NIH/NIDDK DIACOMP Pilot and Feasibility Program, Kidney Health Australia, National health and Medical Research Council of Australia.

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Exome-chip association analyses for diabetic nephropathy and estimated glomerular filtration rate in Chinese patients with type 2 diabetes

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Background and aims: Diabetic nephropathy (DN) is the leading cause for end stage renal disease. Despite evidence showing that genetic susceptibility plays a role in the development of DN, the genetic determinants of DN remains poorly understood. This study aimed to identify novel single nucleotide polymorphisms (SNPs) associated with DN and a quantitative measure of renal function, the estimated glomerular filtration rate (eGFR), among Chinese patients with type 2 diabetes.
Materials and methods: Exome-chip association analyses for DN and eGFR were conducted by genotyping 1689 DN cases and 1227 non-DN controls from the Hong Kong West Diabetes Registry (HKWDR). DN cases were defined as subjects with the presence of either microalbuminuria or macroalbuminuria, as indicated by the patient's urinary albumin-to-creatinine ratio in two random urine samples, collected within 6 months, according to the American Diabetes Association criteria; or having an abnormal eGFR<60 mL/min/1.73m^2. Those with normoalbuminuria and an eGFR≥60 mL/min/1.73m^2 were classified as non-DN controls. All subjects were genotyped using the custom Asian Exomechip. Single variant association analyses were conducted on 77,343 and 78,026 SNPs for DN and eGFR, respectively. The associations of SNPs with DN and eGFR were examined by multiple logistic and linear regression analyses, respectively, with adjustment for age, sex, duration of diabetes and presence of hypertension.
Results: A total of 7 SNPs located in 6 loci showed evidence of association with DN ($P<1x10^{-4}$). The strongest association was identified at rs7315438 ($P=8.32x10^{-6}$; OR[95%CI]: 0.76[0.68-0.86]), an intergenic variant located between the *TBX3* and *MED13L* genes. A suggestive association was also identified at rs9313307 ($P=5.07x10^{-5}$; OR[95%CI]: 1.46[1.22-1.75]), located upstream of the *TENM2*. In the analysis for eGFR, 12 SNPs located in 10 loci were associated with eGFR at $P<1x10^{-4}$. The *TENM2* rs9313307, which also demonstrated an association with DN, was found to be significantly associated with eGFR ($P=7.04x10^{-7}$; β[95%CI]: -0.2[-0.28, -0.12]). Several low-frequency missense variants at *ZKSCAN2* (rs61746620; P=9.75x10-6; β[95%CI]: -1.67[-2.41, -0.93]), *BRICD5* (rs199775669; $P=6.20x10^{-5}$; β[95%CI]: -1.64[-2.43, -0.84]), and MUC17 (rs150088438; $P=7.53x10^{-5}$; β[95%CI]: -0.88[-1.32, -0.44]) also demonstrated suggestive associations with eGFR.
Conclusion: We have identified the associations of several novel loci with DN and/or eGFR in the exome-chip association analyses among Chinese patients with type 2 diabetes. Although genome-wide significance was not found for any of the identified SNPs, several genes, such as *TBX3*, *TENM2* and *MUC17,* are potential functional candidates for DN and altered eGFR. Further replication studies in independent cohorts would serve to validate our findings.
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Disclosure: **C.Y.Y. Cheung:** None.

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Interleukin-21 receptor (IL-21R) blockade reduces the development of albuminuria in a uninephrectomised db/db mouse model of diabetic nephropathy

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Background and aims: Chronic kidney disease (CKD) encompasses a range of disorders affecting kidney structure and function that affect an estimated 10% of the world's population. Current treatments are limited to blockade of the renin-angiotensin system, however, the identification of novel mechanisms contributing to CKD provide the potential for new classes of therapies. Here we explore a role for blocking interleukin-21 receptor (IL-21R) signalling in a murine model of diabetic nephropathy (DN). IL21R is a type I cytokine receptor and has been detected on glomeruli and tubules in both mouse and man. IL21R has been reported to play a role in intrarenal T-cell accumulation and is upregulated in murine models of DN. Similarly, genetic perturbation of IL21R signalling has protective effects against kidney disease in murine models of accelerated autoimmune disease.
Materials and methods: Here we utilise the uninephrectomized type 2 diabetic *db/db* mouse model to test the effect of a blocking murine IL21R-Fc fusion protein (mIL21R-Fc) on markers of CKD. Mice underwent uninephrectomy at 7 weeks of age and were randomised by urine albumin to creatinine ratio (UACR) at 15 weeks of age. mIL-21R-Fc (400ug/mouse 3x week; n=14) or control (n=14) was administered intraperitoneally (i.p.) from 16-21 weeks of age. Blood and urine was collected at 15, 18 and 20 weeks, and terminal tissues were collected at 21 weeks of age. Plasma glucose, plasma creatinine, urine albumin, and urine creatinine were measured to determine *in vivo* efficacy of mIL21R-Fc on renal function. Protein levels of kidney damage markers and cytokines were also assayed *ex vivo* in parallel with an immunohistochemical assessment of glomerular damage.
Results: At 15 weeks of age the UACR levels were 245 mg/g ± 21 for the control group and 249 mg/g ± 35 for the mIL21R-Fc group. Treatment with mIL21R-Fc significantly attenuated the progression of albuminuria at 18 and 20 weeks compared to vehicle control (% change in UACR; treatment 27% ± 9 versus control 47% ± 9 at 18 weeks; treatment 24% ± 9 versus control 65% ± 14 at 20 weeks, p = 0.035). These therapeutic effects were associated with a reduction in renal protein levels of the inflammatory cytokine macrophage inflammatory protein 3alpha (MIP-3a; control 130 pg/ml ± 18 v treatment 78 ± 6 pg/ml p=0.041). mIL21R-Fc also reduced renal vascular endothelial growth factor (VEGF) protein levels (control 6 ± 1 pg/ml versus treatment 4.1 ± 0.6 pg/ml p=0.014, n=9-12). mIL21R-Fc did not affect histopathological scoring of glomerular damage and no differences in blood urea nitrogen (BUN) or plasma glucose were detected between treatments.
Conclusion: In summary, blockade of IL-21R signalling attenuates albuminuria in a diabetic nephropathy model and may act by reducing MIP-3a levels. MIP-3a is a chemokine that has been reported to attract T lymphocyte, and previous reports that intrarenal $CD4^+$ T cell accumulation occurs via an IL-21R-dependent pathway support this interpretation. Silencing VEGF signalling has been reported to improve DN in mice suggesting that IL-21R blockade may also act by reducing VEGF signalling. Blockade of IL-21R signalling may provide a novel pathway for the treatment of diabetic nephropathy.
Disclosure: **A. Seth:** None.

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Renal TNF-alpha-induced protein 2 is altered in diabetic nephropathy

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Background and aims: Diabetic nephropathy (DN) is characterised by increased glomerular permeability to proteins. Hyperglycemia, hypertension, and inflammatory processes are believed to play a key role in the pathogenesis of the complication by inducing podocyte injury. The cytosolic protein TNF-α-Induced Protein 2 (TNFAIP2), also known as B94, is induced by TNF-α, an inflammatory cytokine implicated in the pathogenesis of DN, and by retinoic acid, a key regulator of podocyte phenotype. This raises the possibility that TNFAIP2 may play a role in DN and represent a new target for intervention; however, there is no information on TNFAIP2 in diabetic or other kidney diseases. Our aim was, thus, to establish if TNFAIP2 expression is altered in DN and to investigate if DN-related insults modulate TNFAIP2 in cultured podocytes.
Materials and methods: Human kidney biopsies: TNFAIP2 protein expression was studied in kidney biopsies from type 2 diabetic patients (n=6) and control non-diabetic subjects (n=6) by immunohistochemistry. Experimental diabetes: diabetes was induced in eight-week-old male C57BL6 mice by intraperitoneal injection of streptozotocin at a dose of 55 mg/kg in citrate buffer. Control animals were injected with citrate buffer alone. Diabetic (DM n=15) and control (ND n=15) animals were euthanized 14 weeks following induction of diabetes and expression of TNFAIP2 was studied by either immunohistochemistry or immunofluorescence in the renal cortex. Prior to sacrifice, blood samples were taken and urine collected over 18-hours for urinary albumin concentration measurement by ELISA. In a subgroup of animals, glomeruli were isolated using a modified Dynabead method and TNFAIP2 levels assessed by real time PCR and immunoblotting. In vitro experiments: cultured podocytes were exposed to high glucose (25 mM), mechanical stretch (10% elongation), TNF-α (50 ng/mL), or retinoic acid (1μM) for various time periods and TNFAIP2 expression analysed real-time PCR and immunoblotting. Control cells were studied in parallel.
Results: Only few glomerular cells stained positively for TNFAIP2 in normal human kidney biopsies, but immunostaining for TNFAIP2 was significantly enhanced in patients with DN (ND: 1.42±0.44; DM: 7.21 ±1.35; mean ± SD; $p<0.05$ DM vs. ND). In mice, blood glucose, glycated albumin, and albumin excretion rate were significantly higher in the presence of diabetes, confirming the development of DN. Glomerular TNFAIP2 expression was significantly increased in diabetic mice compared to controls as assessed by immunohistochemistry (ND: 2.00±0.18; DM: 6.09±0.66; mean ± ESM; $p<0.05$ DM vs. ND) and real time PCR (ND: 037±0.05; DM: 1.04±0.17; $p<0.05$ DM vs. ND). Furthermore, podocytes were the predominant glomerular cell type expressing TNFAIP2 as suggested by staining distribution and confirmed by immunostaining for TNFAIP2 and podocin on serial cortex sections. Cultured podocytes constitutively express TNFAIP2 at both mRNA and protein level. TNFAIP2 expression was enhanced in podocytes exposed to high glucose (vehicle: 1.00±0.01; HG: 2.60±0.36, $p<0.01$), TNF-α (vehicle: 1.00±0.01; TNF-α: 2.31±0.24; $p<0.01$), and retinoid acid (vehicle: 1.00 ±0.1; RA: 1.75±0.06; $p<0.01$), while no changes were observed in podocytes exposed to mechanical stretch.
Conclusion: Glomerular TNFAIP2 expression was enhanced in both human and experimental DN, particularly in podocytes. Furthermore, DN-related insults increased TNFAIP2 expression in podocytes, providing a potential underlying mechanism of TNFAIP2 overexpression.
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Disclosure: **F. Barutta:** None.

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Atg5 deficiency in endothelial cells contributes to the development of IL-6 dependent endothelial to mesenchymal transition and kidney fibrosis

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Background and aims: Autophagy is a catabolic process that preserves cellular homeostasis via autophagic clearance. Dysregulation of autophagy is well known contributor in the development of organ dysfunction. There is considerable understanding of the molecular and biological functions of autophagy in pathophysiological conditions. There are some reports showing autophagy deficiency contributes to endothelial dysfunction in patients with diabetes. However, the role of autophagy within the vasculature is ambiguous.
Materials and methods: In this study to test the role of autophagy within endothelium, we utilized human microvascular endothelial cells (HMVECs) and endothelial specific Atg5 knockout mice ($atg5^{endo}$, Atg5f/f; Cdh5Cre/+). HMVECs were treated with Atg5 siRNA and $atg5^{endo}$ mice were treated with streptozotocin (STZ) or high fat diet (HFD).
Results: Inhibition of autophagy in human microvascular endothelial cells (HMVECs) and in the endothelial specific $atg5^{endo}$ mice are associated with the induction of IL-6 and increased rate of endothelial-to-mesenchymal transition (EndMT), which contributes in kidney fibrosis. IL-6 secretion was remarkably higher in Atg5 siRNA-transfected HMVECs culture medium; neutralization of IL-6 by specific antibody completely inhibited EndMT in Atg5 siRNA-transfected HMVECs. Atg5 siRNA-transfected HMVECs exhibited significantly enhanced TGF-β/p-Smad3 signaling; unexpectedly EndMT in Atg5 siRNA-transfected HMVECs was independent from TGF-β/p-Smad3 pathway revealed by specific neutralizing antibody experiment. Similar to the in vivo data, $atg5^{endo}$ mice displayed kidney fibrosis when compared to littermate control associated with higher level of plasma IL-6, induction of EndMT, and increased TGF-β/p-Smad3 levels. Such fibrosis in $atg5^{endo}$ mice was accelerated when mice were either treated STZ or fed with HFD.
Conclusion: These results revealed that the essential role of autophagy in endothelial cell homeostasis and the disruption of such endothelial autophagy could lead to IL-6 dependent EndMT and kidney fibrosis associated with diabetes and metabolic syndrome.
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Disclosure: **T. Yuta:** None.

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Targeting the C5a-C5aR1 signalling axis in diabetic kidney disease

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Background and aims: The complement system plays a central role in the activation of innate immunity, inflammation and tissue remodelling. The complement activation fragment C5a is the most potent pro-inflammatory effector molecule of the complement system. Although previous studies indicate an association between activation of complement and diabetic kidney disease (DKD), the role of C5a and its receptor, C5aR1, in mediating injury in the kidney in diabetes has yet to be determined. The aim of this study was to investigate the role of complement C5a and its receptor, C5aR1 in the development of diabetic kidney disease.
Materials and methods: The spontaneously diabetic Ins2-Akita mouse model was used to characterise the changes in complement activation products associated with DKD (n=8 mice/group). Streptozotocin-induced diabetic C57BL/6 mice were treated with the highly selective

C5aR1 antagonist, PMX-53 (2mg/kg/day), in drinking water for 24 weeks (n=8-15 mice/group). Kidney injury was assessed by urine albumin excretion. Complement was examined by qRT-PCR, ELISA or immunohistochemistry (IHC). C5a was measured in plasma from patients with diabetes (n=16) or non-diabetic controls (n=40) by ELISA. C5aR1 was determined in renal biopsies from patients with DKD (n=23) and non-diabetic controls (healthy donors, n=6) by IHC.

Results: Ins2-Akita mice displayed upregulated gene expression of C5 (1.27±0.31 vs 6.35±2.10 AU; p=0.05) and C5aR1 in the renal cortex (0.95±0.08 vs 1.34±0.11 AU; p=0.02) compared to wildtype littermates. Urinary C5a was increased in Ins2-Akita mice (50±9 vs 151±32 pg/24 hr, p<0.01). Blockade of C5a-C5aR1 signalling with PMX-53 resulted in decreased urinary albumin in diabetic mice when compared to vehicle-treated diabetic controls (83±18 vs 30±9 μg/24 h; p=0.02). Plasma levels of C5a were increased in patients with type 1 diabetes compared to age-matched non-diabetic controls (110±23.5 vs 35±2ng/ml; p<0.0001). Examination of C5aR1 in human kidney by IHC revealed upregulation in the glomeruli and tubulointerstitium of renal biopsies from subjects with DKD compared to non-diabetic controls (15.7±1.8 vs 5.1±1.9; p=0.003).

Conclusion: The C5a-C5aR1 signalling axis is activated in human and mouse DKD. A pilot study using PMX-53 indicates that blockade of C5a-C5aR1 signalling attenuates albuminuria in experimental diabetes. Further studies are required to validate C5aR1 as a therapeutic target in diabetic kidney disease.

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PS 092 Novel aspects of classical risk factors

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Prognostic value of glycosuria amount during hyperglycaemia in patients with type 1 diabetes and risk for diabetic nephropathy (DN)

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Background and aims: Only a fraction of T1DM patients with poor glycemic control develop DN due to selective haemodynamic changes, maybe in tubulo-glomerular feed-back. We examined the prognostic value of changes produced by hyperglycemia in Glomerular Filtration Rate (GFR) and solute clearances (glucose, lithium, sodium, total osmols) in T1DM patients at risk for DN: glomerular hyperfiltration (hyperGFR), and/or microalbuminuria (Urinary Albumin Excretion (UAE) 30-300mg/24 h) compared to low risk controls (normal GFR and UAE).

Materials and methods: In 1990-92, 108 T1DM patients without DN attending a University Hospital were screened for microalbuminuria and hyperGFR (>mean+ 2SD of age-matched healthy controls; ^{51}CrEDTA plasma disappearance technique). 32 underwent renal tests described below: 21 at high risk for DN (9 with isolated hyperGFR, 11 with microalbuminuria, including 4 with both), and 11 at low risk (normal GFR and UAE despite poor glycaemic control, including 6 with T1DM duration >20 years). GFR and Effective Renal Plasma Flow (ERPF, Iodothalamate and Hippurate infusion techniques), Fractional Clearances (Fract Cl) of glucose, lithium (marker of proximal tubular reabsorption), sodium, and total osmols were measured during 2 clamp-periods in the same test: normoglycaemia, then hyperglycaemia. Follow-up was until 2016, with time to occurrence of DN (UAE>300mg/24h), eGFR<60 mL/min/1.73m^2, End Stage Renal Disease, or all-cause death as primary end point.

Results: At baseline, 53 patients were at high risk (36 with hyperGFR, 10 with microalbuminuria, 7 with both), and 55 ones at low risk. The participants to renal tests at high risk were comparable to low risk ones for age (32(SD13) years), sex ratio (53% women), T1DM duration (median15 (interquartiles 9-22) years), and HbA1c (9.1 (2.1)%), all representative of the whole cohort. Glycaemia rose similarly in both groups from 6.1(2.0) to 15.6(5) mM. Their GFR and ERPF were similar in normoglycaemia (139(31) and 631(219) mL/min/1.73m$^{2)}$. On hyperglycaemia, GFR increased by 4(median, ranges -6, 18)% and ERPF by 6(-7, 24)% in the high risk group, vs decreases of -6(-13, 1)% for GFR and 1(-15,6)% for ERPF in the low risk group (p<0.05 for both). On hyperglycaemia, glycosuria was twice lower in the high risk group than in the low risk one (0.341(0.247) vs 0.611(0.435) mmol/min; p<0.05), with reduced changes in Fract Cl of glucose (2.2(1.5)x10^{-3} vs 5.1(3.9) x10^{-3}(p< 0.01), lithium (-1.1(30.1)vs +32 (47); p<0.05), osmols (124(166) vs 284(267); p< 0.05), and sodium (-0.54(39) vs +35(62); p=0.06).Participants with isolated hyperGFR and those with microalbuminuria were not different. At follow-up (median duration 22 (interquartiles 12-26) years), 15 high risk patients underwent primary end points, vs 6 low risk ones (OR 3 .47 (95% CI 1.14-11.96; p=0.03 after adjustments on age, sex, T1DM duration, and follow-up duration).

Conclusion: Reduced glycosuria during hyperglycaemia is a risk for DN in T1DM patients.

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Serum uromodulin predicts a decline in kidney function independently from the presence of type 2 diabetes

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Background and aims: Uromodulin is the most abundant protein excreted in urine. Low uromodulin has been found to be associated with type 2 diabetes (T2DM) as well as with chronic kidney disease (CKD). Whether it also predicts a future decline in kidney function is not known and is addressed in the present study.
Materials and methods: We measured serum uromodulin in 529 patients undergoing coronary angiography for the evaluation of established or suspected coronary artery disease (CAD).
Results: Uromodulin was lower in patients with T2DM than in non-diabetic subjects (148±70 vs. 171±79; p=0.001) and significantly correlated with estimated glomerular filtration rate (eGFR; r=0.242, p<0.001) and, inversely, with the albumin creatinine ratio (ACR; r=-0.120, p=0.012). It was significantly lower in patients with CKD (eGFR <60 ml/min/1.73 m^2) than in those with normal kidney function (72±29 vs. 169±76 ng/ml; p<0.001), and also in patients with albuminuria than in patients without increased albumin excretion (149±72 vs. 168±78 ng/ml; p=0.008). Further, uromodulin at baseline was significantly lower in patients who developed an eGFR <60 ml/min/1.73 m^2 during 4 years of follow-up compared to those who did not (127±42 vs. 180±79ng/ml, p=0.003). It was inversely associated with declining eGFR even after full adjustment including ACR, baseline CAD and the presence of T2DM (OR=0.354 [95%CI 0.131-0.957], p=0.041). The inclusion of uromodulin to a basic prediction model for CKD increased the model performance (C-statistic 0.844 vs. 0.804, p=0.049).
Conclusion: In conclusion, we for the first time show that serum uromodulin predicts a decline in kidney function independently from conventional risk factors including T2DM.
Disclosure: **E. Brandtner:** None.

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Normoalbuminuric renal impairment in type 2 diabetes is associated with a high risk of death. The Renal Insufficiency And Cardiovascular Events (RIACE) study

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Background and aims: Nonalbuminuric renal impairment has become the prevailing phenotype of diabetic kidney disease (DKD) in patients with type 2 diabetes and an estimated glomerular filtration rate (eGFR) <60 ml/min/1.73m^2, though a few data are available on death rate in these individuals. This study aimed at assessing the rate and determinants of all-cause death in the nonalbuminuric phenotype, as compared with the albuminuric ones.
Materials and methods: The Renal Insufficiency And Cardiovascular Events (RIACE) Italian Multicenter Study is an observational, prospective, cohort study enrolling 15,773 consecutive non-dialytic patients with type 2 diabetes in 2006-2008 in 19 outpatients Diabetes Clinics in Italy. Baseline cardiovascular risk factors, complications and treatments were recorded. Based on baseline albuminuria and eGFR, patients were classified as having: no DKD (nonalbuminuric non-chronic kidney disease, CKD), albuminuria alone (albuminuric non-CKD), reduced eGFR alone (nonalbuminuric CKD), or both (albuminuric CKD). Vital status on October 31, 2015 was retrieved for 15,656 patients (99.26%).
Results: After a follow-up of 7.4±2.1 years, death rate was 31.0 per 1,000 person-years (95% CI 30.01-32.04). There was a gradual increase in crude mortality rates from nonalbuminuric non-CKD (19.87 [18.87-20.86]) to albuminuric CKD (90.25 [83.41-97.08]), with a higher death rate for nonalbuminuric CKD (61.49 [56.59-66.40]) than for albuminuric non-CKD (36.79 [34.23-39.35]). Unadjusted HRs were 1.696 (95% CI 1.528-1.883), 3.020 (2.630-3.467), and 5.366 (4.623-6.227) for albuminuric non-CKD, nonalbuminuric CKD, and albuminuric CKD, respectively. Differences between albuminuric non-CKD and nonalbuminuric CKD disappeared after adjustment for age and multiple confounders. Trends were similar in males and females. However, when patients were stratified by age, in the youngest category (<55 years) subjects with nonalbuminuric CKD had the highest crude mortality rates and unadjusted and adjusted HRs, even higher than subjects with albuminuric CKD. In normoalbuminuric subjects with an eGFR <45 ml/min/1.73m^2, especially with low albuminuria (10-29 mg/day), risk was higher than in microalbuminuric and similar to macroalbuminuric individuals with preserved eGFR. At recursive partitioning and amalgamation analysis, prevalent CVD and lower HDL cholesterol were the most relevant correlates of mortality in all phenotypes. Other correlates differed among DKD phenotypes; albuminuria >8.3 mg/day and higher waist circumference were associated with a higher likelihood of death in the nonalbuminuric form.
Conclusion: Mortality risk is elevated in nonalbuminuric renal impairment, particularly so in younger individuals and in those with an eGFR <45 ml/min/1.73m^2 and low albuminuria. Correlates of death partly differ from those of the albuminuric phenotypes.
Clinical Trial Registration Number: NCT00715481
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Nonalbuminuric diabetic kidney disease in type 1 diabetes: association with the risk of major vascular outcomes and all-cause mortality

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Background and aims: Different diabetic kidney disease (DKD) phenotypes has been described not only in type 2, but also in type 1 diabetes. Despite improvements in care, individuals with type 1 diabetes, mostly those with DKD, have higher mortality and reduced life expectancy (LE) compared with the general population. Recently, a prognostic model to assess the risk of major outcomes and all-cause death in type 1 diabetes has been developed from data of the EURODIAB Prospective Complication Study (PCS). Here we evaluated the association of different DKD phenotypes with the EURODIAB PCS risk score and with all-cause mortality in a cohort of 774 type 1 diabetic individuals.
Materials and methods: The EURODIAB PCS absolute risk, stratified as low- (LR, score up to 15), intermediate- (IR, score 16 to 20), and high- (HR, score 20 or higher), the distribution of the different DKD phenotypes [no DKD, stages 1-2 DKD, nonalbuminuric stage ≥3 DKD (Alb-

DKD), albuminuric stage ≥3 DKD (Alb+DKD)] and all-cause mortality in a mean follow-up of 8.25±2.34 years (median 7.58 years, IQR 6.47-9.73) have been evaluated in a cohort of 774 individuals with type 1 diabetes belonging to a single center (men/women 52.6/47.4%, 40.2 ±11.7 year-old with a mean diabetes duration of 19.3±12.2 years and HbA1c of 7.83±1.18%, 62.1±12.9 mmol/mol). Adjudications of DKD phenotypes were made by blinded investigators.

Results: Out of 774 subjects, 692 (89.4%) had no DKD, 53 (6.8%) had DKD stages 1-2, 17 (2.2%) had Alb-DKD stage ≥3 and 12 (1.6%) had Alb+DKD stage ≥3. Risk score distribution was: LR n. 466 (60.2%), IR n. 205 (26.5%) and HR n. 103 (13.3%) in the whole cohort, and LR n. 461 (62.9%), IR n. 195 (26.6%) and HR n. 77 (10.5%) after exclusion of 41 type 1 diabetic individuals (5.3%) who already had major outcomes. Prevalence of subjects with high score was: no DKD, 9.1%; DKD stages 1-2, 34.0%; Alb-DKD, 64.7%; Alb+DKD, 91.7% ($p<0.0001$). All cause mortality increased through DKD phenotypes: no DKD, 3.0%; DKD stages 1-2, 15.1%, HR 4.504 (95%CI: 1.992-10.186); Alb-DKD, 29.4%, HR 8.573 (3.222-22.815); Alb+DKD, 50.0%, HR 20.683 (8.292-51.587) (Kaplan-Meier, logrank $p<0.0001$). Accounting in Cox regression for age (HR 1.070; 1.044-1.098, $p<0.0001$) and sex (men: HR 1.922; 0.969-3.813, $p=0.061$), HRs for all-cause mortality compared to no DKD were: DKD stages 1-2, 3.841 (1.686-8.750, $p=0.001$); Alb-DKD, 2.970 (1.018-8.662, $p=0.046$); Alb+DKD, 7.441 (2.781-19.911, $p<0.0001$). In a second Cox model, accounting for sex and the EURODIAB score (intermediate risk: HR 3.346, 1.201-9.321, $p=0.021$; high risk: HR 11.736, 4.437-31.044, $p<0.0001$), HRs for all-cause mortality compared to no DKD were: DKD stages 1-2, 2.571 (1.113-5.939, $p=0.027$); Alb-DKD, 2.772 (0.968-7.943, $p=0.058$); Alb+DKD, 4.584 (1.691-12.421, $p=0.003$). Trends in trajectories of both Kaplan-Meier analysis of all-cause mortality as well of each model of the Cox regression were confirmed after exclusion of the 41 individuals who have had previous MACE.

Conclusion: In type 1 diabetes, the Alb-DKD phenotype has a prevalence of 2.2% and accounts for 20.7% of all DKDs and is independently associated with an increased risk of major vascular outcomes and an high all-cause mortality rate, to the same extent as the albuminuric stages 1-2 DKD. The highest risk and the highest mortality rate were observed in Alb+DKD. Intensive cardiovascular prevention strategies should be applied to these subjects.

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Disclosure: **M. Garofolo:** None.

1072

Diabetes Kidney Disease (DKD) severity classifications and therapy impact in a real-world setting across 5EU/US

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Background and aims: The strong correlation between type 2 diabetes mellitus (T2DM), cardiovascular and renal complications is well documented and by addressing multifactorial risks, it is claimed that diabetes-related complications can be reduced. This analysis explores the co-existence of these conditions amongst T2DM patients, clinical versus physician-driven diabetes kidney disease (DKD) severity label assignment and subsequent impact on therapy class choice by DKD severity stratification.

Materials and methods: Data were drawn from the 2016 Diabetes Disease Specific Programme in the US and EU. Diabetes specialists and primary care physicians (PCPs) completed forms for the next 10 consulting T2DM patients, including current antidiabetic therapy, HbA_{1c}, estimated glomerular filtration rate (eGFR) test value, physician-reported diagnosis of DKD (categorized as [1] mild renal or [2] moderate/severe renal), congestive heart failure (CHF) and body mass index (BMI) derived from weight and height reporting. Physicians did not report eGFR stages or BMI classifications and these were retrospectively applied to the data based on clinical criteria. DKD labelling via derived eGFR scores was defined as stages 1-5. Obesity labelling via BMI was ≥30 kg/m^2.

Results: A total of 352 specialists, 501 PCPs, and 8,523 T2DM patients were included across US and 5EU. Of these, 41% had T2DM-only, 30% T2DM+obesity, 14% T2DM+DKD (eGFR stage 1-5), 13% T2DM+DKD+obesity and only 1% with T2DM+DKD+obesity+HF. 28% of patients had an eGFR-confirmed DKD diagnosis compared to only 9% of patients receiving a physician-confirmed DKD diagnostic label. When comparing the physician severity labelling vs. eGFR score, 33% of patients were not classified with the same DKD stage. When looking at which therapy class is prescribed at which eGFR stage, there is evidence of some contraindicated usage across DKD stages in a real-world setting predominantly with metformin, sulfonylurea (SU) and dipeptidyl peptidase-4 inhibitors (DPP-4i) therapy classes.

Conclusion: DKD prevalence is high amongst T2DM patients in a real-world setting, with around two-thirds of DKD patient cases not being recognized by the physician according to derived eGFR staging. As a result, there is evidence that some contraindicated therapy options may be being prescribed at various DKD stages. Further education around DKD severity classifications could address these issues.

Disclosure: **V. Higgins:** None.

1073

Evolution of renal function in type 2 diabetics: a retrospective study of the last 12 years

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Background and aims: Albuminuria is associated with progression to chronic kidney disease, is a strong predictor of cardiovascular risk and is associated with an increase in all-cause and cardiovascular mortality. It is more relevant in patients at higher risk, such as type 2 diabetics. Our aim was to evaluate the variation of the renal function rate in type 2 diabetic patients, between 2005 and 2016, considering albuminuria and other potentially relevant factors, such as the duration of the disease, presence of comorbidities and treatments.

Materials and methods: Retrospective observational study in 1031 consecutive patients with type 2 diabetes mellitus followed for at least three years. We collected 39 clinical, laboratory and therapeutic variables from our electronic dataset. A total of 7371 determinations of albuminuria/creatininuria ratio were performed in our lab and estimated GFR (eGFR) was based on the CKD-EPI formula. Patients younger than 18 years, with other types of diabetes, impaired kidney function at first determination (eGFR < 60 mL/min/1.73 m^2) or transplanted were excluded. Results are presented by median and standard error. We applied descriptive statistics methods, Pearson's correlations, Student T test for continuous variables and the chi-square in the categorical variables.

Results: A total of 1031 patients were evaluated, with a predominance of female gender (59.3%). The initial age of the patients was 62.5 ± 0.3 years. The mean follow-up time was 5.9 ± 0.8 years. The first albuminuria was 12.4 ± 9.7 mg/mg and the eGFR decreased an initial value of 87.0 ± 0.4 to 74 ± 0.6 mL/min/1.73m^2. There was great prevalence of comorbidities: dyslipidemia (97.1 %), hypertension (91.7%), retinopathy (64.1%), obesity (41.8%) and mortality in this period was 5.8% (60 patients) and there were 22.7% dropouts. We found significant correlations between the eGFR variation and

the initial albuminuria ($p<0.01$), age ($p<0.01$) and HbA_{1c} ($p=0.01$). During follow-up we found correlations with mean albuminuria ($p<0.01$) and blood pressure, but especially mean pulse pressure ($p<0.01$). Patients who died were older ($p<0.01$), had lower inicial eGFR ($p<0.01$) and larger decline of eGFR ($p<0.01$).

Conclusion: Albuminuria is associated with the risk of progression to chronic kidney disease, with the initial and the mean value being significantly associated with a higher rate of decline in renal function (eGFR). The same for the initial blood pressure values and their control during the follow-up (median= 6 years).The mortality in our group was low and associated with initial age, with eGFR and its decline, but not with albuminuria or other laboratorial variables in the study population.

Disclosure: **C. Ferrinho:** None.

1074

Decreased plasma kallikrein activity is associated with kidney function decline in patients with type 1 diabetes

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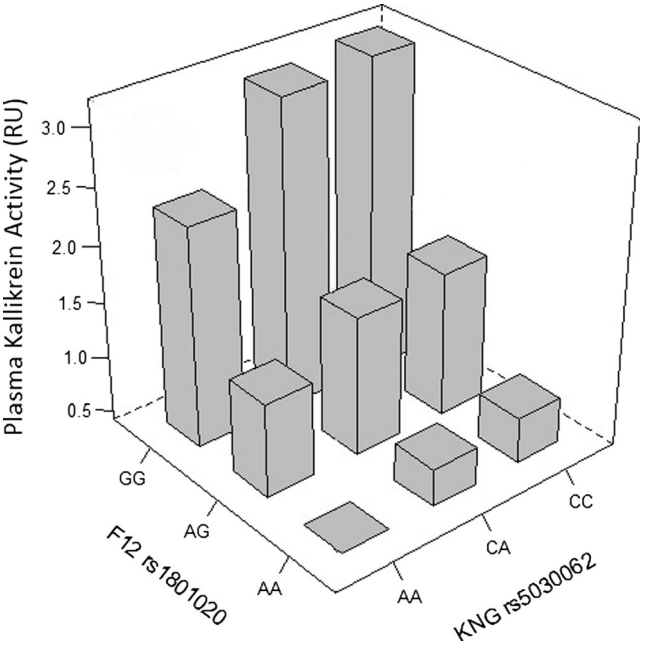
Background and aims: Kallikreins are serine proteases that release kinins from kininogens and may be involved in the renin-angiotensin-aldosterone system by converting prorenin into renin. It has been suggested that kallikreins and kinins, through protease dependent signaling, may play a role in the pathogenesis of kidney disease. We therefore measured plasma kallikrein (KK) activity in patients with type 1 diabetes (T1D) with various stages of diabetic nephropathy (DN). We also assessed the genetic impact of the plasma KK cofactor gene KNG1 and the upstream Factor XII gene F12.

Materials and methods: Plasma KK activity was determined with a chromogenic assay (S-2302, Haemochrom Diagnostica) in 297 patients with T1D. All patients were investigated as part of the Finnish Diabetic Nephropathy (FinnDiane) Study. DN was determined based on urinary albumin excretion rate (AER): normal AER (<30 mg/24 h; n=166), microalbuminuria (≥30 and <300 mg/24 h; n=41), macroalbuminuria (≥300 mg/24 h; n=38), and end-stage renal disease (ESRD; dialysis or transplantation; n=52). Renal function (eGFR) was estimated by the CKD-EPI equation. Genotypic information for the F12 and KNG1 genes was obtained from the FinnDiane GWAS study.

Results: Plasma KK activity decreased by advancing stage of DN: normal AER (median 2.59 [IQR 1.39-4.10]), microalbuminuria (2.04 [1.22-3.35]), macroalbuminuria (1.44 [0.96-2.83]), and ESRD (1.34 [0.71-1.72]; p=1.23e-05). The activity was lower in patients on dialysis compared to patients with kidney transplant (p=0.009). Notably, the plasma KK activity differed by genotypes of the common functional variants F12 rs1801020 ($p=7.16\times10^{-15}$) and KNG1 rs5030062 (p=0.001) in such a way that carriers of the rs1801020 minor (A) and the rs5030062 major (A) alleles had in general lower plasma KK activity as shown below. The activity correlated positively with eGFR ($r_s=0.34$; $p=2.72\times10^{-9}$), and this association remained after adjustment for age, sex, diabetes duration, waist circumference, HbA_{1c}, and with the two functional variants and their interaction (β=0.20 [95% CI 0.08-0.31], p=0.0006).

Conclusion: Plasma KK activity decreases with advancing stages of kidney disease and correlates positively with eGFR in patients with T1D. We also show that the plasma KK activity is associated with functional genetic variants, that have previously been linked to circulating Factor XII, renin and aldosterone protein levels. Whether plasma KK by itself influences the development of DN remains to be elucidated.



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Disclosure: **M. Härma:** None.

1075

Hypertriglyceridaemia, diabetic nephropathy and coronary heart disease in type 1 diabetes: Simple association or causal relationship?

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Background and aims: Dyslipidemia is a major risk factor for diabetic nephropathy and coronary heart disease (CHD), the most common causes of premature death in type 1 diabetes (T1D). Data whether hypertriglyceridemia is causally related to these diabetic complications are scarce. We therefore investigated in patients with T1D, if hypertriglyceridemia is causally related to diabetic nephropathy and CHD.

Materials and methods: This cross-sectional analysis was part of the prospective, ongoing Finnish Diabetic Nephropathy Study (FinnDiane). Between 1994 and 2015 data were obtained from 4481 T1D patients in more than 80 hospitals or health centers across Finland. The potentially causal effect of triglycerides on CHD and stages of nephropathy was explored using Mendelian Randomization (MR) and a genetic risk score (GRS) based on 96 SNPs associated with triglycerides in the literature. The strength of MR is to create a form of a randomized controlled trial. Based on triglycerides, which are strongly affected by genetic variants, patients have been randomly allocated to different groups before birth. Follow up period started at birth and ended at the study visit when clinical endpoints were evaluated (>41 years of follow-up). MR analysis was performed using the GMM estimation of structural mean models for binary and the two-stage least squares method for continuous outcome.

Results: In the entire cohort there were 47.8% females, age was 41±12.7 yrs, diabetes duration 25.1±13.1 yrs, and HbA1c 8.4±1.4%. CHD was present in 8.3% of the patients, 18% had CKD stage 3-5, 12.5% microalbuminuria, 13.8% macroalbuminuria and 9.7% ESRD. GRS was associated with triglycerides ($p<0.001$), and the association remained significant after adjustment for LDL-C and multiple combinations of LDL-C, HDL-C, total cholesterol, ApoA1 and ApoB, sex, age, BMI and systolic blood pressure ($p<0.001$). Triglycerides ($p<0.001$) but not GRS ($p=0.464$) was associated with eGFR. However, instrumental variables regression showed no causal effect of triglycerides on eGFR ($p=0.470$). Triglycerides ($p<0.001$) and GRS ($p=0.002$) was associated with CHD. Notably, MR showed a causal effect of genetically elevated triglycerides on CHD with a 2.5-fold elevated risk for each SD-elevation of logTriglycerides.

Conclusion: In addition to confirming that triglycerides are associated with CHD, our data also suggest that there is a causal link between triglycerides and CHD in patients with T1D. In contrast, we found no causal relationship between triglycerides and diabetic nephropathy in this population.

Disclosure: **L. Stechemesser:** None.

1076

Reduced levels of anti-ageing hormone Klotho are associated with microalbuminuria and predict renal function decline in patients with type 2 diabetes

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Background and aims: Soluble Klotho (sKlotho) is a circulating anti-ageing hormone involved in phosphate metabolism which is renoprotective in animal studies. We have previously demonstrated in type 1 diabetes mellitus an association between microalbuminuria (MA) and reduced levels of sKlotho. It is unknown if sKlotho predicts renal function decline in type 2 diabetes mellitus (T2DM) patients with preserved renal function and there is limited data on sKlotho levels in T2DM patients with MA. The primary aim of our study was to investigate if sKlotho predicts renal function decline in T2DM patients with relatively preserved renal function.

Materials and methods: We studied 101 patients (60% male) with T2DM, mean age (range) 60.3 years (40-82), duration of diabetes mean ± SD, 9.8±6.6 years, and estimated glomerular filtration rate (eGFR)>45 ml/min (mean eGFR 90.4± 19.7 ml/min) with and without residual MA on renin angiotensin system (RAS) blockade. Patients were followed up for a median (range) of 8 years (3-12 years) at Guy's Hospital London with standardised clinical and biochemical measurements. eGFR was determined using the Chronic Kidney Disease Epidemiology Collaboration equation. For each patient, a linear regression model of time on eGFR was created, and slope of the regression line was used to estimate changes in eGFR over time. sKlotho, serum phosphate (sPhos), calcium (sCa) and Fibroblast growth factor 23 (FGF-23) were measured from stored samples collected at baseline. The primary endpoint was >50% decline in eGFR from baseline and/or death.

Results: Of the cohort 21% (n=21) reached the primary endpoint. Cox regression analysis showed that baseline sKlotho level independently predicted the primary endpoint after adjustment for risk predictors including MA, HbA1c, sPhos, sCa, FGF-23, blood pressure (BP) variables, and baseline eGFR (Hazard Ratio per 10-pg/mL increase, 0.47; 95% CI, 0.24-0.98; $P=0.04$). Patients with residual MA (n=55) and without MA (n=46) did not have any significant differences in age, duration of diabetes, FGF-23, sPhos, sCa, eGFR, and glycaemic control. MA patients had higher systolic BP (SBP) and body mass index (BMI) and significantly lower levels of sKlotho median, interquartile range (IQR), 184.7 (131.2 to 274.6) pg/ml compared to patients without MA 231.3 (175.5 to 290.8) pg/ml $p=0.01$ and this significant difference remained after adjustment for SBP, BMI and other variables. Patients in the lowest quartile of sKlotho had significantly faster annual rate of eGFR decline, median (IQR) of -3.3 (-4.5 to - 1.7) ml/min/year compared to the highest quartile, -1.4 (-2.8 to 0.02) ml/min/year, independent of other risk factors in multivariate analyses, $p=0.03$.

Conclusion: We demonstrate in T2DM patients with relatively preserved renal function, reduced levels of sKlotho are associated with MA and predict significant renal function decline. The role of sKlotho as a novel bio-marker of renal dysfunction and potential treatment target for renoprotection in T2DM requires further evaluation.

Disclosure: **N. Fountoulakis:** None.

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1077

Fructosamine 3-kinase and glyoxalase 1 polymorphisms and their association with development of vascular complications in diabetes: a 10-year follow-up study

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Background and aims: Advanced glycation end-products are key players in the pathogenesis of long-term vascular complications in diabetes. Fructosamine 3-kinase (FN3K) and glyoxalase 1 (GLO1) are crucial enzymes preventing excessive glycation processes. The aim of our study was to evaluate an association of FN3K (rs3848403, rs1056534) and GLO1 (rs4746) polymorphisms with development of chronic vascular changes in diabetes.
Materials and methods: Totally, 467 subjects with diabetes were enrolled within the study - 128 Type 1 /T1D/ aged 45 ± 14 yrs and 339 Type 2 /T2D/ aged 64 ± 11 yrs. Mean duration of the study was 10 years. DNA analysis was performed using RealTime PCR and Taqman genotyping method for allelic discrimination (FN3K polymorphisms) or PCR-RFLP method (GLO1 polymorphism). Both at the beginning and at the end of the study basic anthropometrical data, glycated hemoglobin HbA_{1c} (in IFCC units), glomerular filtration rate (using CKD-EPI formula) and (micro)albuminuria (albumin/creatinine ratio) were assessed, and macrovascular endpoints were recorded. χ^2 test was used to compare the qualitative data.
Results: Diabetes control expressed by HbA_{1c} was improved during the study both in T1D and T2D (T1D: 70 ± 14 vs 62 ± 10 mmol/mol, p<0.0001; T2D: 64 ± 20 vs 57 ± 15 mmol/mol, p<0.0001). Glomerular filtration rate was significantly increased in T1D (108 ± 24 vs 122 ± 27 mL/min/1.73 m^2, p<0.0001), whereas no change was observed in T2D (107 ± 28 vs 108 ± 36 mL/min/1.73 m^2, NS). A new development of diabetic nephropathy was observed in 6 % of T1D and 13 % of T2D, whereas 12 % of T1D and 16 % of T2D transformed from micro- to normoalbuminuria. Such improvement was observed neither in retinopathy nor neuropathy. Genotype frequencies of rs3848403, rs1056534 and rs4746 polymorphisms followed the expected frequencies according to Hardy-Weinberg equilibrium and did not differ between T1D and T2D. In T1D FN3K (rs3848403) TT genotype with mutated T allele was significantly associated with development of diabetic nephropathy (p<0.04) or a new microvascular endpoint (new nephropathy, retinopathy or neuropathy) (p<0.02). Such association was not present in T2D. Similarly, FN3K (rs1056534) CC genotype without mutated G allele was significantly associated with development of diabetic nephropathy in T1D (p<0.02), but not in T2D. There was not observed any association of GLO1 rs4746 polymorphism with the development of either micro- or macrovascular damage.
Conclusion: Our 10-year follow-up study showed improvement in diabetes treatment and partial regression of diabetic nephropathy. While FN3K polymorphisms rs3848403 and rs1056534 may have some functional effect in the development of diabetic angiopathy, especially in T1D, the role of GLO1 rs4746 polymorphism is disputable and other factors will act in this scenario.
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Disclosure: **J. Škrha jr.:** Employment/Consultancy; Boehringer Ingelheim GmbH. Lecture/other fees; Eli Lilly.

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Haemorheologic assessment for screening diabetic nephropathy in type 2 diabetes

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Background and aims: Hemorheologic alterations or changes in blood viscosity have been suggested to play a role in the pathogenesis of diabetic microvascular complications. We measured various hemorheologic parameters in type 2 diabetes patients and assessed their possible role as a diagnostic tool for diabetic nephropathy.
Materials and methods: Four hundred-seventy patients with type 2 diabetes were included in this study. Hemorheologic parameters, including erythrocyte deformability, elongation index (EI), critical shear stress (CSS), and aggregation index (AI) were measured using microfluidic hemorheometer. Various metabolic parameters were assessed from fasting blood samples and urinary albumin to creatinine ratio was used to assess diabetic nephropathy.
Results: There were significant differences in Elongation index at 3 Pascal (EI at 3Pa), Fibrinogen/EI, and shear stress among patients in different stages of chronic kidney disease (all p<0.05), EI at 3 Pa, Fibrinogen/EI, and shear stress significantly differed among the groups. Fibrinogen/EI differed between normal or CKD 1 and CKD 2 patients. In multiple regression analysis, Fibrinogen/EI at 3Pa was an independent predictor of albumin to creatinine ratio independent of age, ESR, hematocrit, HbA1c, and body mass index (ß=0.101, p<0.05). Also, critical time, critical stress, fibrinogen/EI at 3Pa, CSS/EI et 3Pa, ad fibrinogen/CSS at 3Pa were significantly different among patients at different stages of diabetic nephropathy (all p< 0.05). Among the variables, Fibrinogen/EI at 3Pa showed area under the ROC curve of 0.721, suggesting 860 mg/dL% as a cut off point for diabetic nephropathy with the sensitivity of 74% and specificity of 62%.
Conclusion: Fibrinogen/EI is a sensitive parameter measured via point-of-care testing for screening diabetic nephropathy in patients with type 2 diabetes.
Disclosure: **S. Lee:** None.

1079

Biomarkers associated with progression of renal disease in type 1 diabetes

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Background and aims: To identify biomarkers associated with and/or predictive of progression of renal disease in subjects with type 1 diabetes mellitus (T1DM).
Materials and methods: The study comprised 926 patients with T1DM recruited from diabetes clinics and primary care into the Scottish Diabetes Research Network Type 1 Bioresource (SDRNT1BIO) and 315 patients recruited as part of the FinnDiane project. FinnDiane participants were oversampled for albuminuria. Retrospective and prospective clinical record measures of risk factors, eGFR and albuminuria and direct measures of eGFR and albuminuria were available. Progression of renal disease was evaluated by approximating eGFR trajectories for each patient by a linear slope and by defining a rapid progressors category by dichotomizing the linear slope according to a threshold of a loss of more than 3 ml/

min/1.73^2 per year. Biomarkers were measured in non-fasting serum samples using the Luminex platform (30 protein biomarkers) and mass spectrometry (94 metabolites). We investigated associations with eGFR slopes through linear regression where each biomarker was evaluated independently in models adjusted for age, sex and diabetes duration. A cross-validated forward selection approach was adopted to create the sparsest panel of biomarkers that would improve prediction.
Results: After setting a Bonferroni-corrected significance threshold of 4x10^{-4}, 17 protein markers and 10 metabolites showed significant association with eGFR slopes beyond age, sex, diabetes duration and study day eGFR (strongest associations with Kidney Injury Molecule-1 (KIM-1) beta=-0.37 (95% CI -0.42, -0.31), CD27 antigen beta=-0.39 (-0.47, -0.31), Alpha-1 Microglobulin beta=-0.30 (-0.36, -0.23)). These associations were also present when considering the binary rapid progressors outcome. KIM-1 was still significantly associated with eGFR slopes and rapid progression even after further adjustment for BMI, systolic blood pressure, diastolic blood pressure, HbA_{1c}, HDL, total cholesterol, smoking status and categorical albumin:creatinine ratio (ACR). Serum KIM-1 was shown consistently to be the most strongly associated biomarker, and was the most predictive marker in forward selection. When predicting rapid progression, KIM-1 alone increased the predictive performance of a model adjusted for age, sex, diabetes duration and study day eGFR, from an AUC of 0.703 to 0.762.
Conclusion: Serum KIM-1 is the biomarker most associated with rapid eGFR loss in patients with diabetes and significantly improves its prediction above clinical data.
Supported by: JDRF
Disclosure: **M. Colombo:** None.

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Individuals with impaired glucose tolerance have a higher risk to develop chronic kidney disease over ten years than individuals with impaired fasting glucose

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Background and aims: Impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) are insulin resistance states but differing in the tissue aetiology of the insulin resistance. In summary, individuals with IFG suffer primarily from beta cell insufficiency, while individuals with IGT suffer primarily from insulin resistance in muscle tissue. The aim of this study was to explore the difference in development of chronic kidney disease stage 3 (CKD) between individuals with IFG and IGT over ten years.
Materials and methods: A random sample of 2816 individuals, 30-75 years old, in a middle-sized town in South-west of Sweden, were examined in a longitudinal study. All participants performed an oral glucose tolerance test and were examined by three specially educated nurses with blood pressure, body weight and height. IFG and IGT were defined by WHO criteria. Blood tests were drawn for S-creatinine and morning urine was collected for urine albumin and creatinine. After a mean follow-up time of 9.7 (7.8-11.6) years 1327 of the original participants were re-examined following the same protocol. In this study we addressed individuals with IFG (n=29) and IGT (n=24) in both examinations. CKD stage 3 was defined by estimated glomerular filtration rate (eGFR) ≤60 ml/min/1.73m^2 according to CKD -epi.
Results: During follow-up a decrease in eGFR was observed in the study population (mean=6.1 ml/min, SD=11.9). At baseline 2.1 % (n=28) of all subjects (n=1327) had chronic kidney disease stage 3, while there at follow-up was an increase to 8.3 % (n=109). The decrease in eGFR was greater in those with IGT when compared to those with IFG; Δ eGFR (IGT-IFG)= 8.5 ml/min, P= 0.003, CI= 2.8-14.2. There was in particular a significant difference between individuals with IFG and those with IGT considering the development of chronic kidney disease stage 3 after ten years, OR = 24.3, P= 0.016, CI= 1.8-327. These differences were still significant after adjustments for age, sex and eGFR at baseline.
Conclusion: Subjects with IGT are at higher risk to develop nephropathy when compared with subjects with IFG. Thus, it is particularly important to identify individuals with IGT to implement lifestyle interventions and when indicated also treatment with pharmaceuticals to prevent the development of nephropathy.
Supported by: GU;(ALF), VGR, FoU, SI.
Disclosure: **S.G.J. Diurlin:** None.

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Non-alcoholic fatty liver disease significantly predicts future decline in kidney function in angiographied coronary patients

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is associated with insulin resistance, type 2 diabetes (T2DM), and an increased risk of cardiovascular events. A potential association of NAFLD with the decline of kidney function over time has not been investigated yet and is addressed in the present study.
Materials and methods: We prospectively investigated a series of 981 consecutive patients referred to coronary angiography for the evaluation of established or suspected coronary artery disease (CAD). NAFLD was diagnosed using the validated fatty liver index, and the glomerular filtration rate (eGFR) was calculated both at baseline and after a follow-up period of 4 years.
Results: At baseline, in patients with NAFLD (n=447; 45.6% of the study cohort) the prevalence of T2DM and of the metabolic syndrome was higher (32.7 vs. 18.8 %; p<0.001 and 72.9 vs. 23.0%; p<0.001, respectively), whereas eGFR was similar (73.2±20.0 vs. 72.9±21.3 ml/min/1.73 m^2; p=0.812) when compared to subjects who did not have NAFLD. Prospectively, NAFLD significantly predicted a decline in eGFR after adjustment for age, gender, and eGFR at baseline (F=8.97; p=0.003) and after further adjustment for smoking, LDL cholesterol, T2DM and the MetS (F=7.35; p=0.007).
Conclusion: We conclude that NAFLD significantly predicts a decline in kidney function over 4 years independently of baseline kidney function in angiographied coronary patients.
Disclosure: **C. Lins:** None.

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Serum galectin-3 predicts progressive kidney disease in type 2 diabetes

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Background and aims: Galectin-3, a member of the multifunctional galectin family, acts a broad-spectrum biological response modifier and is involved in tissue fibrosis, immunity, and inflammatory response. Recent evidence suggests that galectin-3 may play a role in the development of diabetic and non-diabetic nephropathies and serum level of galectin-3 may serve as a biomarker of inflammation and renal fibrosis. We have therefore evaluated whether serum galectin-3 level can predict progressive kidney disease in patients with type 2 diabetes.

Materials and methods: Galectin-3 was measured in the baseline serum samples by ELISA in 590 type 2 diabetic patients with estimated glomerular filtrate rate (eGFR) >30 mL/min/1.73m^2. Progressive kidney disease was defined as a doubling of the serum creatinine concentration, dialysis or transplant during follow up.
Results: 126 subject developed progressive kidney disease over 7.5 ± 4.4 years of follow up. At baseline, serum galectin-3 level significantly correlated with serum creatinine level and eGFR. Subjects with deterioration in renal function had higher baseline serum galectin-3 level than those whose renal function remained stable (9.07 ± 3.02 ng/ml vs 7.73 ± 2.18, p<0.01). Serum galectin-3 was a significant independent predictor of progressive kidney disease even after adjustment for age, gender, smoking, baseline eGFR, systolic blood pressure, HbA1c and duration of diabetes (p= 0.004, odds ratio 1.163, 95% CI 1.049-1.289).
Conclusion: Elevated serum galectin-3 level was associated with progression of diabetic kidney disease in type 2 diabetes and galectin-3 may be a potential useful biomarker to predict renal deterioration.
Disclosure: **K.C.B. Tan:** None.

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Components of metabolic syndrome and their causality for diabetic nephropathy

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Background and aims: The metabolic syndrome (MetS) is a risk factor for diabetic nephropathy (DN), and the individual components of the MetS are associated with DN. However, whether these components are casually related to the pathogenesis of DN is unknown. This work therefore assesses potential causality.
Materials and methods: The study includes 4,533 participants with Type 1 Diabetes (T1D) from the FinnDiane Study. The data on five components of the MetS; systolic blood pressure (SBP), HDL cholesterol (HDL), triglycerides (TG), and waist circumference (WC) were measured at the baseline visit. Fasting glucose (FG) was not measured as all patients had T1D. All participants were genome-wide genotyped. Risk SNPs for components of the MetS were identified from publicly available GWASs. Genetic risk scores (GRS) for each patient were computed for each of the components (including FG). DN was defined as a case-control phenotype: controls as patients with normal albumin excretion rate (n=2,012), and cases as patients with macroalbuminuria or ESRD (n=1,161), using the latest available data (1,360 patients with DN status not compatible with case or control definition were used for GRS quality evaluation only). For the analyses the Multivariate Mendelian randomization (MR) and two-stage least-squares (2SLS) estimation on individual level phenotype/genotype data was used. The first-stage regression was defined as linear multivariate multiple regression using control patients only, and the second-stage was defined as logistic univariate multiple regression, using the predicted values of the risk factors from the model of the first-stage as independent variables. Risk factors demonstrating significant estimates for the second-stage were considered causal for DN.
Results: The created GRSs were strongly associated with the corresponding traits ($P_{GRS\text{-}SBP}=4.6\times10^{-5}$ to $P_{GRS\text{-}HDL}=2.4\times10^{-45}$), and explained approximately 0.4-4.5% of the trait variation. Some GRSs showed significant inter-correlations (GRS_{FG} vs. GRS_{TG}, r=-0.06, $p=4.4\times10^{-5}$ and GRS_{HDL} vs. GRS_{TG}, r=-0.31, $p=2.3\times10^{-104}$), and associations with other risk factors (GRS_{TG} associated with HDL, p=0.02), suggesting plausible pleiotropic genetic effects. GRS_{WC} was also directly associated with DN independently of other GRSs (OR=2.3 for each SD increase in GRS_{WC}, p=0.002). At the second stage of the MR analyses, only genetically predicted WC was associated with the DN outcome (OR=1.22 for each cm increase in genetically predicted WC, p=0.003), demonstrating a causal effect on DN. This association remained even when the first and second stage models were adjusted for sex and age at the baseline visit (OR=1.23, p=0.003).
Conclusion: Components of the MetS partially share genetic background. However, only genetically determined waist circumference seem to be causal for DN, whereas the other components of the MetS demonstrated no significant causal effects. Improving the quality of GRSs by using results from population-specific GWAS studies and/or increasing the number of included risk SNPs will most likely improve the power to find also other factors casually related to DN in the future.
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Disclosure: **I. Toppila:** None.

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Reduced sublingual endothelial glycocalyx in type 1 diabetic patients with diabetic nephropathy

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Background and aims: Glycocalyx is a glycoprotein layer that lines and protects the inner surface of the capillary endothelium. Damage to the glycocalyx may be an early stage in development of microvascular complications in diabetes. Insight into the function and thickness of glycocalyx in vivo, has been limited by the lack of easy and non-invasive quantification tools. The development of capillaroscopy has made it possible to visualize the sublingual capillaries by sidestream dark field imaging. Capillaroscopy estimates the dimensions of the glycocalyx by measuring the sublingual perfused boundary region (PBR). Our aim was to evaluate the glycocalyx thickness non-invasively in type 1 diabetic patients with different levels of historical and current albuminuria.
Materials and methods: Cross-sectional study including 77 type 1 diabetic patients stratified by history of normoalbuminuria (<30 mg/g) (n=26; age (mean±SD) 55.5±11.2 years; 34.6% women); microalbuminuria (30-299 mg/g) (n=27; 62.2±9.3 years; 48.2% women); and macroalbuminuria (>300 mg/g) (n=24; 57.5±9.9 years; 54.2% women). Glycocalyx thickness was assessed by 5 measurements with the GlucoCheck device (GlucoCheck BV, Maastricht, The Netherlands), an in vivo non-invasive hand-held microscope generating video recordings of the sublingual capillaries. The endothelial glycocalyx thickness was estimated from the PBR in capillaries with a diameter range of 5 to 25 μm. Higher PBR indicates smaller glycocalyx width. Urinary albumin-to-creatinine ratio (UACR) was measured in three morning samples.
Results: In normo-, micro-, and macroalbuminuric patients PBR was 2.30±0.22 μm, 2.32±0.25 μm, and 2.49±0.35 μm, respectively. The differences between normo- and macroalbuminuric patients (p=0.020) and micro- and macroalbuminuric patients (p=0.042) were significant, whereas the difference between normo- and microalbuminuric patients was not (p=0.74). After adjustment for age, sex, HbA_{1c}, diabetes duration and systolic blood pressure, the differences between normo- and macroalbuminuric patients (p=0.018) and micro- and macroalbuminuric patients (p=0.004) remained significant. In pooled (n=77) multivariate linear regression, higher level of current UACR (p=0.0007) and longer diabetes duration (p=0.026) were associated with a higher PBR.
Conclusion: In type 1 diabetic patients with a history of macroalbuminuria, measurements with the non-invasive GlucoCheck device revealed significantly higher PBR, suggesting an impaired glycocalyx, compared to patients with normo- or microalbuminuria. Moreover, higher current level of albuminuria was associated with higher PBR.

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PS 094 Hypertension

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Early pregnancy clinical risk factors for preeclampsia in women with type 1 and type 2 diabetes

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Background and aims: To assess the prevalence of pregnancy-induced hypertensive disorders and to identify early clinical, modifiable predictors of preeclampsia in women with type 1 and type 2 diabetes.
Materials and methods: A population-based cohort study of 494 women with pre-existing diabetes (307 type 1 and 187 type 2 diabetes) within a population of 2 million inhabitants, included at their first antenatal visit at 11±6 gestational weeks (mean±SD) from 2012 to 2016. Predictors of preeclampsia present at first antenatal visit were sought identified.
Results: At the first antenatal visit HbA1c was 6.9±2.3 % (51±10 mmol/mol) vs. 6.8±2.6 % (49±14 mmol/mol) and blood pressure 120 ±12/76±8 mmHg vs. 122±14/79±10 mmHg, (p=0.16/p=0.001) in women with type 1 and type 2 diabetes, respectively. Preeclampsia developed in 40 women at 36±3 gestational weeks with delivery 8±9 days later. The prevalence of preeclampsia was 8% (9% vs. 7%) and gestational hypertension 8% (9% vs. 6%). Univariate analysis identified nulliparity, presence of retinopathy or diabetic nephropathy including microalbuminuria and increasing blood pressure as predictors of preeclampsia. At the first antenatal visit, presence of diabetic microangiopathy (nephropathy, microalbuminuria and/or retinopathy) and diastolic blood pressure, were independently, positively associated with the development of preeclampsia, while neither diabetes type or HbA1c were associated with preeclampsia in this group of women with pre-existing diabetes and comparable good glycemic control.
Conclusion: At the first antenatal visit, diastolic blood pressure was the only independent, potentially modifiable risk factor for preeclampsia, in women with pre-existing diabetes regardless of diabetes type.
Disclosure: **S.K. Nørgaard:** None.

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Role of cardiovascular autonomic activity in hypertension associated with obesity

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Background and aims: We previously reported that a defect in vagal activity detected by cardiovascular autonomic reflex tests was associated with a higher rate of hypertension in patients with type 1 or type 2 diabetes, and that vagal activity is often depressed in the non diabetic obese population. Muscle sympathetic activity was found to be greater in hypertensive obese patients compared to normotensive ones. The aim was here to examine the relations between hypertension and cardiac autonomic nervous system activity in obese or overweight patients with normal (NGT), impaired glucose tolerance (IGT) or type 2 diabetes (T2D).
Materials and methods: We included 81 patients (38 NGTs, 28 IGTs and 15 T2Ds), including 25 with well-controlled hypertension. All of them were free of cardiovascular history. Central and peripheral blood pressure was assessed by applanation tonometry (SphygmoCor®). Cardiac sympathetic activity (LF-HR), vagal activity (HF-HR) and sympatho-vagal balance (LF/HF-HR) were determined by spectral analysis of heart rate variations at a controlled breathing rate during 6 minutes (Task Force Monitor® digital plethysmography).

Results: HF-HR, LF-HR and LF/HF-HR did not differ significantly according to glucose status. Compared with normotensive patients, hypertensive patients were older (60.0±12.8 *vs* 40.5±10.5 years, p<0.0001), had similar BMI(35.7±6.6 *vs* 36.4±5.3 kg/m^2) and HbA1c levels (5.89 ±1.15 *vs* 5.61±0.72%), higher systolic and diastolic blood pressure, higher heart rate, lower HF-HR and LF-HR (p<0.01 to p<0.0001). In multivariate analysis hypertension was associated with LF-HR and HF-HR independently of age, BMI and glucose status.
Conclusion: The present data suggest that among overweight or obese patients cardiac vagal and sympathetic activity is lower in those with hypertension whatever their glucose status. Changes in autonomic activity might be involved in hypertension.
Disclosure: **S. Chiheb:** None.

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Disrupted patterns of blood pressure in patients with type 1 diabetes

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Background and aims: Hypertension is a major risk factor for microvascular complications and cardiovascular disease in patients with type 1 diabetes. However, the office-based blood pressure (BP) measurements may not accurately reflect real diurnal BP variation and does not identify disrupted patterns of BP. Therefore, Ambulatory Blood Pressure Monitoring (ABPM) may provide more accurate estimates of BP variability and, thus, lead to better hypertension control. The aim of the study is to estimate the presence of disrupted patterns of blood pressure (sustained, white-coat and masked hypertension) in patients with type 1 diabetes.
Materials and methods: This study comprised 126 patients with type 1 diabetes [52% men, mean age 43.6 ± 10.4 years and diabetes duration 26.0 ± 9.7 years] from the Finnish Diabetic Nephropathy Study (FinnDiane). The thresholds for hypertension of the office based measurement was ≥140/90 mmHg and for the 24-h ABPM ≥130/80 mmHg. Masked hypertension was defined as normal office BP, but elevated ABPM, and white-coat hypertension as elevated office BP, but normal ABPM. 24h ABPM, central BP and pulse wave velocity (PWV) were measured simultaneously by a non-invasive brachial oscillometric device (Mobil-O-Graph).
Results: Of the cohort, 37% were normotensive and 33% hypertensive (of them 45% were untreated), while 5.5% had white-coat and 24% masked hypertension (of them 47% were untreated). Although patients with masked hypertension had similar office systolic BP as those who were normotensive, 24-h systolic BP did not differ from those with sustained hypertension (p=0.1). Markers indicating arterial stiffness, (i.e. 24-h central BP and PWV) were higher in patients with masked hypertension than in those with normotension (p <0.0001).
Conclusion: Detailed evaluation of blood pressure by ABPM identifies one-quarter of the patients with masked hypertension which is associated with arterial stiffness. Further studies are needed to evaluate whether masked hypertension predict micro- and macrovascular complications in patients with type 1 diabetes.

Variable	A. Normotensive	*p*-value A. vs B.	B. Masked hypertension	*p*-value B. vs C.	C. Sustained hypertension
Men/women (n)	12 / 35	0.002	18 /12	0.3	30 /12
Age (years)	38.5 ± 8.4	0.03	42.8 ± 7.9	0.02	48.5 ± 11.5
Antihypertensive drug (%)	36	0.7	40	0.2	55
Office systolic BP	122 ± 13	0.2*	127 ± 10	<0.0001*	154 ± 12
diastolic BP (mmHg)	73 ± 7	0.02*	77 ± 8	<0.0001*	84 ± 7
24-h systolic BP	118 ± 7	<0.0001*	132 ± 12	0.1*	138 ± 11
diastolic BP (mmHg)	73 ± 4	<0.0001*	84 ± 5	0.02*	86 ± 6
24-h central systolic BP	108 ± 6	<0.0001*	121 ± 10	0.04*	126 ± 10
diastolic BP	74 ± 4	<0.0001*	85 ± 5	0.01*	88 ± 6
Pulse Wave Velocity (m/s)	5.9 ± 0.7	<0.0001*	6.7 ± 1.0	0.1*	7.6 ± 1.4

Table 1 Characteristics of the study population; Data are mean ± SD or %; *adjusted for age

Disclosure: **R. Lithovius:** None.

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Blood pressure variability in individuals with and without (pre)diabetes: the Maastricht study

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Background and aims: The mechanisms underlying the associations between (pre)diabetes and cardiovascular disease (CVD) are incompletely understood. We hypothesize that greater blood pressure variability (BPV) may underlie this association, as greater BPV is associated with (incident) CVD. However, data on BPV in (pre)diabetes is scarce. Therefore, we investigated the association between (pre)diabetes and within-visit, 24-hour and 7-day BPV.
Materials and methods: Cross-sectional data from The Maastricht Study (N=3451, 1924 with normal glucose metabolism [NGM], 511 with prediabetes, and 975 with type 2 diabetes [T2DM], 51% men, aged 60±8 years), an observational population-based cohort study enriched with individuals with T2DM. As BPV-indices, we determined standard deviation (SD) for within-visit BPV (n=3244), average real variability (ARV) for 24-hour BPV (n=2699), and SD for 7-day BPV (n=2259) (by office, 24-hour ambulatory, and 7-day home blood pressure measurements, respectively). We additionally analyzed 24-hour BPV divided into day (09:00h - 21:00h) and night (01:00h - 06:00h). Differences in BPV as compared to NGM were assessed with multiple linear regression, adjusted for age, sex, mean systolic or diastolic BP, smoking status, alcohol use, BMI, prior CVD, lipid profile, use of lipid-modifying and antihypertensive medication, and estimated GFR.
Results: In T2DM, the average systolic/diastolic values of within-visit, 24-hour and 7-day BPV were: 4.8/2.6, 10.5/7.3 and 10.4/6.5mmHg, respectively and in prediabetes 5.0/2.6, 10.3/7.0 and 9.4/5.9mmHg, respectively. Adjusted analyses showed that T2DM was associated with greater nocturnal systolic BPV (ARV 0.42mmHg [95%CI: 0.05 - 0.80]) and greater 7-day systolic BPV (SD 0.76mmHg [0.32 - 1.19]) as compared to NGM. Prediabetes was associated with greater within-visit systolic BPV only (SD 0.35mmHg [0.06 - 0.65]) as compared to NGM.
Conclusion: Both prediabetes and T2DM are associated with greater very-short to mid-term BPV. Nevertheless, the slightly greater BPV seen in both prediabetes and T2DM as compared to NGM suggest that very-short to mid-term BPV may explain not more than a small part of the increased CVD risk associated with glucose metabolism status. These findings do not detract from the fact that very-short to mid-term BPV is substantial and important in individuals with and without (pre)diabetes.
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Disclosure: **T. Zhou:** None.

PS 095 Diagnosis and mechanisms involved in complications

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Expression of neutrophil elastase and myeloperoxidase mRNA in patients with newly diagnosed type 2 diabetes

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Background and aims: Neutrophil elastase (NE) and myeloperoxidase (MPO) enzymes actively participate in the formation of neutrophil extracellular trap and protect us from infection by killing pathogens. The impaired activity of NE and MPO may lead to impaired host defense against infection, and exaggerated activity of these enzymes may induce chronic inflammation. Importantly, diabetes mellitus (DM) is known to be associated with impaired host defense and chronic low-grade inflammation. Previous studies in DM found both higher and lower levels/activities of NE and MPO proteins in the serum or leukocytes. The present study was therefore aimed to explore the expressions of NE and MPO mRNA in the peripheral blood leukocytes (PBL) in patients with newly diagnosed type 2 DM.
Materials and methods: A total of 104 subjects were recruited from those who came for diabetes screening at our medical university. Subjects were grouped as control (normoglycemic) and diabetes based on their fasting blood glucose and HbA1c levels following ADA criteria. Subjects with previous history of DM and those suffering from complications of DM, chronic liver and kidney diseases, infection, inflammatory disease and malignancy were excluded. Insulin levels were measured by chemiluminescent microparticle immunoassay. Insulin resistance and beta cell functions were calculated. Total RNA from PBL was extracted and converted to cDNA by standard method. The mRNA levels of NE and MPO genes were quantified relative to reference gene beta-actin by quantitative real-time PCR. Gene expression data were analyzed by comparative Ct method, and expressed as percentage of reference gene.
Results: The control (n=65) and diabetes (n=39) groups were found similar in terms of sex distribution (m=57, f=47) and BMI. However, compared to control group the diabetes group was found aged (34±6 versus 46±8 yrs; $p<0.001$) with elevated systolic and diastolic BP ($p<0.01$). Fasting glucose, HbA1c and CRP levels were found significantly elevated in diabetes compared to control group ($p<0.01$). Fasting insulin levels were found similar between control (10.8±5.7), and diabetes (10.7±6.9 μU/mL) groups. However, HOMA-IR values [median (IQR)] were found 2.0 (1.3-3.1) for control and 3.6 (2.2-6.3) for diabetes groups ($p<0.001$); and HOMA-%B values were found 147% (108-234) for control and 45% (22-69) for diabetes groups ($p<0.001$). The NE mRNA expression was found significantly decreased in diabetes [0.29% (0.12-0.64)] compared to control group [0.45 (0.18-1.99); p=0.039]. Similarly, the MPO mRNA expression was also found significantly decreased in diabetes [0.09 (0.02-0.22)] compared to control group [0.16 (0.03-0.48); p=0.023]. The NE and MPO mRNA levels showed a negative trend with fasting glucose levels in all subjects (r= - 0.17, p=0.08 and r= - 0.16, p=0.10, respectively). However, the NE or MPO mRNA levels did not show any significant correlation with HbA1c, fasting insulin, HOMA-IR and HOMA-%B neither in diabetes group nor in all the study subjects.
Conclusion: The NE and MPO mRNA levels decrease in patients with newly diagnosed type 2 DM and show a negative trend with fasting glucose level. This finding may be considered as a probable molecular mechanism of increased susceptibility to infection in DM.
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Disclosure: **S.K. Biswas:** Grants; BMRC; HEQEP CP-3073.

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Relationship of chronic vascular complications with beta cell dysfunction and insulin resistance in newly-diagnosed type 2 diabetes. The VNDS Study

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Background and aims: Type 2 diabetes (T2D) is a heterogeneous disorder resulting from the variable combination of impaired insulin sensitivity (IS) and defective beta-cell function (BF). Severe chronic vascular complications that ensue from exposure to chronic (sub)diabetic hyperglycemia may be present long before overt diabetes is ultimately diagnosed and they may be associated with the underlying pathogenic phenotype. This study aimed at assessing the prevalence of vascular complications and their relationships with beta cell function (BF) and insulin sensitivity (IS) defects in a large cohort of patients enrolled in the Verona Newly-Diagnosed Type 2 Diabetes Study (VNDS).
Materials and methods: In 712 GADA-negative, drug naïve, consecutive VNDS participants we measured: 1) IS, by euglycemic insulin clamp; 2) BF, by mathematical modeling of 5h-OGTT; 3) microvascular complications (MIC): diabetic retinopathy, cardiac autonomic and sensory-motor neuropathy (SMN, by biothesiometer and tendon reflexes), e-GFR_{MDRD}<60 mL/min/1.73 m^2 or albuminuria >30 mg/24h; and 4) macrovascular complications (MAC): prior cardiovascular disease, ischemic ECG, lower-limb artery stenosis or carotid stenosis >40% at US-scans. Thresholds for defective BF and IS were the 25th percentiles of BF and IS assessed with the same methods of VNDS in subjects with normal glucose regulation of the GENFIEV (n=340) and GISIR (n=386) studies, respectively.
Results: The prevalence of combined BF and IS defects was 78.9%, while that of isolated BF and IS was 10.8% and 8.8%, respectively. Overall, the prevalence of MIC and MAC was 48.2% and 18.6%. The most frequent MIC and MAC were SMN (28.5%) and prior cardiovascular disease (11%), respectively. Notably, the prevalence of isolated or combined IS and BF defects did not significantly differ between patients with and without complications. Logistic regression analysis revealed that reduced IS, older age, male sex and smoking independently predicted MAC, but not MIC, after adjusting for BMI, blood pressure, LDL-cholesterol and A1c. BF was not associated with any complication.
Conclusion: Our study showed that patients with newly-diagnosed T2D have a high prevalence of MIC and MAC, the latter being independently associated with reduced IS. These findings highlight the clinical importance of a systematic screening for chronic complications in the earliest disease stages.
Clinical Trial Registration Number: NCT00879801, NCT01526720
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Disclosure: **M. Dauriz:** None.

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Compensatory protective effect of cardiotrophin-1 on hyperglycaemic crisis

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Background and aims: High glucose generates reactive oxygen species (ROS), and contributes to glucotoxicity in hepatocytes. Hyperglycemia causes functional changes of the liver to promote liver injury. However, only a mild hepatic dysfunction observed in hyperglycemic crisis, implying a factor that exerts a hepatic protective effect in hyperglycemic crisis. Cardiotrophin-1 (CT-1) is a novel cytokine that modulates insulin sensitivity, and also exerts a hepatic protective activity in non-alcoholic fatty liver diseases. However, the role of CT-1 in hyperglycemic crisis-induced hepatic dysfunction remains unknown. Thus, the aim of this study is to investigate the hepatic protection effects of CT-1 in hyperglycemic crisis.
Materials and methods: Plasma CT-1 levels, and routine biochemistry were measured in 29 patients with hyperglycemic crisis before and after standard treatments. The effects of CT-1 on hepatic functions were evaluated in streptozotocin-induced hyperglycemic mice (STZ mice). HepG2 cells were used to clarify the possible mechanisms in the regulation of CT-1 expression.
Results: Plasma CT-1 concentrations were significantly decreased in subjects with hyperglycemic crisis after standard treatment, in accompanied with improved hepatic functions. Correction of hyperglycemia in STZ mice decreased the hepatic CT-1 expression. Injection of recombinant CT-1 improved hepatic dysfunctions, and liver architecture in STZ mice with increased anti-oxidative proteins, including hepatic superoxide dismutase-1 (SOD1). Moreover, CT-1 increased SOD1 expression through a STAT3-dependent pathway to decrease methylglyoxal-induced ROS production, and improved cell viability.
Conclusion: These findings highlight the protective activity of CT-1 in hyperglycemic crisis.
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Disclosure: **C. Chang:** None.

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Impaired pulmonary function in type 2 diabetes: Is there a role for advanced glycation end-products measured by skin autofluorescence? Data form the ILERVAS project

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Background and aims: Tissues that are rich in collagen and elastin, such as the lung, can be target of non-enzymatic glycation. Therefore, the production of advanced glycation end-products (AGEs) would collaborate in the deterioration of lung function previously described in type 2 diabetes (T2D). Our aim was to assess the relation between AGEs and pulmonary function because little information exits in these patients.
Materials and methods: For this purpose, we designed a cross-sectional study including 747 subjects from the ILERVAS project (58.1±6.4 years; 51.3% men; 6.3% with T2D) without known pulmonary disease, who underwent a baseline spirometry (Datospir©). The ILERVAS project is a interventional prospective study evaluating subclinical atherosclerosis and hidden kidney disease in subjects without previous cardiovascular disease. Restrictive pattern was defined by GOLD's guidelines pattern as forced vital capacity (FVC) <80% and forced expiratory volume in the first second (FEV1)/FVC ratio >70%. Skin autofluorescence (AF), a non-invasive assessment of subcutaneous AGEs accumulation, was measured (AGE Reader; DiagnOptics Technologies, The Netherlands).
Results: Patients with T2D showed less FVC [77.0 (38.0-114.5) vs. 91.1 (31.0-154.0) % predicted, p<0.001], FEV1 [80.1 (28.0 to 124.5) vs. 90.1 (28.0 to 143) % predicted, p=0.001], and a higher percentage of restrictive pattern (44.7% vs. 19.5%, p<0.001) compared to non-diabetic subjects. In the entire population, subjects with a restrictive pulmonary pattern showed an increased skin AF compared with subjects with normal lung function (2.3±0.6 vs. 2.0±0.5 arbitrary units, p<0.001). This difference persisted when only patients with T2D were evaluated (3.0±0.6 vs. 2.6 ±0.7AU, p=0.048). Univariate analysis showed that skin AF negatively correlated with FVC (r=-0.233, p<0.001) and FEV1 (r=-0.213, p<0.001). A multivariate regression analysis showed that skin AF (together with age, gender, BMI and packs of cigarettes per year) independently predicted FVC (R^2=0.174) and FEV1 (R^2=0.183).
Conclusion: In conclusion, skin AF is correlated with impaired pulmonary function. This is the first clinical evidence that suggest the role of AGEs as a mechanism to explain the deleterious effect of T2D in lung function.
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Disclosure: **A. Lecube:** None.

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Antagonistic regulation of filtration barrier permeability by PKGIalpha-AMPK signalling

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Background and aims: The podocytes with their foot processes are important cellular layer of glomerular barrier involved in the regulation of glomerular permeability. Disturbance of podocytes function has a central role in the development of proteinuria in diabetic nephropathy. Retraction of the podocyte foot processes forming slit diaphragm is a common feature of proteinuria. The correlation of retraction with the development of proteinuria in not well understood. Protein kinase G type I alpha (PKGIα) is an intracellular target for vasorelaxant factors. We showed the relationship between oxidative stress, PKGIα activation, actin reorganization and changes in permeability to albumin across the filtration layer. AMPK-activated protein kinase (AMPK) is central in controlling the metabolism of glucose and fatty acid, its role in obesity and type 2 diabetes is major importance. AMPK also plays an important role in modulating of cell polarity, permeability and cytoskeleton reorganization. Here we investigated the interplay between PKGIα and AMPK signaling in cultured rat podocytes and its influence on filtration barrier permeability.
Materials and methods: We measured glomerular capillary permeability to albumin in isolated glomeruli from Wistar rats and transmembrane albumin flux in cultured rat podocytes. We examined the mutual interaction between AMPK and PKGIα activities and their influence on podocytes permeability by knocking down PKGIα and AMPK with small interference RNA (siRNA). The PKGIα-AMPK interaction was confirmed by co-immunoprecipitation and immunofluorescence. All experimental procedures were conducted in accordance with directive 2010/63/EU.
Results: Glomerular permeability to albumin and transmembrane albumin flux were decreased in the presence of AMPK activators (metformin, AICAR) or increased in the presence of PKGI activators (8-Br-cGMP, hydrogen peroxide). We demonstrated that PKGIα and AMPK mutually regulated in podocytes. We observed that PKGIα protein co-immunoprecipitated with AMPKα1 and AMPKα2 proteins. Using siRNA directed against PKGIα we also observed the activation of AMPK-dependent signaling pathways. We showed that decrease of PKGIα expression (about 56%) induced increase in basal levels of phosphorylated AMPK (from 0.536±0.012 to 0.821±0.048, n=4, P<0.05) and Acetyl-CoA carboxylase (ACC, from 0.307±0.028 to 0.635±0.032, n=4, P<0.05). The same effect was observed in the reverse procedure. Using siRNA against AMPKα2 (expression decreased by about 40%) we observed increase in basal level of phosphorylated myosin phosphatase target subunit 1 (MYPT1, from 0.160±0.001 to 0.416±0.077, n=3, P<0.05) and decrease in the phosphorylated myosin light chain 2 (MLC2, from 0.644 ±0,018 to 0.545±0.014, n=4, P<0.05). Moreover decrease of AMPKα1 expression by siRNA did not influence PKGIα activity.

Conclusion: Thus we propose that interplay between PKGIα and AMPKα2 activity regulate contraction apparatus and permeability to albumin in podocytes. Overall we have identified a potentially important new mechanism that may be injurious during diabetes in podocytes and affect filtration barrier permeability.
Supported by: grant from the NSC (2014/14/E/NZ4/00358).
Disclosure: **A. Piwkowska:** None.

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TRPC6 regulates the insulin effect on podocyte cytoskeleton dynamics

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Background and aims: Podocytes are cells uniquely sensitive to insulin, demonstrating similarities to skeletal muscle and adipocytes with respect to insulin stimulated glucose uptake kinetics and the expression of glucose transporters (GLUTs). Recent studies have shown that insulin could dynamically remodel the actin cytoskeleton of podocytes, what was critically important in maintenance of the glomerular filtration barrier integrity. Rho family GTPases, dynamic modulators of the actin cytoskeleton, are expressed in cells forming glomerular filtration barrier. Thus changes of Rho GTPases activity may affect glomerular permeability to albumin, leading to albuminuria/proteinuria, a sign of kidney disease and important risk factor of the renal failure progression. Cumulative evidence has suggested that podocyte function and insulin signaling are central to the development of diabetic nephropathy, but the underlying mechanism is unclear. In addition, accumulating evidence suggests that TRPC6 channels are crucial mediators of podocyte calcium handling, involved in the regulation of glomerular filtration. Here we investigated whether TRPC6 is involved in insulin-dependent regulation of cytoskeleton dynamic in cultured rat podocytes.
Materials and methods: We assessed insulin-induced changes in glomerular permeability by measuring albumin flux in cultured rat podocyte. Expression and phosphorylation of proteins associated with actin cytoskeleton reorganization (PAK, Rac1/cdc42, cofilin) were confirmed in the podocytes using Western Blotting and immunofluorescence. F-actin network was labeled and visualized by fluorescence microscopy. All experiments were conducted in accordance with Directive 2010/63/EU and were approved by the Local Ethics Committee.
Results: Insulin induced the activation of proteins associated with actin cytoskeleton reorganization. We observed that insulin increased phosphorylation of PAK by about 46% (from 0.879±0.018 to 1.280±0.102, n=4, $P<0.05$) and decreased phosphorylation of cofilin (S71) by about 28% (from 1.998±0.106 to 1.450±0.033, n=4, $P<0.05$). We found that TRPC channel inhibitor SKF96365 or siRNA knockdown of TRPC6 abolished this effects and attenuated insulin-induced rearrangement of the actin cytoskeleton.
Conclusion: Taken together, our data suggest a key role of TRPC6 channels in the mediation of insulin-dependent regulation of actin dynamics in podocytes and identify a potential mechanism explaining disturbances in filtration barrier permeability in diabetes.
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Disclosure: **D. Rogacka:** None.

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Diabetes induced changes in circulating and kidney mitochondrial DNA: a potential novel pathway of renal damage

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Background and aims: We recently showed that circulating mitochondrial DNA (MtDNA) levels are an independent risk marker in patients with diabetic nephropathy (DN) and proposed that diabetes induced early systemic changes in MtDNA may be involved in DN. To test this hypothesis, in the current study we investigated the early effect of diabetes on circulating and renal MtDNA levels in the Streptozotocin (STZ) induced diabetic mouse model.
Materials and methods: C57Bl/6 mice were defined as diabetic based on blood glucose levels of >20mM 4 days post STZ injection. Blood samples were obtained from control and diabetic mice after 1 week and 4 week of diabetes and kidney tissue after 4 weeks. Diabetic mice were cured using islet transplantation and subsequently cured mice were rendered diabetic by the removal of the islet graft to examine the effect of reversion to diabetes, and blood samples from cured and reverted mice were obtained. Total DNA was isolated from blood/tissue samples and absolute MtDNA copy numbers were measured using real time qPCR.
Results: MtDNA levels were significantly increased in the blood after 1 week and remained elevated after 4 weeks of diabetes, however kidneys from 4 week diabetic mice showed decreased MtDNA (n=3-6, $P<0.05$). To establish if the increased circulating MtDNA was a direct consequence of the diabetes, diabetic mice were treated by islet transplantation and this reduced their elevated circulating MtDNA to normal levels. Subsequent reversion to diabetes by the removal of the islet graft was accompanied by a concomitant increase in circulating MtDNA.
Conclusion: These data show that diabetes can directly lead to increased circulating MtDNA and that this increase can be normalised by correcting blood glucose levels. The loss of renal MtDNA in diabetic mice suggests that the increase circulating MtDNA may have originated from organs. Diabetes induced loss of renal MtDNA indicates loss of renal mitochondria which as an early event may predispose the diabetic kidney to bioenergetic deficit and contribute to the risk of DN. Furthermore, since MtDNA can act as an inflammatory molecule, the demonstration of diabetes induced increase in circulating MtDNA could explain the chronic inflammation often seen in diabetes patients. These data suggest that diabetes induced MtDNA changes may play important roles in the risk of diabetic complications and need further investigation.
Supported by: EFSD/Janssen
Disclosure: **A.N. Malik:** None.

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The AGEs inhibitor pyridoxamine prevents kidney injury and dysfunction in mice fed high-fat high-fructose diet

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Background and aims: Recent evidence suggests a key role of the local accumulation of diet-derived Advanced Glycation End-Products (AGEs) in evoking kidney injury/dysfunction associated with chronic exposure to hypercaloric diets. Pyridoxamine, a structural analog of vitamin B6 that exerts anti-glycative effect by interfering with oxidative macromolecular damage, is now in phase 3 clinical efficacy trial to delay chronic kidney disease progression in patients with type 1 diabetes. However, so far, the potential beneficial renoprotective effects of pyridoxamine in type 2 diabetes and obesity have not yet been investigated. Thus, we aimed to study the role of dietary supplementation with piridoxamine as preventive strategy to counteract the deleterious renal effects evoked by hypercaloric diet in mice.
Materials and methods: C57Bl/6J mice were fed a standard diet (SD, n = 16) or a diet enriched in fat (40%) and fructose (45%) (HFHF, n=16) for

12 weeks. At week 3, two subgroups of SD and HFHF mice started to receive pyridoxamine supplementation (150 mg/kg/day) in the drinking water. At week 12, mice were sacrificed and urine, plasma and kidneys were collected for Western blot, ELISA, histological and immunohistochemical analysis.

Results: When compared to SD mice, HFHF fed mice showed increased body weight (25,2±1,1 vs. 33,7±2,1 g, p<0,001) and impaired glucose homeostasis (fasting glycaemia 72,80±18,89 vs. 138,80±12,68 mg/dL, p<0.001). Pyridoxamine administration significantly improved fasting glycaemia (104,60±9,71 mg/dL, p<0,05) but not body weight (34,7±3,5 g). Renal function was strongly impaired by hyper-caloric supplement (serum creatinine 0.70±0.06 vs 1.13±0.15 mg/dL p<0.05; urine albumin 74.44±14.18 vs 265.07±15.43 μg/mL, p<0.001) and, most notably, pyridoxamine significantly prevented the renal function derangements (serum creatinine 0.66±0.03, p<0.05 vs HFHF; urine albumin 195.28±34.68 μg/mL, p<0.05 vs HFHF). Kidney morphology of HFHF fed mice presented strong vacuolar degeneration and loss of tubule brush border, both clearly attenuated by pyridoxamine administration. The HFHF-induced morphological and functional derangements were associated with a robust increase in the local expression of AGEs receptor (RAGE) and pro-fibrogenic markers (fibronectin, vimentin, SMAD2/3) as well as a significant activation of the pro-inflammatory NF-kB and Rho/ROCK signaling pathways. Interestingly, piridoxamine prevented the diet-induced overexpression of RAGE and pro-fibrogenic markers as well as the activation of the inflammatory signaling cascades.

Conclusion: The present study demonstrated for the first time that the administration of the anti-glycative compound pyridoxamine counteracted diet-dependent kidney injury and dysfunction by interfering with local AGEs accumulation, thus resulting in reduced activation of the pro-fibrotic and inflammatory cascades.

Disclosure: **M. Collino:** None.

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Arteriell stiffness and progression of cerebral white matter lesions in asymptomatic patients with type 2 diabetes and healthy controls: a 5-year cohort study

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Background and aims: Stroke is a frequent and feared complication in patients with type 2 diabetes. Arterial stiffness may improve current suboptimal risk prediction in this patient group, however, studies in diabetes populations are lacking. We aimed to determine the association between arterial stiffness and the progression of cerebral white matter lesions (WML), a marker of stroke risk, in patients with type 2 diabetes and healthy controls. We tested the a priori hypothesis that progression of arterial stiffness is associated with progression of WMLs.

Materials and methods: We conducted a 5-year cohort study. Observers were blinded to patient status. Arterial stiffness and WMLs were assessed at both baseline and follow-up visits. Arterial stiffness (carotid-femoral pulse wave velocity [PWV]) was obtained by applanation tonometry. WMLs were assessed by cerebral T2-FLAIR MRI and quantified by Breteler score. WML progression was defined as an upward change in category during follow-up. Data from 49 patients newly diagnosed with type 2 diabetes and no previous history of cerebrovascular disease and 58 age- and sex-matched controls were available for analysis. At baseline, participants had a mean (±SD) age of 59±10 years and patients had a median (range) diabetes duration of 1.8 (1.0-3.2) years. Fifty-two (49%) were males.

Results: Patients with type 2 diabetes had a higher PWV than controls at both baseline (9.2±2.2 vs. 7.9±1.4m/s, p<0.01) and follow-up (9.8±2.4 vs. 8.6±1.9m/s, p=0.01). Breteler scores and WML progression were similar in the two groups (p>0.05). PWV progression was associated with WML progression in the total cohort (adjusted for age, sex, diabetes, baseline PWV and systolic blood pressure progression: OR 1.58 per 1m/s [95%CI: 1.09-2.28], p=0.02). We found no interaction between diabetes and PWV progression on WML progression.

Conclusion: PWV progression is associated with WML progression in patients with type 2 diabetes and healthy controls. PWV candidates as a new risk marker for stroke.

Carotid-femoral pulse wave progression and WML progression

WML progression OR per 1m/s increase in PWV (95% CI)						
	All (n=107)	p	DM (n=49)	p	Controls (n=58)	p
Crude model	1.51 (1.07-2.12)	0.02	1.27 (0.84-1.93)	0.26	1.95 (1.20-3.18)	0.01
Adjusted model [a]	1.58 (1.09-2.28)	0.02	2.02 (1.15-3.54)	0.01	1.83 (1.14-2.95)	0.01

[a]Adjusted model: age, sex, diabetes status (yes/no), progression in ambulatory systolic BP and baseline PWV.
WML = Cerebral white matter lesions.
PWV = Pulse wave velocity.

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Association of mannose-binding lectin levels with progression in carotid artery intima-media thickness in type 2 diabetic patients: a 7-year follow-up

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Background and aims: Mannose-binding lectin (MBL), a weak acute phase reactant protein activates the "lectin" pathway of complement system independently of the antibodies and plays an important role in the innate immunity. It has been suggested that MBL may be involved in the pathogenesis of micro- and macrovascular complications of diabetes and MBL levels may be used as a prognostic marker for these complications. Only a few data about the role of MBL in the development of subclinical atherosclerosis (carotid artery intima-media thickness - IMT) in diabetes mellitus were reported. According to these results low MBL levels enhance the progression in IMT, while high MBL levels have protective role. Our aim was to analyse a possible association between serum MBL levels and changes in IMT after a 7-year follow-up in Type 2 diabetic (T2DM) patients.

Materials and methods: Serum MBL levels (ng/ml) and IMT (mm) were measured in a total of 106 Type 2 diabetic patients (56 male and 50 female). Regarding stability of MBL serum levels they were determined once at the end of the study with a sandwich ELISA (BioPorto Diagnostics). IMT was measured at the beginning of the study and after a 7-year follow-up. It was carried out on both sides using 2D echo. Data were collected and analysed statistically using repeated measures ANOVA with Newman Keuls post-hoc tests.

Results: Progression in IMT was significantly higher in patients with absolute MBL deficiency (MBL < 100 ng/ml, n=13, ΔIMT: 0.109 ± 0.143 mm, p < 0.001) compared to those above this threshold (MBL ≥ 100 ng/ml, n=93, ΔIMT: 0.039 ± 0.134 mm, p = 0.15). Taking into consideration that thiazolidine-dions (TZD) may decrease IMT, calculations were also performed after exclusion of patients having been treated with TZD agents. In this setting progression in IMT was significant in

both groups but, with much stronger elevation in MBL deficient group (n=11, ΔIMT: 0.138 ± 0.103 mm, p < 0.001) compared to normal and elevated MBL levels group (n=67, ΔIMT: 0.039 ± 0.134 mm, p = 0.03). We also evaluated changes in IMT according to the initial IMT values. We could observe progression in patients with MBL < 100 ng/ml in both IMT < 0.8 mm and IMT ≥ 0.8 mm groups (n=8, ΔIMT: 0.122 ± 0.099 mm, p < 0.01 and n=3, ΔIMT: 0.184 ± 0.121 mm, p < 0.001, respectively). However, patients in the group of MBL levels ≥ 100 ng/ml neither with initial IMT < 0.8 mm (n=50, ΔIMT: 0.099 ± 0.106 mm, p=0.06), nor with initial IMT ≥ 0.8 mm (n=17, ΔIMT: -0.047 ± 0.146 mm, p=0.4) showed significant progression. There was no significant difference in HbA1c and lipid parameters of patients and all patients were treated with ASA 100 mg/day.

Conclusion: Our study shows that low MBL levels partially depending on the initial IMT values increase the progression of IMT in T2DM during a 7-year follow-up and MBL probably may be one of the markers for subclinical atherosclerosis.

Supported by: Hungarian Diabetes Association

Disclosure: **M. Kaplar:** None.

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Protective effect of SOCS1-based therapy against renal and vascular oxidative stress in diabetic mice

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Background and aims: Oxidative stress resulting from excessive production of reactive oxygen species (ROS) or impaired antioxidant defenses is a key pathogenic factor in diabetic complications, including nephropathy and atherosclerosis. Chronic activation of Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) signaling pathway contributes to diabetic complications by inducing genes involved in cell proliferation, fibrosis, inflammation and oxidative stress. Suppressors of cytokine signaling (SOCS) family controls both magnitude and duration of JAK/STAT pathway and has emerged as a new target for therapeutic intervention. This study investigates the beneficial effect of SOCS1-based therapies to combat oxidative stress and tissue injury in a mouse model of diabetes.

Materials and methods: Streptozotocin-induced diabetic apolipoprotein E-deficient mice received either a single dose of recombinant adenovirus expressing SOCS1 ($1x10^{12}$ viral particles/kg, i.v.) or 3 weekly injections of SOCS1 peptidomimetic (3 mg/kg/day, i.p.). After 6-10 weeks of intervention, kidney, aorta, serum and urine samples were used to evaluate clinical parameters, oxidative stress markers, morphology, and gene expression.

Results: Diabetes induction in mice resulted in a progressive alteration of the redox balance, as demonstrated by increased ROS levels and decreased antioxidant activity, which ultimately led to renal dysfunction and vascular injury. Interestingly, these molecular and pathological alterations in diabetic mice were partially reversed by SOCS1 gene transfer and, more efficiently, by SOCS1 peptidomimetic. Compared with untreated controls, kidney and aorta from mice treated with SOCS1 peptidomimetic exhibited significantly lower levels of superoxide anion (% vs control: 59±4% and 64±4%, respectively), DNA oxidation marker (44±6% and 58±8%) and NADPH oxidase subunits (Nox1, 41±6% and 36 ±3%; Nox4, 47±4% and 33±7%), along with higher levels of antioxidant genes (heme oxygenase-1, 197±22% and 238±46%; superoxide dismutase, 244±37% and 254±37%; catalase, 150 ±28% and 239±51%) and urinary total antioxidant capacity (183 ±10%). These trends remained throughout the course of the treatment period and correlated with a reduction in parameters of renal dysfunction (albuminuria, mesangial expansion and fibrosis), atherosclerosis (lesion size and lipid content) and inflammation (leukocyte infiltration and chemokine expression). In cultures of renal tubuloepithelial cells, macrophages and vascular smooth muscle cells, SOCS1 attenuated ROS generation induced by cytokines and/or high-glucose. This effect was associated with a decrease in NADPH oxidase activity and expression of catalytic (Nox1, Nox2 and Nox4) and regulatory (NoxA1 and NoxO1) subunits, and was mediated by both STAT1- and PI3K-dependent mechanisms.

Conclusion: SOCS1-based therapies have a protective role against increased oxidative stress under chronic hyperglycemia, by altering the expression of enzymes associated with the induction and resolution of oxidative stress in diabetic mice.

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Disclosure: **L. Lopez-Sanz:** None.

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Novel insights into the protective role of Nrf2 activation in diabetes-associated atherosclerosis

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Background and aims: The intertwined relationship between metabolism, oxidative stress and pro-inflammatory events in the vascular system is determinant in the pathogenesis of diabetic cardiovascular disease. Therefore, novel approaches to limit vascular inflammation and to restore redox balance are of clinical interest. Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a stress-sensitive transcriptional regulator of genes involved in inflammatory and oxidative stress responses, and has emerged as a promising therapeutic target. This study investigates the underlying mechanisms of the protective effect of Nrf2 induction in an experimental model of diabetes-associated atherosclerosis.
Materials and methods: Streptozotocin-induced diabetic apolipoprotein E-deficient mice were randomized to receive 3 weekly *i.p.* injections of the Nrf2 activator tert-butyl hydroquinone (tBHQ, 50 mg/kg/day) or vehicle. After 6 weeks of treatment, we evaluated atherosclerotic lesions (size and composition) in the aorta and the expression levels of markers for inflammation, redox balance and autophagy.
Results: Administration of tBHQ effectively increased the number of Nrf2-activated cells within the atherosclerotic lesions of mice, colocalizing with both macrophages and vascular smooth muscle cells. Nrf2 induction by tBHQ treatment resulted in a significant decrease in the size and extension of atherosclerotic lesions in diabetic mouse aorta (% reduction *vs* vehicle: 39±9) and also reduced the lipid content of plaques, without affecting hyperglycemia and serum lipid profile. Nrf2 activator attenuated atherosclerotic plaque inflammation in diabetic mice, by reducing total macrophage content (23±6%), M1/M2 phenotype balance (44±9%), foam cell size (34±4%) and chemokine gene expression (*Ccl2*, 76±6%; *Ccl5*, 72 ±6%). Atheroprotection was accompanied by systemic and local antioxidant effects, characterized by lower levels of oxidative stress markers (8-hydroxydeoxyguanine and superoxide anion) and higher levels of systemic total antioxidant capacity and antioxidant genes (heme oxygenase-1, 346 ±47%; superoxide dismutase, 307±43%; catalase, 246±29% *vs* vehicle). Interestingly, tBHQ treatment upregulated autophagy-related gene (*Atg5, Beclin1, Atg7, Lc3* and *p62*) and protein (LC3-II, 184±10%; p62, 193 ±15%) expression in aorta and liver of diabetic mice. A parallel *in vitro* study confirmed that tBHQ induced autophagy in vascular smooth muscle cells under both starving and normal serum culture conditions.
Conclusion: Nrf2 induction is a promising therapeutic approach needed to tackle diabetes-driven vascular damage, according to its protective effects on inflammation, oxidative stress and autophagy.
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Disclosure: **I. Lazaro:** None.

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Carnosinase inhibition by thiol-containing compounds

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Background and aims: Carnosinase 1 (CN1) activity is up-regulated under diabetic conditions by post-translational modifications. Supplementation of diabetic mice with carnosine mitigates diabetic complications. The cysteine residue at position 102, but not at position 229, regulates CN1 activity. We now tested whether thiol-containing compounds interact with CN1 cysteine residue at position 102 by cysteinylation and modify CN1 activity and therefore cytoprotective carnosine levels.
Materials and methods: The dynamic behavior of CN1 before and after addition of cysteine was examined by characterizing enzyme kinetics of the recombinant and renal enzyme and by using molecular dynamic (MD) simulations.
Results: Recombinant CN1 (rCN1) efficiency for carnosine degradation (5.2±0.2 μmol/mg/h/mM) was reduced by addition of thiol-containing compounds, such as reduced glutathione (3.2±0.4 μmol/mg/h/mM; p=0.05), cysteine (1.6±0.2 μmol/mg/h/mM; p=0.05) and N-acetylcysteine (2.0±0.3 μmol/mg/h/mM; p=0.05) whereas L-glutamic acid, glycine and GSSG (oxidized glutathione) had no effect on recombinant CN1 activity. Increased renal CN1 activity in diabetic (db/db) mice was reduced and normalized by adding GSH to renal tissue samples in a concentration-dependent manner. Substitution of rCN1 cysteine residues at position 102 (Mut 1^{C102S}) and 229 (Mut 2^{C229S}) showed that only cysteine at position 102 is mandatory for regulation of CN1 activity by thiols. Whereas Mut 1^{C102S} was not influenced, the addition of thiols to rCN1 Mut 2^{C229S} lowered catalytic efficiency (3.4±0.5 μmol/mg/h/mM; p=0.05 compared to control), indicating that the inhibitory effect is due to S-cysteinylation of cysteine at position 102 but not at position 229. MD simulation confirmed a conformational rearrangement of the negatively charged residues surrounding the zinc ions by S-cysteinylation of cysteine 102 causing a partial shift of the carnosine ammonium head with the consequent distancing of the carbonyl group, resulting in a less effective pose of the substrate within the catalytic cavity and a decrease in CN1 activity.
Conclusion: We provide evidence for a novel mechanism of CN1 regulation. Kinetic parameters and MD simulations revealed that inhibition by thiol-containing compounds is due to allosteric interactions through S-cysteinylation. Inhibition of circulating and tissue CN1, resulting in higher carnosine levels, may represent a valuable therapeutic strategy for mitigation of complications associated with diseases such as diabetes mellitus.
Supported by: SFB 1118 (DDG)
Disclosure: **T. Weigand:** None.

1102

Loss of glyoxalase 1 in various mammalian cells is associated with an increased resistance against environmental stressors

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Background and aims: In contrast to other enzymes involved in the detoxification of xenobiotics, the glyoxalase system consisting of glyoxalase 1 and 2 (GLO1 and 2) has an extremely limited substrate specificity. In fact it is mainly responsible for the detoxification of methylglyoxal (MG), a toxic dicarbonyl compound formed spontaneously during glycolysis. Given this background MG has gained a lot of attention within the context of dicarbonyl induced damage under hyperglycemic conditions such as diabetes. Therefore since decades the glyoxalase system was meant to be indispensable to maintain cellular viability in eukaryotic cells. We have established three mammalian cell lines with a total loss of GLO1 to proof this hypothesis. Major aim of this approach was to look at metabolic side effects in those three *in vitro* model systems regarding cellular viability and resistance against xenobiotics.

Materials and methods: GLO1 knock-out in murine cardiac endothelial, kidney mesangial and neuronal Schwann cells was established using the CRISPR-Cas9 technique. Intracellular levels of MG and MG-modifications were measured by MS. The LD_{50} for various xenobiotics and UV-radiation was determined using MTT assay. Enzyme kinetics and total GSH/GSSG content were analyzed spectrophotometrically. Protein concentration and mRNA content were assesed using western blot and qPCR. All data are expressed as mean values ± standard error and were analyzed for significance using unpaired t-test with *Welch`s* correction. Differences were considered to be significant at $p<0.05$.
Results: The loss of GLO 1 was neither linked to increased levels of MG nor MG-modified proteins. The mRNA and protein expression in three different $GLO1^{-/-}$ cell models revealed an upregulation of several subtypes of aldo-keto-reductase (AKR) and aldehyde dehydrogenase (ALDH). Kinetic profiles for the upregulated enzymes present in the cytosolic fractions of $GLO1^{-/-}$ cells revealed that those oxidoreductases were able to detoxify formaldehyde, 4-hydroxynonenal, malondialdehyde and acrolein with higher efficiency compared to wild-type (WT) cells. This was confirmed by increased LD_{50}-values for those xenobiotics, hydrogen peroxide and ultraviolet radiation in a toxicity assay. Additionally $GLO1^{-/-}$ cells showed significantly improved ratios of GSH to GSSG compared to appropriate WT cells.
Conclusion: The loss of GLO1 in three mammalian *in vitro* models was compensated effectively regarding the MG metabolism, even under high glucose growth conditions. The upregulation of various oxidoreductases (AKR and ALDH) in $GLO1^{-/-}$ was associated with increased resistance against harmful environmental stressors such as ultraviolet radiation and exposure to hydrogen peroxide or several xenobiotics. Furthermore, the improved ratio of GSH to GSSG in $GLO1^{-/-}$ reflects an optimization in the potential oxidative stress response. Consequently we claim the intriguing hypothesis that the loss of GLO1 has protective character due to the upregulation of various enzymes of the xenobiotic defense metabolism. Further *in vivo* studies are required to prove the concept if GLO1 is fundamentally necessary for life or just a remnant of evolution.
Supported by: SFB1118 (DFG)
Disclosure: **J. Morgenstern:** Grants; SFB1118.

1103

Perivascular adipose tissue and endothelial dysfunction in an aged model of obesity

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Background and aims: Obesity is a growing problem worldwide. In this context, perivascular adipose tissue (PAT) has recently been recognized as a novel factor in vascular biology, with implications in the pathophysiology of cardiovascular disease. The main goal of this study was to investigate the effects of high-fat diet on PAT and study its impact on endothelial function of mesenteric arteries of obese animal models.
Materials and methods: Eight-month-old male Wistar (W) rats were randomly divided in two subgroups: group 1) W control group fed with standard diet (W12m); group 2) W rats fed with high fat diet during 4 months (WHF) and compared with six-month-old male W rats (W6m). Glucose, lipids, leptin, adiponectin and HbA_{1c} concentrations were measured on blood samples. Vascular contraction and functional endothelial-dependent and independent vasorelaxation was evaluated in isolated mesenteric ring preparations from the different groups. Oxidative stress, inflammatory biomarkers, and adipocytokines were also evaluated in arteries and PAT of the different groups of rats.
Results: High fat diet induced significantly increased body weight, glucose at 2h after a glucose load and systemic levels of free fatty acids, leptin and leptin/adiponectin ratio. It also significantly reduced the efficacy of NO-dependent and independent vasorelaxation (by 60 and 22%, respectively) in mesenteric arteries accompanied by 2-fold increment in vascular oxidative stress. In WHF group, PAT significantly increased the expression of the receptor for advanced glycation end products (RAGE), intercellular adhesion molecule-1 (ICAM-1), chemokines (as monocyte chemotactic protein-1/CCL2, RANTES/CCL5) and pro-inflammatory adipokines (as resistin, leptin and lipocalin-2) compared to W6m, probably important contributors of endothelial dysfunction.
Conclusion: Inflammation in PAT directly impacts vascular disease of the underlying artery, perhaps contributing to the endothelial dysfunction underlying atherosclerosis.
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Disclosure: **A.R. Costa:** None.

1104

Position of oxidative stress and microcirculation parameters in artificial neuronal network for assessment of chance for type 2 diabetes development

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Background and aims: Prevalence of type 2 diabetes (T2D) is on the rise that explains search of approaches to diagnosis and prophylaxis of disease. Oxidative stress (OS) and microcirculation (MC) firstly exhibit changes during deviations of glycaemia. Study changes in conjunctival MC and OS parameters in patients with T2D to create new configuration of artificial neuronal network (ANN).
Materials and methods: We included 155 patients and 41 almost healthy person and divided into 4 groups: 1 - almost healthy, 2 - overweight and obesity I st. (56 patients), 3 - prediabetes (59 patients), 4- T2D (40 patients). Computer-assisted intravital conjunctival microscopy was used to evaluate 20 parameters of MC. Activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP), glutathione reductase (GR) and concentrations of oxidized glutathione (GSSG), reduced glutathione (GSH) and thiobarbituric acid reactive substances (TARS) and total antioxidant activity (tAOA) were assessed as markers of OS. All patients underwent biochemical tests for HbA1c, lipid profile, GFR was calculated and eyegrounds examination was conducted. New configuration of ANN was created and included 38 input values and 5 output values.
Results: MC changes in study groups were identical some way compared to control group and included increased count of arteriolo-venular anastomosis (AVA), enhanced meandric tortuosity of venules ($Mean_v$) ($p<0,05$). Additionally identical for prediabetes and T2D arteriolo-venular diameter ratio (AVD) was decreased and tela was lost (Net) compared to control group ($p<0,05$). In T2D to all listed above changes we revealed enhanced meandrous tortuosity of capillaries ($Mean_c$) and inequalities in vessels caliber (Cal) ($p<0,05$ compared to other groups). Markers of OS were changed on case-by-case basis in groups. tAOA was increased in patients with prediabetes (69,48 (59,09;85,93)% vs 62,24 (47,60;71,39)% in patients with T2D ($p<0,05$). Overweight and obesity were associated with increased GSSG concentrations (0,36 (0,31;0,39) mmol/l vs 0,33 (0,31;0,37) mmol/l). T2D was characterized by decreased activity of SOD (65,24 (30,55; 89,47) RVU/ml vs 86,43 (36,49;130,36 RVU/ml in controls ($p<0,05$) and decreased GSH concentration (2,08 (2,08;2,92 mmol/l vs (2,53 (2,29;3,15) mmol/l ($p<0,05$)). According to estimated changes in MC and OS parameters and including other risk factors for T2D and microangiopathy development we created new configuration of ANN that showed high values of included parameters. During education of ANN several deviations were revealed. Detailed analyze of this patients (deviations) disclosed changes in MC and OS specific to T2D.

Conclusion: We observed increasing changes in microcirculation from overweight and obesity to prediabetes and diabetes and different changes of OS parameters in study groups. ANN can optimize work with large count of parameters and reveal patients with increased risk for T2D development. This can be done by receiving results from already educated ANN.
Disclosure: **V.M. Shyshko:** None.

1105

Glycaemic variability is associated with oxidative damage independently from glycaemic control in type 1 diabetes

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Background and aims: Oxidative stress contributes in diabetic organ damage. Oxysterols as indicators of oxidative stress have been shown to play critical roles with cytotoxic and proinflammatory actions in the course of Diabetes Mellitus. Currently the impact of glycemic variability on oxidative stress whether dependently or independently on glycemic control is a controversial issue. We aimed to investigate an association between oxysterol levels and glycemic variability as well as glycemic control in type 1 diabetic patients.
Materials and methods: Thirty adult patients with Type 1 diabetes mellitus (T1DM), (28 K, 2 E, mean age 32.4 ± 10.1 years, median 13 years, HbA1c mean: 8.1 ± 1.2%) who were on insulin pump were included in the study. The mean amplitude of glucose excursion (MAGE) of the second day, as a sign of intraday glycemic variability, and the mean of daily differences (MODD), as a sign of interday glycemic variability (consecutive 3 days) calculated by using72-hour Continuous Glucose Monitoring System (cGMS) data. Serum samples for oxysterols (7-ketosterol, cholestane-3β, 5α, 6β-triol (cholestanetriol))measurements were taken from patients at 0, 24 and 72 hours of cGMS measurements. Quantification of oxysterols were accomplished using high sensitivity and specific LC-MS / MS (tandem-mass spectrometry) method.
Results: There was a significant correlation between MAGE of the second day (24-48. hour, mean: 112.3 ± 57.6 mg / dl) and 7-ketosterol and 7-ketosterol/total cholesterol levels of the subsequent day (72nd hour) (r:0,558, p:0.001; r:0.468; p:0.009). There was no significant correlation between MODD and oxysterol levels. There was no significant correlation between HbA1c and oxysterol levels.
Conclusion: This study with continous glucose monitoring system data of the patients with advanced T1DM on insulin pump, showed that intraday glycemic variability is associated with oxidative stress independently of glycemic control. Accordingly, while attempting to provide glycemic control in these patients, insulinization strategies avoiding glycemic variability should be preferred
Supported by: Hacettepe University, Research Funding
Disclosure: **S. Dagdelen:** None.

1106

What predicts early glycaemic control in people with type 2 diabetes?

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Background and aims: It has been demonstrated that early glycaemic control substantially reduces the risk of microvascular complications in type 2 diabetes (T2D) and may also impact on subsequent macrovascular risk. Despite the evidence of the relationship between optimal glucose control and reduction in cardiovascular risks, a significant proportion of those with T2D fail to achieve early good glycaemic control. It is not clear which patient characteristics are associated with failure to achieve early glycaemic improvement. We evaluated the association between a range of patient characteristics and early glycaemic change.
Materials and methods: We performed a retrospective cohort analysis using a primary care sentinel network in England (Royal College of General Practitioners Research and Surveillance Centre). We identified people with new onset (incident) T2D between 1st January 2006 and 31st July 2016 and both an HbA1c measurement at the time of diagnosis and one year after diagnosis. We performed a linear regression analysis to identify factors associated with the change in HbA1c value from diagnosis to one year. Factors included for analysis were; HbA1c at diagnosis, patient age, gender, ethnicity, socioeconomic status, smoking status, body mass index (BMI), comorbidities, and medication use within the first year. Socioeconomic status was measured using the official national measure; index of multiple deprivation (IMD). Associations are reported as beta coefficients (mmol/mol change in HbA1c per unit change in model factor) with associated 95% confidence intervals (CI) and p values.
Results: From 72,910 people with T2D we identified 13,873 people with incident T2D and HbA1c measurement at diagnosis and one year post diagnosis. The mean HbA1c was 62.4 (SD 23.3) mmol/mol at diagnosis, 51.9 (SD 13.5) mmol/mol at one year. The mean change in HbA1c was -12.9 (SD 22.3) mmol/mol. Greater reduction in HbA1c was seen in those with higher HbA1c at diagnosis (-0.84; 95% CI -0.80 to -0.88; p=0.000) and increasing age (-0.13; -0.02 to -0.23; p=0.020). Compared to those of White ethnicity people of Asian ethnicity had less reduction in HbA1c at one year (difference 5.59; 9.88 to 1.30; p=0.011). Gender, socioeconomic status, smoking status, BMI, and co-morbidities did not significantly affect the change in glycaemic control. Insulin use within the first year was associated with the greatest reduction in HbA1c (-4.97; -1.36 to -8.59; p=0.007). The model accounted for a moderate proportion of the variation seen in glycaemic change (multiple R-squared = 0.650).
Conclusion: A large variation in glycaemic improvement within the first year of diagnosis can be seen in practice. Those who are younger or of Asian ethnicity have a smaller reduction in HbA1c after adjusting for initial glycaemic control. Whilst we have not determined the reasons for these differences these data suggest these groups should be targeted for more intense monitoring and aggressive intervention. The most effective treatment for early glycaemic control was insulin.
Supported by: Real World Evidence diabetes projects funded by Eli Lilly and Company
Disclosure: **A.P. McGovern:** Other; University of Surrey-Eli Lilly and Company Real World Evidence (RWE) Centre in Diabetes.

1107

The ratio of glycated albumin to HbA_{1c} as a haemoglobin glycation index with intra-familial correlations

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Background and aims: The mean value of glycated albumin (GA)/HbA_{1C} (A1C) ratio over time was reported to be individually consistent and highly inversely correlated with the glycation gap (g-g) as a hemoglobin glycation index (HGI) in pediatric type 1 diabetes patients (T1D). This study aimed to clarify that GA/A1C ratio was also a HGI in non-diabetic subjects and had intra-familial correlations.
Materials and methods: The subjects in this cohort from 2008 to 2012 were Japanese children and adolescents with TID (n=544) and their non-diabetic family (NDF) (n=433). Mean value of GA/A1C ratio using simultaneous measurement of GA and A1C more than five times was calculated in each diabetic patient and the value at enrollment was used in non-diabetic family. Since GA/A1C ratio showed different results due

to a constituent as each A1C using the National Glycohemogobin Standardization Program (NGSP) unit, the International Federation of Clinical Chemistry (IFCC) unit and the KO500 unit as the sustainable method in Japan Diabetes Society, the analyses using each A1C unit were as follows. Correlations between GA and A1C and between GA/A1C ratio and A1C itself were examined in T1D and NDF.

Results: NDF revealed a significant and negative correlation between GA/A1C ratio and A1C itself (r=-0.37, -0.63 or -0.57, using NGSP, IFCC and KO500 units, respectively, all $p<0.0001$), while the correlation between GA and each A1C unit was lower in NDF than in T1D. The mean (SD) of GA was 23.9 (4.05) % and 13.9(1.09) %, in T1D and NDF, where each A1C in T1D and NDF was 8.05(0.98) and 5.20(0.27) %, 6.46(1.07) and 3.35(0.25) mmol/mol and 5.36(0.84) and 2.94(0.20) %, in NGSP, IFCC and KO500 units, respectively. Thus each GA/A1C ratio in NDF exhibited a HGI within narrow glycemic ranges of one fourth in SD of GA and each A1C in T1D. The GA/A1C ratio was 2.96(0.25), 0.37(0.03) and 4.47(0.37) in T1D, and 2.67(0.22), 0.42(0.04)) and 4.76(0.45) in NDF, using A1C-NGSP, A1C-IFCC and A1C-KO500, respectively. Overlap rates of SD range of GA/A1C ratio in T1D by that in NDF were 40.6, 41.7 and 64.0%, using NGSP, IFCC and KO500 units respectively. The GA/A1C ratio in T1D was correlated with that in father (121 pairs, r=0.40, 0.45 and 0.48; all $p<0.0001$), mother (137 pairs, r=0.25, $p=0.0028$, r=0.32, $p=0.0001$ and 0.29, $p=0.0006$) or sibling (91 pairs, r=0.21, $p=0.0512$, r=0.32, $p=0.0022$ and r=0.29, $p=0.0046$), using NGSP, IFCC and KO500 units, respectively, indicating the intra-familial correlations.

Conclusion: We propose the GA/A1C ratio as a useful HGI both in T1D and non-diabetic subjects. The lower a GA/ A1C ratio, the higher a hemoglobin glycation (HGI) is. While the g-g may be used in a fixed population, the GA/A1C ratio will be applied to a modifier of glycemic target and a predictor of diabetic complications in addition with A1C and be comparable between on-going populations regarding diabetic types, races, non-diabetic family et.al. Although each A1C standardization can be useful in common practice, GA/A1C ratio using IFCC and KO500 units may be more accurate as a HGI than that using NGSP unit. The role of hereditary and biochemical factors on HGI remains to be elucidated.

Supported by: JDF

Disclosure: **S. Amemiya:** None.

PS 097 Biomarkers for cardiovascular mortality

1108

Symmetric and asymmetric dimethylarginine as risk markers of cardiovascular disease, mortality and deterioration in kidney function in patients with type 2 diabetes

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Background and aims: To evaluate symmetric dimethylarginine (SDMA) and asymmetric dimethylarginine (ADMA) as risk markers of cardiovascular disease, all-cause mortality and deterioration in renal function in a well characterised type 2 diabetic population with microalbuminuria and without symptoms of coronary artery disease.

Materials and methods: 200 participants followed for 6.1 years. SDMA and ADMA were measured at baseline. Endpoints included 1) composite cardiovascular endpoint (n=40); 2) all-cause mortality (n=26); and 3) decline in eGFR of >30% (n=42). Cox models were unadjusted and adjusted for traditional risk factors (sex, age, systolic blood pressure, LDL-cholesterol, smoking, HbA_{1c}, creatinine and urinary albumin excretion rate). To assess if SDMA or ADMA improved risk prediction beyond traditional risk factors we calculated c-statistics and relative integrated discrimination improvement (rIDI).

Results: Higher SDMA was associated with increased risk of all three endpoints (unadjusted: $p\leq0.001$; adjusted: $p\leq0.02$). Higher ADMA was associated with all-cause mortality (unadjusted: p=0.002; adjusted: p=0.006), but not cardiovascular disease or decline in eGFR ($p\geq0.29$).The c-statistics was not significant for any of the endpoints for either SDMA or ADMA ($p\geq0.10$). The rIDI for SDMA was 15.0% (p=0.081) for the cardiovascular endpoint, 52.5% (p=0.025) for all-cause mortality and 48.8% (p=0.007) for decline in eGFR; for ADMA the rIDI was 49.1% (p=0.017) for all-cause mortality.

Conclusion: In persons with type 2 diabetes and microalbuminuria higher SDMA was associated with incident cardiovascular disease, all-cause mortality and deterioration in renal function. Higher ADMA was associated with all-cause mortality. SDMA and ADMA significantly improved risk prediction for all-cause mortality, and SDMA for deterioration in renal function beyond traditional risk factors.

Supported by: EFSD, CLINICAL RESEARCH GRA

Disclosure: **T. Hansen:** None.

1109

Serum uromodulin predicts mortality independently from the presence of type 2 diabetes

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Background and aims: Uromodulin is produced exclusively by the kidneys and present in urine and blood. Low serum uromodulin has recently been demonstrated to be associated with chronic kidney disease and type 2 diabetes (T2DM). Whether serum uromodulin also predicts mortality is unknown and is addressed in the present study.

Materials and methods: We measured uromodulin in a series of 529 patients who underwent coronary angiography for the evaluation of

established or suspected stable CAD and prospectively recorded mortality during a follow-up of up to 8 years.

Results: Uromodulin significantly correlated with eGFR (r=0.242, p<0.001) and, inversely, with age (r=-0.208, p<0.001), fasting glucose (r=-0.161, p<0.001), C-reactive protein (CRP; r=-0.133, p=0.002) and proBNP (r=-0.164, p=0.002); it was significantly lower in patients with T2DM than in nondiabetic subjects (148±70 vs. 171±79; p =0.001). Prospectively, we recorded 95 deaths; mortality was significantly higher in patients with T2DM than in those who did not have diabetes (28.2 vs. 14.6 %; p <0.001). Serum uromodulin proved protective for overall mortality in univariate analysis (HR=0.56 [95%CI 0.43-0.72]; p<0.001), and also after multivariate adjustment for standard risk factors including diabetes, eGFR, proBNP and presence of CAD as well (adj. HR=0.57 [95%CI 0.37-0.89]; p=0.014).

Conclusion: We conclude that serum uromodulin predicts mortality independently from kidney function and the presence of T2DM.

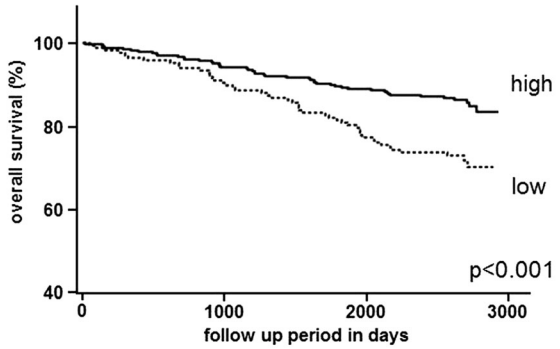

Figure 1: Survival with respect to uromodulin concentration.
The Kaplan Meier plot indicates the overall survival in the total study population of patients with low and high uromodulin concentration according to the low tertile cut-off (125.3 ng/ml). The low uromodulin group is represented by a dashed and the group with respectively higher uromodulin concentrations by a solid line. The log Rank p-values were <0.001.

Disclosure: **J. Ebner:** None.

1110

Levels of soluble ST2 are associated to diabetes and cardiovascular mortality in patients from Tor Vergata Atherosclerosis Registry

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Background and aims: To identify novel biomarkers for cardiovascular diseases we studied a cohort of 399 patients with a chronic atherosclerotic disease and different degree of impaired glucose metabolism.

Materials and methods: 399 patients with atherosclerotic disease were enrolled in the Tor Vergata Atherosclerosis Registry (TVAR) from 2007 to 2014. All the patients had a major CV event or underwent a vascular procedure for significant vascular stenosis. We evaluated lipid profile, liver and kidney function, markers of inflammation and performed an Oral Glucose Tolerance Test in those patients with a negative history of type 2 diabetes (n=325). The follow up was performed every year by phone interview. The primary endpoint was major cardiovascular events (cardiovascular death, nonfatal stroke, nonfatal myocardial infarction, and peripheral vascular surgical procedures) and the secondary endpoint was death for any other disease.

Results: We divided the 399 patients in four groups depending on the glucose tolerance status**.** 118 were normal glucose tolerant (NGT), 138 were impaired glucose tolerant (IGT), 69 had a new diagnosis of type 2 diabetes (nT2D) and 74 were diabetic at enrollment (oT2D). The four groups did not differ for age, sex or BMI. Among the several metabolic and inflammatory variables, we interestingly found that the levels of soluble ST2 (sST2), the receptor for the inflammatory cytokine IL33, were significantly increased from NGT to oT2D (NGT 14420.3±8747.9 vs IGT 14303.1±6838.3 vs nT2D 15260.3±6639.0 vs oT2D 19707.9 ±8378.2, ANOVA p>0.00001). Circulating sST2 levels increased with age (R=0.229, p=0.00001) and reduced kidney function (GFR, R=-0.204, p=0.00001). We also found that levels of sST2 were significantly correlated with fasting plasma glucose (R=0.164, p=0.002), Hba1c (R=0.167, p=0.002), and HOMA index (R=0.159, p=0.004) after adjusting for age, sex and GFR. We didn't find any significant correlation between sST2 and lipid profile, inflammatory biomarkers or insulin secretion. We performed a follow up for all-cause and cardiovascular mortality for a mean of 61,08±34,80 months on 371 patients. During follow-up 66 deaths (20,7%) occurred, 27 of which were due to cardiovascular causes (8,5%). We divided the 371 patient in tertiles of sST2 levels and found that patients within the highest tertile of sT2 showed an increased rate of all-cause and cardiovascular mortality, as shown in figure 1 (all-cause mortality, log rank test, p<0.007 and CVD mortality log rank test, p=0.05).

Conclusion: Some previous evidences showed that the sST2/IL-33 pathway might be relevant for vascular disease and diabetes in animal models but its role in human is still under debate. In our cohort with chronic atherosclerosis and different degrees of detective glucose metabolism we found that sST2 is associated with diabetes and increased all-cause and cardiovascular mortality. Further mechanistic studies determining how sST2 is linked to atherosclerosis and diabetes pathways may clarify its role in cardiovascular diseases.

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Disclosure: **M. Cardellini:** None.

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Higher plasma methylglyoxal levels are associated with incident cardiovascular disease in individuals with type 1 diabetes: a 12-year follow-up study

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Background and aims: Methylglyoxal (MGO), a major precursor for advanced glycation endproducts (AGEs), is increased in diabetes. In diabetic rodents, inhibition of MGO prevents cardiovascular disease (CVD). Whether plasma MGO levels are associated with incident CVD in people with type 1 diabetes is unknown.

Materials and methods: We included 159 individuals with persistent normoalbuminuria and 162 individuals with diabetic nephropathy (DN) from the outpatient clinic at a diabetes center. We measured MGO at baseline with tandem mass spectrometry. We recorded fatal and non-fatal CVD over a median follow-up of 12.3 years (interquartile range 7.6-12.5). Data were analyzed with Cox regression, with adjustment for sex, age, HbA1c, DN, diabetes duration, smoking, systolic blood pressure, anti-hypertensive medication and BMI.

Results: During follow-up, 73 individuals suffered at least one CVD event (36 fatal and 53 non-fatal events). Higher MGO levels were associated with total- (HR: 1.47; 95%CI: 1.13-1.91), fatal- (HR: 1.42; 95%CI: 1.01-1.99) and nonfatal incident CVD (HR: 1.46; 95%CI: 1.08-1.98). A similar trend was observed for total mortality (HR: 1.24; 95%CI: 0.99-1.56).

Conclusion: This study shows, for the first time, that plasma MGO levels are associated with cardiovascular events in individuals with type 1 diabetes. MGO may thus explain the increased risk, at least in part, for CVD in type 1 diabetes.

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1112

Association of dehydroepiandrosterone with severity of type 2 diabetes: the Rotterdam study

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Background and aims: Emerging evidence shows that high levels of dehydroepiandrosterone (DHEA) are associated with reduced risk of type 2 diabetes (T2D). It is not known whether DHEA is associated with diabetes severity. We examined whether higher levels of DHEA were associated with less complications of T2D, later initiation of insulin therapy and reduced risk of mortality.
Materials and methods: We included 1130 men and women with T2D from the Rotterdam Study, a prospective follow-up study. DHEA levels were measured at baseline. Complications of T2D included chronic kidney disease (CKD), hypertension (HTN), and stroke. Prevalent CKD was defined as estimated glomerular filtration rate <60 ml/min/1.73 m^2. Prevalent HTN was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or use of blood pressure lowering medication. Incident stroke events and insulin therapy initiation were obtained on the basis of medical records. Mortality data were obtained by notification from the municipal administration. Logistic regression (for CKD, HTA and insulin therapy initiation) and Cox proportional hazards models (for stroke and mortality) with adjustment for relevant confounders were used to calculate odds ratios (ORs) and hazard ratios (HRs), and their 95% confidence intervals (CIs).
Results: Higher levels of DHEA were associated with lower odds of having CKD (OR=0.66, 95%CI: 0.47-0.92) and HTA (OR=0.66, 95%CI: 0.49-0.90). During 19 years of follow-up, we identified 164 incident cases of insulin initiation, 110 cases of stroke and 540 deaths. Higher levels of DHEA were associated with later initiation of insulin therapy (OR=0.61, 95%CI: 0.41-0.93) and reduced risk of all-cause mortality (HR=0.81, 95%CI: 0.69-0.95), whereas no association was found between DHEA levels and risk of stroke (HR=0.83, 95%CI: 0.58-1.18). Further adjustment for glucose, insulin and downstream hormone levels such as total estradiol and testosterone did not affected any of these associations.
Conclusion: These findings suggest that high levels of DHEA are associated with fewer complications in diabetics. It is at present unclear whether medications or lifestyle factors that alter DHEA metabolism can be effectively used in prevention of diabetes complications.

Table 1 The association between dehydroepiandrosterone levels, diabetes complications, initiation of insulin therapy and risk of mortality*.

	Model 1 Relative risk(95%CI)*#[1]	Model 2 Relative risk(95%CI)*#[2]	Model 3 Relative risk(95%CI)*#[3]
Chronic kidney disease†	0.66(0.47, 0.92)	0.65(0.47, 0.92)	0.62(0.47, 0.88)
Hypertension†	0.67(0.49, 0.90)	0.67(0.50, 0.92)	0.64(0.50, 0.91)
Insulin therapy†	0.61(0.42, 0.92)	0.60(0.40, 0.91)	0.60(0.39, 0.90)
Stroke incidence‡	0.83(0.58, 1.18)	0.83(0.58, 1.19)	0.82(0.57, 1.18)
Mortality‡	0.81(0.69, 0.95)	0.81(0.69, 0.95)	0.79(0.68, 0.93)

†Odds ratios estimated by using logistic regression models
‡Hazard ratios estimated by using Cox's proportional hazard models
*Result are reported per one unit increase in natural log transformed dehydroepiandrosterone
Relative risks are odds ratio (for chronic kidney disease, hypertension and insulin therapy) or hazard ratios (for stroke and mortality)
[1]Model 1: adjusted for age, sex, cohort, progesterone, sex-hormone binding globulin, cortisol, body mass index, alcohol, smoking, physical activity, hormone replacement therapy, prevalent cardiovascular disease, serum total cholesterol, statin use, prevalent oral medication for diabetes, prevalent insulin use.
[2]Model 2: additionally adjusted for glucose and insulin.
[3]Model 3: additionally adjusted for estradiol and testosterone.

Disclosure: **N. Kastrati:** None.

1113

GDF-15 and FGF-23 are associated with mortality in type 2 diabetic patients with microalbuminuria

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Background and aims: We evaluated two biomarkers (growth differentiation factor 15 (GDF-15) and (FGF-23) reflecting different aspects of renal pathophysiology as determinants of decline in estimated glomerular filtration rate (eGFR), incident cardiovascular disease (CVD) and all-cause mortality in patients with type 2 diabetes (T2D) and microalbuminuria, but without clinical coronary artery disease.
Materials and methods: Prospective study including 200 patients. GDF-15 and FGF-23 were measured at baseline and were available in 191 patients. Adjusted Cox models included sex, age, LDL cholesterol, smoking, HbA_{1c}, creatinine, systolic blood pressure and urine albumin excretion rate (UAER). A decline in eGFR of >30%, which has recently been suggested as a valid renal outcome, at any time point during follow-up was the predefined endpoint of CKD progression. Hazard ratios (HR) are provided per 1 SD increment of log-transformed values of the urinary biomarkers.
Results: Patients were (± SD) 59 ± 9 years old, eGFR 91.1 ± 18.3 ml/min/1.73m^2 and UAER (IQR) 103 (39-230) mg/24-h. During a median 6.1 years follow-up, there were 40 incident CVD events and 26 deaths and a total of 42 patients reached the predefined CKD progression endpoint after 4.9 years (median). Higher GDF-15 was a determinant of decline in eGFR >30% in unadjusted (HR (95% CI) 1.7 (1.3-2.4); p=0.001) and adjusted (HR 1.7 (1.1-2.5); p=0.018) models, a predictor of CVD in the unadjusted model (HR 1.4 (1.0-1.9); p=0.034) but not in the adjusted model (HR 1.3 (0.9-1.8); p=0.25) and of all-cause mortality in unadjusted (HR 1.8 (1.3-2.6); p<0.001) and adjusted (HR 1.9 (1.2-2.9); p=0.003) models. Higher FGF-23 was not associated with decline in eGFR >30% or CVD, but was associated with all-cause mortality in unadjusted (HR 1.5 (1.1-2.0); p=0.010) and adjusted (HR 1.6 (1.1-2.2); p=0.011) models.
Conclusion: In patients with T2D and microalbuminuria, GDF-15 was independently associated with decline in kidney function and all-cause mortality, and higher FGF-23 was associated with all-cause mortality.
Disclosure: **M. Frimodt-Møller:** None.

1114

Plasma matrix metalloproteinases are associated with incident cardiovascular disease and all-cause mortality in patients with type 1 diabetes: a 12-year follow-up study

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Background and aims: The pathophysiological mechanisms leading to cardiovascular disease (CVD) in type 1 diabetes have only been partly elucidated. Altered regulation of extracellular matrix remodeling by matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinase (TIMP) may contribute to vascular complications in type 1 diabetes. Therefore, we investigated associations between plasma MMP-1, -2, -3, -9, -10 and TIMP-1, and cardiovascular events and all-cause mortality in type 1 diabetic patients.
Materials and methods: We prospectively followed 337 type 1 diabetic patients (mean age 41.4 years (9.6), 39% female), 170 with and 167 without diabetic nephropathy, with median follow-up of 12.3 years.

Survival analyses were applied to investigate differences in plasma MMP-1, -2, -3, -9, -10, and TIMP-1-levels in patients with and without a cardiovascular event and in those who died vs survivors. All analyses were adjusted for age, sex, duration of diabetes, HbA1c, nephropathy and for other conventional cardiovascular risk factors. In addition, we investigated the extent to which low-grade inflammation (LGI), endothelial dysfunction (ED) and renal dysfunction (eGFR and albuminuria) contributed to these associations.

Results: After adjustment for potential confounders, higher MMP-2 plasma levels were significantly associated with higher incidence of cardiovascular events [HR 1.49 (95%CI 1.11;1.99)], and higher plasma levels of MMP-1 [1.38 (1.07;1.78)], MMP-2 [1.60 (1.19;2.15)] and MMP-3 [1.39 (1.05;1.85)] were associated with all-cause mortality. All associations were independent of LGI and ED as estimated by plasma markers. Associations between MMP-2 and cardiovascular events and between MMP-3 and mortality were attenuated after further adjustment for eGFR and changes in eGFR.

Conclusion: In patients with type 1 diabetes followed for a median of 12.3 years, higher plasma MMP-2 levels are associated with incident CVD and higher plasma MMP-1, MMP-2, MMP-3 are associated with all-cause mortality. These associations are independent of cardiovascular risk factors, LGI and ED. However, baseline eGFR and decline in eGFR during follow-up attenuated the association between MMP-2 and CVD as well as the association between MMP-3 and all-cause mortality; eGFR may thus, in part, mediate these associations.

Disclosure: **S.A. Peeters:** None.

1115

Visceral adipose tissue volume is associated with ^{18}F-FDG-PET assessed arterial inflammation in male patients with early type 2 diabetes

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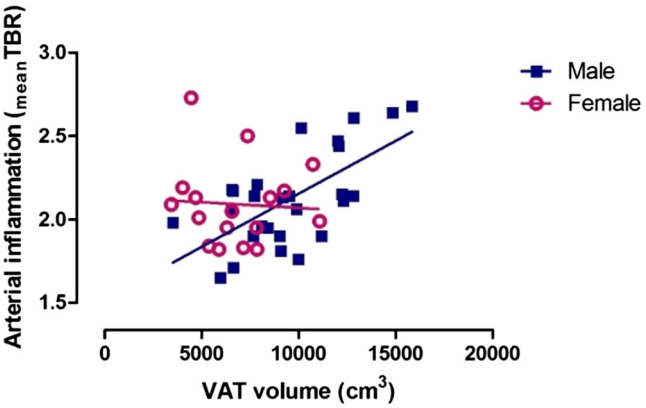
Background and aims: Already early in the course of their disease, patients with type 2 diabetes (T2D) have a considerably increased cardiovascular risk, irrespective of glycemic control. Visceral adipose tissue (VAT) is thought to play an important role, by inducing insulin resistance (IR) and non-alcoholic fatty liver disease (NAFLD) and by being metabolically active and producing adipocytokines. Its contribution to early atherosclerosis development is not fully understood. We investigated the association between VAT volume and subclinical arterial inflammation in early T2D patients by using ^{18}F-fluordeoxyglucose (FDG) positron emission tomography (PET) with low dose computed tomography (CT) and performed a pilot to explore the value of FDG-uptake in VAT as a proxy for its metabolic activity.

Materials and methods: In 44 patients with T2D (age 63 [54-66] years, BMI 30.4 [28-36], HbA1c (%) [6.3±0.4]), without cardiovascular disease or glucose lowering medication an FDG-PET was performed. VAT and subcutaneous adipose tissue (SAT) volumes (cm^3) were quantified at L1-L5 with a semi-automatic method based on CT. Arterial inflammation was quantified by glucose corrected target-to-background ratio ($_{mean}$TBR) of FDG standardized uptake value $(SUV)_{max}$ of the aorta, carotid, iliac, and femoral arteries versus blood pool activity. HOMA-IR (index of IR), ALT (proxy of NAFLD), and adiponectin were measured in plasma. Finally, FDG-uptake in VAT was measured (VAT-SUV_{mean}).

Results: Fat distribution (VAT/SAT ratio) differed between males (n=27; [1.2 (0.94-1.7)]) and females (n=17; 0.6 [0.4-0.7] (p<0.0001)). VAT volume correlated with HOMA-IR (β=0.37, p=0.015), ALT (β=0.57, p<0.001), and adiponectin (β=-0.39, p=0.008). Since VAT volume and gender interacted in relation to $_{mean}$TBR (p=0.003), men and women were investigated separately. VAT volume, but not SAT volume correlated with $_{mean}$TBR in males (β=0.652 (p<0.001)) but not in females (β=-0.059 (p=0.821)). This association was independent of HOMA-IR, ALT, and adiponectin. Unexpectedly, VAT-SUV_{mean} correlated negatively with VAT volume in both sexes (males: β=-0.509, p=0.007, females: β=-0.598, p=0.011).

Conclusion: VAT volume is associated with FDG-PET assessed arterial inflammation in early T2D males, independent of markers for IR and NAFLD, and adiponectin. These findings underscore the role of VAT, in contrast to SAT, in early atherosclerosis development in T2D. The FDG uptake in VAT needs further study and validation.



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Disclosure: **M.R. Reijrink:** None.

1116

Combined effect of high sensitive C-reactive protein and serum amyloid component P on mortality rate in patients with type 2 diabetes

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Background and aims: Though the role of some inflammatory acute phase proteins (APPs) on cardiovascular (CV) mortality in type 2 diabetes (T2D) is known, that of all APPs as considered as a whole has never been addressed. We aimed at investigating the combined effect of of high sensitive C-reactive protein (hs-CRP), fibrinogen, serum amyloid component P (SAP), haptoglobin and alfa-2 macroglobulin, on both all-cause and CV mortality rate.

Materials and methods: All five APPs were assayed using multiplex detection 5-plex kit from Bio-Rad (Hercules, CA) in 334 patients with T2D from the Gargano Heart Study-prospective design (follow-up=5.4 ±2.5 yrs; 73 all-cause and 51 CV-mortality events).

Results: In a model comprising all 5 log-transformed and standardized APPs, only hs-CRP and SAP, were associated with all-cause (HRs; 95%Cs=1.9; 1.5-2.5 and 0.8; 0.6-0.9, respectively) as well as CV (HRs; 95%CIs=1.4; 1.2-1.8 and 0.3; 0.1-0.7, respectively) mortality. Patients were then stratified according to relatively low and high hs-CRP and SAP levels (< or > the median value: 1.4 mg/l and 36.2 μg/ml, respectively), so to obtain four groups: low/high (1; i.e. presumably the most protected individuals), low/low (2), high/high (3), and high/low (4; i.e. presumably the most at risk individuals) hs-CRP/SAP levels. As compared to group 1, group 2, 3 and 4 had HRs; (95%CIs) of 2.6 (0.8-8.9), 4.8 (1.5-15.7) and 10.0 (3.0-33.4), for all-cause and 2.7 (0.6-11.9), 4.5 (1.0-19.2) and 12.2; (2.8-52.9), for CV mortality, respectively. Similar results

were observed after adjusting for age, sex, smoking habit, BMI, HbA1c, diabetes duration and all ongoing treatments.

Conclusion: In conclusion, in patients with T2D: i) hs-CRP and SAP show an independent effect on both all-cause and CV mortality; ii) when the two markers are combined together, a greatly increased predicting ability on both outcomes is observed. This suggests that the simultaneous use of hs-CRP and SAP may prove to be very much effective for clinical use in predicting mortality rate in T2D. Further studies in larger diabetic prospective cohorts, are needed to both confirm our finding and better address the intrinsic nature of the interwoven effect between hs-CRP and SAP.

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Disclosure: **C. Menzaghi:** None.

1117

High sensitivity C-reactive protein, LDL cholesterol and cardiovascular outcomes in patients with type 2 diabetes and acute coronary syndrome from EXAMINE

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Background and aims: High-sensitivity C-reactive protein (hsCRP) level is known to be an independent predictor for future development of cardiovascular disease. The aim of this study was to assess the risk of major adverse cardiovascular events (MACE) of cardiovascular death, nonfatal myocardial infarction (MI) and stroke according to baseline hsCRP level in patients with type 2 diabetes and recent cardiovascular events enrolled in the EXAMINE trial.

Materials and methods: Study participants were stratified by baseline hsCRP values using established decision-limits (<1, 1-3, and >3 mg/l) for prediction of CV outcomes. Study participants were also divided into 4 groups according to both baseline hsCRP (≤3 mg/l, >3 mg/l) and achieved LDL cholesterol <70 mg/dl or ≥70 mg/dl. Multivariate Cox proportional hazards models were used to analyze the independent association between baseline hsCRP level and MACE as well as urgent revascularization due to unstable angina, hospitalization for heart failure, and death from any cause.

Results: At baseline, patients with higher hsCRP were more likely to have higher blood pressure, fasting glucose, glycated hemoglobin levels, and LDL cholesterol levels. During 30 months of follow-up, cumulative rates of MACE were 11.5% (119 events), 14.6% (209 events), and 18.4% (287 events) in patients with baseline hsCRP <1 mg/l, 1-3 mg/l, and >3 mg/l, respectively (P<0.001). Patients with baseline hsCRP >3 mg/l showed independent associations with future MACE, nonfatal MI, hospitalization for heart failure, and death from any cause compared to patients with baseline hsCRP <1 mg/l (Table). Additionally, the rates of MACE were 11.0% (128 events), 14.4% (100 events), 15.6% (194 events), and 21.3% (182 events) in patients with both low LDL cholesterol and hsCRP, low LDL cholesterol and high hsCRP, high LDL cholesterol and low hsCRP, and both high LDL cholesterol and hsCRP, respectively (P<0.001).

Conclusion: Levels of hsCRP were associated with secondary cardiovascular events in patients with type 2 diabetes and recent acute coronary syndrome and this association appears to be independent of and additive to the achieved LDL cholesterol.

Table. Hazard ratios* (95% confidence interval) for cardiovascular outcomes according to baseline hsCRP concentration in EXAMINE

	<1 mg/l** (n=1278)	1-3 mg/l (n=1963)	>3 mg/l (n=2139)	P-value
MACE	1	1.11 (0.88, 1.40)	1.42 (1.13, 1.78)	0.002
Death from cardiovascular causes	1	0.97 (0.67, 1.40)	1.40 (0.98, 2.00)	0.062
Nonfatal myocardial infarction	1	1.14 (0.85, 1.54)	1.40 (1.04, 1.89)	0.025
Nonfatal stoke	1	1.62 (0.81, 3.22)	1.57 (0.79, 3.13)	0.195
Urgent revascularization due to unstable angina	1	1.22 (0.72, 2.08)	0.91 (0.52, 1.61)	0.754
Hospitalization for heart failure	1	1.30 (0.83, 2.04)	2.04 (1.34, 3.11)	<.001
Death from any cause	1	1.12 (0.80, 1.55)	1.77 (1.29, 2.42)	<.001

*Adjusted for treatment group, age, sex, body mass index, current smoking, total cholesterol, estimated glomerular filtration rate, systolic blood pressure, diastolic blood pressure, glycated hemoglobin, and diabetes duration

** reference group

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PS 098 Stroke and other vascular complications

1118

Carotid preclinical atherosclerosis in Mediterranean individuals with type 1 diabetes: prevalence and association with the Steno T1 Risk Engine

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Background and aims: Specific cardiovascular risk (CVR) scores could help tailor cardioprotective treatment to patients with type 1 diabetes (T1D), and, also, to decrease their main cause of mortality: cardiovascular disease (CVD). We aimed to assess the relationship between CVR score "Steno T1 Risk Engine (Steno-Risk)" and preclinical carotid atherosclerosis in our Mediterranean T1D patients.
Materials and methods: T1D patients without CVD from a tertiary hospital were selected. Inclusion criteria were as follows: ≥40 years, diabetic nephropathy, or diabetes duration ≥10 years with at least one additional CVR factor. Intima-media thickness (IMT) and plaque presence (IMT ≥1.5 mm) were assessed by B-mode ultrasonography at the common carotid (CC), bulb, and internal carotid (IC). Abnormal IMT-CC was defined as IMT above age-and-sex specific 75th percentile. The Steno-Risk was used to estimate 10-years CVR (<10% low; 10-20% moderate; ≥20% high risk). This score includes: age, gender, diabetes duration, HbA1c, systolic blood pressure, LDLc, albuminuria, glomerular filtration rate, smoking habit and regular exercise (≥3.5 h/week).
Results: We included 90 patients (37% men, age 48.7±9.3 years, duration of diabetes 24.9±6.3 years). Prevalence of abnormal IMT-CC and carotid plaque were 54% and 42%, respectively, with no sex differences. The Steno-Risk was directly associated with mean IMT-CC (r=0.357; p=0.001) and mean IMT-Bulb (r=0.228; p=0.033). Patients' characteristics according to CVR are shown in the table. There was a higher prevalence of microvascular complications in individuals with high vs. low risk: nephropathy, 29 vs. 0%; retinopathy, 46 vs. 7%, respectively (both p<0.05). Abnormal IMT-CC (26.7, 56.9, 66.7%; p=0.022) and carotid plaque presence (26.7, 39.2 and 58.3%; p=0.043) increased with increasing risk (for low, moderate and high risk, respectively). IC plaque presence (6.7, 25.5 and 37.5%) and presence of ≥3 plaques (6.7, 7.8 and 20.8%, for low, moderate and high risk, respectively) were also positively associated with Steno-Risk (both p<0.05).
Conclusion: We found a high prevalence of preclinical atherosclerosis in our primary prevention sample of Mediterranean T1D patients with no sex differences. The Steno-Risk score was significantly associated with preclinical carotid atherosclerosis. Optimization of cardiovascular prevention management in T1D warrants additional studies including the use of specific scales of CVR, early detection strategies of preclinical atherosclerosis and cardiovascular clinical outcomes.

	Steno-Risk <10% (n=15)	Steno-Risk 10-20% (n=51)	Steno-Risk ≥20% (n=24)	p for lineal trend
Age (years)	36.9±9,3	48.2±5.0	57.2±8.0	<0.001
Men	5 (33.3)	20 (39.2)	8 (33.3)	0.848
Diabetes duration (years)	18.9±7.9	25.7±3.8	27.2±7.5	<0.001
Systolic blood pressure (mmHg)	120±14	127±13	132±14	0.007
Smoking habit	3 (20)	16 (31.4)	7 (29.2)	0.552
Statin use	5 (33.3)	19 (37.3)	16 (64.0)	0.034
LDL-c (mg/dL)	110±25	109±21	114±24	0.513
HbA1c (%)	7.6±1.7	7.7±0.9	8.1±0.9	0.101
IMT-CC (mm)	0.56±0.14	0.69±0.11	0.76±0.14	<0.001
IMT-Bulb (mm)	0.76±0.30	0.81±0.15	0.91±0.26	0.033
IMT-CI (mm)	0.58±0.15	0.70±0.24	0.71±0.28	0.132

Data are shown as mean ± standard deviation or n (percentage)

CC: common carotid, CI: internal carotid; IMT: intima-media thickness

Disclosure: **A.J. Amor:** None.

1119

Type 2 diabetes, chronic kidney disease, and mortality in patients with established cardiovascular disease

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Background and aims: Both type 2 diabetes (T2DM) and chronic kidney disease (CKD) are associated with a high risk of cardiovascular disease (CVD) and premature death. We aimed at investigating the single and joint effects of T2DM and of CKD on all-cause mortality in high-risk patients with established CVD.
Materials and methods: We prospectively investigated 2108 patients with established CVD (1789 with angiographically proven coronary artery disease and 319 with sonographically proven peripheral artery disease) over 7.0±2.7 years.
Results: Deaths occurred more frequently in T2DM patients (n=652) than in non-diabetic subjects (38.2% vs. 19.6%; p <0.001) and in patients with CKD (eGFR <60ml/min/1.73m²; n=357) than in those with an eGFR ≥60ml/min/1.73m² (48.8% vs. 19.8%; p <0.001). When both, T2DM and CKD were considered, 1248 subjects had neither T2DM nor CKD, 503 had T2DM but not CKD, 208 did not have diabetes but had CKD, and 149 had both diabetes and CKD. When compared with mortality among patients with neither T2DM nor CKD (16.1%), mortality was significantly higher in patients with T2DM who did not have CKD (30.5%; p<0.001) as well as in non-diabetic patients with CKD (40.1%; p<0.001) and was highest in patients with both, T2DM and CKD (62.4%; p<0.001), in whom mortality was higher than in those with T2DM but no CKD (p<0.001) or those without T2DM but with CKD (p=0.045); mortality was higher in non-diabetic CKD patients than in diabetic patients who did not have CKD (p=0.013).
Conclusion: We conclude that CKD in patients with established CVD confers an even higher mortality risk than T2DM. Mortality is extremely high in CVD patients with the combination of CKD and Diabetes.
Disclosure: **C.H. Saely:** None.

1120

The gut microbiom and cardiovascular complications in patients with type 2 diabetes

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Background and aims: The composition of the gut microbiom seems to reflect not only the development of bowel disease but also the course of systemic diseases like obesity and diabetes. We investigated the relationship between the composition of the gut microbiom and the prevalence of cardiovascular complications (CVC) in patients with type 2 diabetes.
Materials and methods: Demographic characteristics, routine lab results, prevalence of CVC and composition of the gut microbiom assessed by 16S rRNA sequencing of fecal samples were collected from 60 patients with type-2-diabetes. After exclusion of bacterial types with a low abundance (median proportion of 0%), the relationship between bacterial type abundance and CVC rate was investigated by logistic regression.
Results: 28 patients presented with CVC (coronary heart disease 57 %, stroke 14 %, peripheral artery occlusive disease 29 %). Differences regarding demographic factors and lab results between patients with and without CVC were only observed for gender (females with CVC: 25%, without CVC: 59 %). Microbiota differences at phylum and class-level did not reach 5% statistical significance. At order-level the average proportion of Coriobacteriales was higher for patients with versus without CVC (0.29% vs 0.13%; p= 0.002). The odds ratio of CVC for patients in the highest quartile of Coriobacteriales abundance (>0.30%) was 9.6 (95%CI 1.86 to 49.5) compared to patients in the lowest quartile (abundance < 0.08%). Logistic regression analysis revealed a 200-fold increased risk of CVC for every 0.01% increase in Coriobacteriales abundance (p=0.01). This association was also present in lower classification levels (species Collinsella aerofaciens). Compared with patients without CVC, patients with CVC showed a 25% lower abundance of butyrate producing bacteria (1.35 ± 1.40% vs 1.69 ± 1.60%; p=0.34) and a 51% higher abundance of TMAO producing bacteria (0.59 ± 0.42% vs 0.39 ± 0.28%; p=0.03).
Conclusion: Coriobacteriales spp. abundance was associated for the first time with the risk for CVC in type 2 diabetes patients. Furthermore, patients with CVC showed a higher amount of TMAO producing bacteria than patients without CVC.
Supported by: Dietmar Hopp Stiftung, St. Leon Rot, Germany
Disclosure: **E. Siegel:** None.

1121

Administration of VCP, a new p38 MAPK inhibitor, promoted recovery in diabetic stroke

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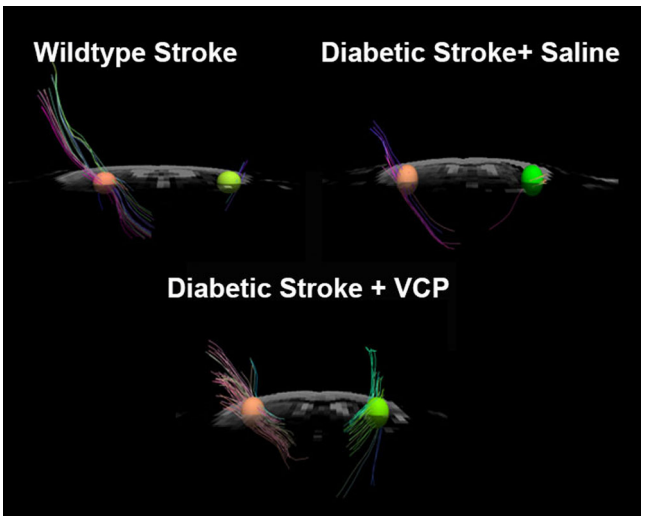
Background and aims: Type 2 Diabetes is associated with a doubling risk for ischemic stroke and have less favorable clinical outcomes. Several studies in animal models had shown that the activation of p38 MAPK increased in ischemic brain tissue.On the other hand, MAPK pathways are upregulated with insulin resistance and hyperglycemia induced MAPK phosphorylation. P38 MAPKs is linked to increased production of inflammatory mediators such as proinflammatory cytokines and matrix metalloproteinases. Activation of P38 MAPKs in T2DM will worsen the brain damage after stroke . In this study, we administrated diabetic stroke mice with a new p38 MAPK inhibitor-VCP, which is a highly selective inhibitor of the p38-α and p38-βMAPK isoforms and is more potent in vitro than standard p38 kinase inhibitor RWJ67657.
Materials and methods: All animal experiments were approved by the Institutional Animal Care Committee of Medical School of Southeast University. T2 diabetes mellitus was induced in C57BL/6(8-week-old) mice by administration of high-fat diet in combination with intraperitoneal injection of streptozotocin. Wild-type and diabetes mice were induced in ischemic stroke through a photochemical reaction. The animals were scanned on day 14 post ischemia using diffusion tensor imaging (DTI).
Results: The infarct volumes are shown as hyperintensity on T2-weighted images. Diabetic stroke mice treated with VCP presented smaller infarct volume compared with other groups on day 14 (wildtype group: 6.65%±1.33%,diabetes saline group: 20.2%±6.6%,diabetes VCP group: 14.4%±2.3%, $p<0.05$). In addition, diabetic stroke mice treated with VCP showed significant reductions in neurological functional deficits compared with control mice according to the modified neurological severity score (wildtype group:2.75±0.43; diabetes saline group: 4.33 ±0.47, diabetes VCP group:1.33±0.47, $p<0.05$). In vivo DTI analysis revealed that VCP treatment significantly increased fractional anisotropy (FA) in the lesion of infarction on day 14 compared with the other groups. The fiber counts in the lesion of the mice treated with VCP were significantly increased (Figure.1).Immunofluorescent staining showed that the expression of MMP-9 were down regulated in group treated by VCP.
Conclusion: Administration of VCP,a new p38MAPK inhibitor, in T2DM mice significantly improves recovery from diabetic stroke, which might have been caused by enhancing WM axonal and decreasing MMP-9 expression.



Supported by: NSFC No. 81525014
Disclosure: **Y. Cai:** None.

1122

Coronary artery disease or history of stroke triples the odds of having diabetes

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Background and aims: Current estimates show that at least a third of people with diabetes are unaware of their disease. Unfortunately, still too often diabetes is found as late as at the occurring of acute myocardial infarction or stroke. We hypothesized that in general population the history of coronary artery disease (CAD) or stroke are significant risk factors for diagnosing diabetes.
Materials and methods: In 2014-2015 we conducted a primary care physician (PCP)-based nationwide screening programme aiming at identifying individuals with undiagnosed diabetes or prediabetes. 561 PCPs enrolled 21,726 subjects. No earlier diagnosis of diabetes or prediabetes and the presence of at least one risk factor (age>45 yrs, family history of diabetes, sedentary lifestyle, smoking, presence of fatty liver disease, hypertension, hyperlipidemia, CAD, peripheral artery disease, obstructive sleep apnoea syndrome, polycystic ovary syndrome, history of stroke, gestational diabetes or having a child of birth weight >4 kg, BMI>25 kg/m^2 or waist circumference >80 cm [women] or 94 cm [men]) were inclusion criteria. All individuals underwent fasting plasma

glucose (FPG) measurement, and if it exceeded 125 mg/dl, second FPG measurement was conducted. The subjects with first FPG measurement 100-125 mg/dl had an oral 75 g glucose tolerance test (OGTT) performed.
Results: Diabetes was diagnosed in 4,221 (19.4%) subjects, and having had a stroke or CAD tripled the risk for having diabetes, and their effect was greater than that of abdominal obesity, fatty liver disease or lifestyle or family history of diabetes (Table 1).
Conclusion: In conclusion, non diabetes subjects with coronary artery disease or history of stroke are at high risk of having diabetes and should be actively screened towards it.

Table 1. Odds ratio for having diabetes.

	Odds Ratio	95% Confidence Interval
History of stroke	3.310	2.948-3.716
CAD	3.158	2.934-3.398
Abdominal obesity	2.828	2.615-3.059
Fatty liver disease	2.655	2.461-2.864
BMI>25 kg/m2	2.626	2.425-2.844
Sedentary lifestyle	1.882	1.744-2.032
Family history of diabetes	1.780	1.661-1.908

Supported by: TEVA Pharmaceuticals Polska Sp. z o.o.
Disclosure: **E. Szymanska-Garbacz:** None.

1123

Evaluation of different neurological scores and scales to predict fatal stroke in hospitalised stroke patients with type 2 diabetes

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Background and aims: Stroke is one of the most common causes of death worldwide and the most frequent cause of permanent disability. Diabetes mellitus type 2 (T2DM) is a well-known traditional stroke risk factor. Various scales and scores have been established for the neurological evaluation and prognosis regarding the outcome of stroke patients. The purpose of this study is a comparative assessment of various neurological scores and scales to predict a fatal outcome in stroke hospitalized patients with or without T2DM.
Materials and methods: The study included a total of 96 hospitalized stroke patients (40 men, 46 with T2DM), during the period January to December 2016. Anthropometric, clinical, and laboratory data were estimated for each participant. Furthermore, NIHSS (NIH Stroke scale), RRE-90 (Recurrence Risk Estimator), ESRS (Essen Stroke Risk Score), SPI-II (Stroke Prognosis Instrument II), and GCS (Glasgow Coma scale) were also calculated. Discrimination capability was assessed based on the area under the receiver operating characteristic curve (Area Under the Curve, AUC), sensitivity and specificity, positive (PPV) and negative (NPV) predictive values were calculated.
Results: Fatal endpoint was found in 6.5% of all hospitalized patients (5.3% and19.2% in the group of individuals without and with T2DM respectively). In patients with T2DM, receiver operating characteristic analysis revealed that for NIHSS scale a cutoff of ≥10 showed sensitivity: 80%, specificity: 71%, AUC: 0,767 an for RRE score a cutoff of ≥1,5 showed sensitivity 20%, specificity: 80%, AUC: 0.55. ESRS score for a cutoff of ≥4,5 showed sensitivity: 80%, specificity: 58%, AUC: 0,674. SPI-II score for a cutoff of ≥7,5 showed sensitivity: 80%, specificity: 47.4% AUC: 0,60, while the GCS scale for a cutoff of ≥10 showed sensitivity: 80%, specificity: 14.3%, AUC: 0,276. Respectively for stroke patients without T2DM, NIHSS scale for a cutoff of ≥11 showed sensitivity 100%, specificity: 80,6%, AUC: 0,889, while RRE score for a cutoff of ≥1,5 showed sensitivity: 50%, specificity: 52.9% , AUC: 0.397. In addition, ESRS score for a cutoff of ≥3,5 showed sensitivity 20%, specificity: 58,8%, AUC: 0,206, SPI-II score for a cutoff of ≥7,5 showed sensitivity 20%, specificity: 93 9%, AUC: 0,121, and GCS scale for a cutoff of ≥10 showed sensitivity: 50%, specificity: 8,3%, AUC: 0,111.
Conclusion: The results of the present study showed that NIHSS scale displays the best sensitivity and specificity as a predictor of fatal outcome in patients hospitalized with stroke regardless of the presence or not of T2DM. Moreover, SPI-II and ESRS scores showed equally good sensitivity and specificity as predictors of fatal outcome in stroke patients with T2DM.
Disclosure: **A. Angelidi:** None.

1124

Comorbidity prevalence among patients with established cardiovascular disease and type 2 diabetes

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Background and aims: The presence of comorbidities in type 2 diabetes (T2D) can impact a physician's management approach, including selection of antihyperglycemic agents. While the prevalence of comorbidities has been reported widely, limited information is available on the prevalence among patients with cardiovascular disease (CVD) and differences from those without CVD. Therefore, the current study sought to determine the prevalence of comorbidities in these populations.
Materials and methods: A retrospective study was conducted using the Quintiles Electronic Medical Record database. Patients ≥18 years diagnosed with T2D (or prescribed an oral T2D medication) who had ≥1 encounter from October 2014 to September 2015 and ≥1 year of medical history available were included.
Results: Of the 1,514,966 eligible patients, 54% were female, median age was 65 years, and median A1c was 6.7%. 25% of patients had CVD, with coronary artery disease being the most commonly diagnosed form. Median A1c was similar for those with and without CVD (6.8% and 6.7%). The prevalences of comorbidities by CVD type are shown in the table below. The most common conditions for those with and without CVD included hypertension (98% and 91%), overweight/obesity (81% in both groups), hyperlipidemia (95% and 79%), chronic kidney disease (39% and 19%), and heart failure (20% and 4%). Use of ICD-9 codes for comorbidity assessment might have resulted in underreporting.
Conclusion: Patients with T2D and CVD have higher rates of common comorbidities compared to those without CVD.

Comorbidity	No CVD (n=1,136,438)	Any CVD (n=378,528)	CAD only (n=229,532)	PAD only (n=43,853)	Cerebrovascular disease only (n=33,643)
Hypoglycemia	1.4%	2.1%	1.7%	2.7%	2.5%
Chronic Kidney Disease	18.9%	38.6%	35.3%	40.9%	37.0%
Neuropathy	8.6%	13.7%	10.8%	21.8%	15.0%
Congestive Heart Failure	4.3%	20.3%	20.5%	12.0%	11.8%
Genital Mycotic Infection	2.6%	1.7%	1.4%	2.6%	2.5%
Overweight/Obese	80.5%	80.5%	82.6%	78.1%	75.7%
Urinary Tract Infection	1.8%	1.8%	1.5%	2.5%	2.7%
Pancreatitis	0.2%	0.2%	0.2%	0.4%	0.3%
Liver Disease	3.8%	3.0%	2.8%	4.0%	3.2%
Retinopathy	0.7%	0.7%	0.5%	1.0%	0.8%
Hypertension	91.1%	98.3%	98.5%	97.1%	96.9%
Hyperlipidemia	78.5%	94.8%	95.8%	89.7%	89.7%

Footnote: CVD=cardiovascular disease; CAD=Coronary artery disease; PAD=peripheral artery disease. CKD was defined based on ICD-9 diagnosis or eGFR <60 mL/min/1.73m². If not already estimated in the database, eGFR was calculated using the MDRD Study equation. Neuropathy, heart failure, genital mycotic infections, UTIs, pancreatitis, liver disease and retinopathy were defined using ICD-9 diagnoses. Overweight/obesity was defined by either the presence of an ICD-9-CM code or a most recent body mass index (BMI) measure ≥25 kg/m². Hypoglycemia was assessed using a modified algorithm by Ginde et al. Hypertension was defined using ICD-9 diagnoses or SBP ≥140 or DBP ≥90 mmHg using the most recent blood pressure measurement prior to the index date or use of medications to treat hypertension. Hyperlipidemia was defined using ICD-9 diagnoses, by an LDL-c ≥100 mg/dL or use of medications for lowering cholesterol.

Disclosure: **H. Hannachi:** Employment/Consultancy; Merck & Co., Inc. Stock/Shareholding; Merck & Co., Inc.

1125

Impact of past and current smoking on the risk of future cardiovascular events in angiographied coronary patients with type 2 diabetes

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Background and aims: Smoking is a major risk factor for heart disease. When combined with other risk factors (such as unhealthy blood cholesterol levels, high blood pressure, and overweight or obesity) smoking further raises the risk of heart disease. The impact of smoking on cardiovascular event risk in angiographied coronary patients is unclear and is addressed in the present study.
Materials and methods: We enrolled 1804 consecutive patients undergoing coronary angiography for the evaluation of established or suspected stable coronary disease (CAD). Patients who had smoked within 30 days prior to angiography were considered current smokers. Prospectively, cardiovascular events were recorded over a mean follow-up time of 6.3 ±3.7 years.
Results: At baseline, both in patients with T2DM (n=522; 28.9% of the study population) and in nondiabetic subjects the prevalence of a past (44.1 and 39.5%; p=0.035), and, albeit less so, of current smoking (18.0 and 17.7%; p = 0.247) was high. Among patients with T2DM the prevalence of significant CAD with lumen narrowing ≥50% was 61.0% in those who had never smoked vs. 70.9%, p=0.032 and 70.8%, p=0.101 in past and current smokers, respectively. Among nondiabetic patients the corresponding prevalence rates of significant CAD were 45.9% vs. 63.8%, p<0.001 and 53.7%, p=0.046, respectively. Prospectively, current smoking independently predicted cardiovascular events after multivariate adjustment including baseline CAD in patients with diabetes (HR 1.93 [1.20-3.08]; p=0.006) as well as in nondiabetic patients (HR 1.50 [1.07-2.09]; p=0.019), whereas past smoking neither in patients with T2DM nor in nondiabetic subjects was associated with cardiovascular events (HRs 1.07 [0.74-1.55]; p=0.715 and HR 1.11 [0.87-1.41]; p=0.415). An interaction term diabetes x current smoking was not significant (p=0.350), indicating that current smoking was equally predictive of cardiovascular events in patients with T2DM and in nondiabetic subjects.
Conclusion: We conclude that current but not past smoking strongly increases cardiovascular event risk in angiographied coronary patients with diabetes independently from the baseline CAD state.
Disclosure: **R. Gansch:** None.

PS 099 Cardiovascular complications: prevalence and treatment initiation

1126

Global prevalence of type 2 diabetes complications in 14,391 patients initiating second-line therapy: the DISCOVER study

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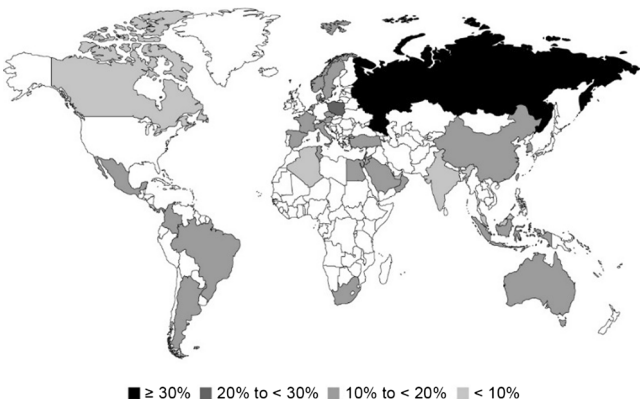
Background and aims: The global prevalence of diabetes-related complications is currently not well described. DISCOVER is a global, prospective, observational study involving 14 391 patients from 37 countries with type 2 diabetes initiating second-line glucose-lowering therapy.
Materials and methods: Patients were recruited from primary and specialist healthcare settings. Data were collected using a standardized case report form. Macrovascular complications were defined as a composite of coronary artery disease, heart failure, stroke and peripheral artery disease. Microvascular complications were defined as a composite of retinopathy, nephropathy and neuropathy. Prevalence estimates were standardized for age and sex using a hierarchical logistic model.
Results: Median time since diagnosis was 4.1 years (interquartile range: 2.0-7.9 years). The overall adjusted prevalence of macrovascular complications was 13.0%, ranging from 4.1% to 46.6% across countries (Figure). The overall adjusted prevalence of microvascular complications was 17.7%, ranging from 5.5% to 41.1% across countries.
Conclusion: The global burden of macrovascular and microvascular complications is substantial, even among patients initiating second-line treatment who are presumably still at low risk. Particularly high rates were seen in Eastern European, Middle Eastern and African countries.

Figure. Age- and sex-adjusted prevalence of macrovascular complications at baseline.



Clinical Trial Registration Number: NCT02322762
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Disclosure: **M.V. Shestakova:** Employment/Consultancy; AstraZeneca, Eli Lilly, Merck Sharp &Dohme, Novo Nordisk, Sanofi, Boehringer Ingelheim. Grants; Sanofi.

1127

Mortality and life expectancy according to cardiovascular comorbidity among 41,161 adults with type 2 diabetes: a Korean national sample cohort study, 2002 to 2013

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Background and aims: Although type 2 diabetes is a strong risk factor for cardiovascular disease and mortality, information on its association with mortality and life expectancy according to cardiovascular comorbidities is limited, especially in Asia. Thus, this study aimed to assess mortality and reductions in life expectancy associated with cardiometabolic multimorbidity.
Materials and methods: A total of 569,831 participants aged more than 30 years from Korean National Health Insurance Service-National Sample Cohort (NHIS-NSC) were enrolled between 2002 and 2006 and were followed for the median of 12.0 years. They were categorized into 5 mutually exclusive groups according to the baseline disease status: none (reference group); diabetes only; diabetes and stroke; diabetes and myocardial infarction (MI); and diabetes, stroke and MI. Mortality rates and hazard ratios (HRs), reductions of life expectancy, and age-specific contributions to life expectancy were calculated by constructing life tables.
Results: The mortality rates per 1000 person-years were 6.85, 19.86, 67.17, 66.34, and 115.52 in the reference, diabetes only; diabetes and stroke; diabetes and MI; and diabetes, stroke, and MI groups, respectively. The corresponding HRs for all-cause mortality were 1.58 (95% CI 1.54-1.62), 3.53 (95% CI 3.21-3.89), 3.37 (95% CI 2.90-3.92), and 5.25 (95% CI 3.36-8.21), compared with the reference group. The estimated reductions in life expectancy were greater at younger ages, and markedly increased with more cardiometabolic comorbidities.
Conclusion: Young type-2 diabetic Asians, especially those with cardiovascular comorbidity lived less than their non-diabetic equivalents. Thus, these individuals require special attention to prevent further reductions in life expectancy.
Disclosure: **Y. Kang:** None.

1128

Prevalence of cardiovascular disease and evaluation of standard of care in type 2 diabetes: a nationwide study in primary care

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Background and aims: Cardiovascular disease (CVD) complicates type 2 diabetes (T2D). Empagliflozin and liraglutide have demonstrated improved survival in patients with T2D and CVD. We assessed prevalence and standard of care (SoC) of patients with T2D and established CVD managed in primary care.
Materials and methods: 129 general practitioners, from both rural and urban areas, responsible for 348373 patients, identified T2D patients from the clinic's electronic patient record system (EPRS) based on ICPC-2 coding system. Patients with concomitant CVD were subsequently identified and characterized.
Results: 17113 patients (4,9%) had T2D. T2D with established CVD was found in 3665 patients (21,4%). A maximum of 20 patients form each gp (2003 patients were futher analyzed for concommittant CVD: Mean age was 72 yrs; 34,6% were women, see table 1. SoC was good; HbA1c was 52,3 mmol/mol, BP 131,4/75,7 mmHg and LDL-cholesterol 2,0 mmol/l. Mean eGFR was 68,2 ml/min.; 32,2% had micro or macroalbuminuria. Almost 80% were in antiglycemic drug treatment predominantly with metformin in mono or combination therapy (63,4%), insulin was used in 19,5%. 21,8% were treated non-pharmacological. 64,9 % had an ACEI/ARB, 78,3% a statin and 96,1% were in antithrombotic therapy, of these 53% with acetylsalicylic acid; 66,9% were in diuretic treatment.
Conclusion: In a nationwide database study in primary care the prevalence of CVD in T2D patients were high (21,4%). SoC was in accordance with local guidelines. Identifying this high-risk group of T2D patients and optimizing treatment might add further CV benefits as suggested in recent cardiovascular outcomes trials. Identification of eligible patients is possible with existing EPRS.

TABLE 1

	N=2003
	no. (%)
Diagnosis	
Ischemic heart disease with angina pectoris	659 (32,9)
Ischemic heart disease without angina pectoris	411 (20,5)
Acute myocardial infarct	515 (25,7)
Heart failure	446 (22,3)
Transient ischemic attack/stroke	613 (30,6)
Atherosclerosis/peripheral vascular disease	387 (19,3)
Hypertension	1327 (66,3)
Atrial fibrillation	376 (18,8)

Supported by: Boehringer-Ingelheim, Denmark
Disclosure: **J. Rungby:** Employment/Consultancy; Consultancy honoraria, Boehringer-Ingelheim.

1129

Baseline characteristics of the DECLARE-TIMI 58 trial population

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Background and aims: Dapagliflozin (DAPA) is a SGLT-2 inhibitor approved for use in patients with type 2 diabetes (T2D), having met pre-marketing safety requirements. Cardiovascular (CV) outcomes trials permit well-powered assessment of the CV safety and efficacy of new anti-hyperglycemic agents. We hereby describe the design and patients baseline characteristics at the DAPA Effect on CV Events (DECLARE-TIMI 58) trial.
Materials and methods: The DECLARE-TIMI 58 is a multinational phase 4, randomized, placebo-controlled trial in patients with T2D and established CV disease (ECVD) or with multiple risk factors (MRF) for CV disease (define as: men: age ≥55 women: ≥60 and at least 1 of: dyslipidemia, hypertension, or smoking). The objective of the trial is to determine the effect of DAPA on CV outcomes relative to placebo when added to current background therapy. The first step is to show that DAPA does not increase the risk for the composite of CV death, MI, or ischemic stroke (MACE). The second step is to test superiority of DAPA in reducing the co-primary efficacy endpoints of MACE and the composite of CV death or hospitalization due to heart failure.

Results: The study randomized 17,276 patients with T2D including 6,978 patients with ECVD and 10,228 patients with MRF (table). The trial will continue until 1,390 patients experience the adjudicated composite endpoint of MACE.

Conclusion: DECLARE-TIMI 58 trial is expected to provide conclusive data on the effect of DAPA relative to standard of care on CV outcomes in T2D patients at high risk for CV events.

		Total (N=17276)	CVD (N=6978)	MRF (N=10228)
Gender – no. (%)	Male	10813 (62.6%)	5028 (72.1%)	5743 (56.1%)
Age (years) - mean± SD		63.8± 6.81	62.5± 8.08	64.7± 5.61
Race – no. (%)	White	13761 (79.7%)	5588 (80.1%)	8113 (79.3%)
	Black/African American	610 (3.5%)	211 (3%)	394 (3.9%)
	Asian	2304 (13.3%)	936 (13.4%)	1365 (13.3%)
	Other	601 (3.4%)	243 (3.5%)	356 (3.4%)
Region – no. (%)	Europe	7660 (44.3%)	3250 (46.6%)	4383 (42.9%)
	North America	5551 (32.1%)	2351 (33.7%)	3174 (31%)
	Latin America	1878 (10.9%)	493 (7.1%)	1373 (13.4%)
	Asia/Pacific	2187 (12.7%)	884 (12.7%)	1298 (12.7%)
BMI (kg/m²)- mean± SD		32.0± 6.02	32.1± 6.02	32.0± 6.01
CV history and risk factors – no. (%)	myocardial infarction	3583 (20.7%)	3583 (51.3%)	0 (0%)
	hypertension	15454 (89.5%)	6123 (87.7%)	9311 (91%)
	Angina pectoris	2781 (16.1%)	2103 (30.1%)	674 (6.6%)
	Congestive heart failure	1670 (9.7%)	1115 (16%)	552 (5.4%)
HbA1c (%) - mean± SD		8.29± 1.20	8.33± 1.24	8.26± 1.18
Glucose-lowering therapy – no. (%)	Metformin	13515 (78.2%)	5181 (74.2%)	8283 (81%)
	Sulfonylurea	6914 (40%)	2599 (37.2%)	4298 (42%)
	Insulin	6764 (39.2%)	3072 (44%)	3674 (35.9%)
	DPP-IV inhibitor	2524 (14.6%)	983 (14.1%)	1533 (15%)
	GLP1-agonist	725 (4.2%)	289 (4.1%)	433 (4.2%)
CV medication – no. (%)	ACEI/ARB	10751 (62.2%)	4558 (65.3%)	6173 (60.4%)
	Beta-blockers	7920 (45.8%)	4635 (66.4%)	3274 (32%)
	Statins	12217 (70.7%)	5714 (81.9%)	6486 (63.4%)
	Acetylsalicyclic Acid	8902 (51.5%)	4943 (70.8%)	3936 (38.5%)
	Clopidogrel	1863 (10.8%)	1714 (24.6%)	149 (1.5%)
Estimated glomerular filtration rate mL/min/1.73m² – no. (%)	<60	1570 (9.1%)	763 (10.9%)	799 (7.8%)
	>=60 - <90	8784 (50.8%)	3588 (51.4%)	5172 (50.6%)
	>=90	6921 (40.1%)	2627 (37.6%)	4256 (41.6%)

Clinical Trial Registration Number: NCT01730534
Supported by: AstraZeneca
Disclosure: **I. Raz:** Employment/Consultancy; Diabetes Medical Center (Tel Aviv). Honorarium; AstraZeneca, Eli Lilly and Company, Merck Sharp & Dohme Limited, Novo Nordisk Inc., Sanofi, Orgenesis, SmartZyme Innovation Ltd, Labstyle Innovations Ltd, Boehringer Ingelheim, Johnson & Johnson, Novartis Pharma AG, Teva. Lecture/other fees; AstraZeneca, Insuline Medical, Gili Medical, Kamada Ltd, FutureRx Ltd, Teva, Comtec Ltd. Stock/Shareholding; Insuline Medical, Labstyle Innovations, Orgenesis, Glucome Ltd.

1130

Cardiovascular hospitalisation among type 2 diabetes patients by stages of eGFR and albuminuria

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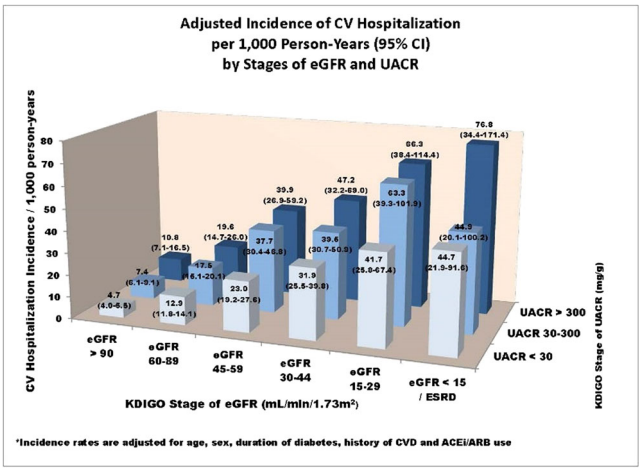
Background and aims: Up to 40% of type 2 diabetes (T2D) patients have chronic kidney disease (CKD) as defined by low estimated glomerular filtration rate (eGFR) (eGFR < 60 mL/min/1.73 m^2) and/or presence of albuminuria. Both low eGFR and albuminuria are known to independently increase cardiovascular (CV) risk but longitudinal studies that include both measures of CKD are scarce. We aimed to compare the 11-year incidence of CV hospitalizations in T2D patients with and without low eGFR and/or with and without albuminuria.

Materials and methods: We used the electronic medical records of an integrated delivery system in the USA to identify 17,054 patients with diagnosed T2D for whom we could calculate eGFR (CKD-EPI formula) from the first available serum creatinine value from 2006-2012 (baseline), confirmed by a second eGFR three months later if the first was < 60 mL/min/1.73 m^2. We required patients to have a UACR within +/- 6 months of the creatinine test. We used KDIGO stages of eGFR (G1: ≥90mL/min/m2; G2: 60-89; G3a: 45-59; G3b: 30-44; G4: 15-19; G5: <15 or ESRD) and albuminuria (A1: <30 mg/g; A2: 30-300; A3: >300) to define 18 possible categories of kidney function. The outcome of interest was incidence per 1,000 person-years (p-y) of first CV hospitalization (primary diagnosis of ischemic heart disease, stroke, or heart failure) estimated over 11 years of follow-up (through 2016). Incidence rates were adjusted for age, sex, duration of diabetes, prior history of CV disease, and use of an angiotensin-converting-enzyme inhibitor or an angiotensin II receptor blocker using generalized linear models with Poisson errors and person-time as an offset to account for differential follow-up.

Results: Nearly two-thirds of patients (n=10,915; 64%) had normal kidney function (stages G1or G2 and A1), 14% had eGFR < 60 mL/min/m^2 (Stage G3A or higher), 28% had albuminuria (stage A2 or A3), and 6% both eGFR < 60 mL/min/m^2 and albuminuria There were 1,696 events over 86,738 person-years of follow-up. Adjusted CV hospitalization rates were lowest among patients with normal kidney function (4.7/1000 p-y, 95% CI 4.0-5.5) and highest among patients at stages G5 and A3 (76.8, 34.4-171.4). Incidence was higher at each eGFR stage as albuminuria stage increased. For example, incidence was over 50% higher at stage G1/A2 (7.4, 6.1-8.1), and over twice as high at stage G1/A3 (10.8, 7.1-16.5). Incidence was also higher at each albuminuria stage as eGFR stage increased. For example, stage G3a/A1 incidence was 23.0 (19.2-27.6) and 41.7 (25.8-67.4) at stage G4/A1.

Conclusion: Low eGFR and albuminuria each independently increase CV hospitalization risk. The combination of higher stages eGFR and albuminuria is particularly potent.



Diclosure: **G.A. Nichols:** Grants; Boehringer-Ingelheim, Amarin Corporation.

1131

Short-term treatment with linagliptin reduces plasma levels of nitrate in parallel with the reduction in FDG-PET-assessed arterial inflammation in early type 2 diabetes

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Background and aims: We showed that the dipeptidyl peptidase-4 (DPP-4) inhibitor linagliptin reduces vascular stiffness (pulse wave velocity (PWV)) and arterial inflammation (arterial ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography (PET) uptake) in early type 2 diabetes (T2D) patients in a randomized controlled trial (RELEASE study). The underlying mechanisms are incompletely understood. One potential pathway may be via improvement in redox tone. We compared plasma levels of reactive nitrogen oxide species (nitrite, nitrate, and nitrosylation products (RXNO)) and free thiols (FT, redox balance) between T2D patients and controls, assessed the effect of linagliptin monotherapy, and whether changes were paralleled by changes in vascular stiffness and arterial inflammation.
Materials and methods: 45 treatment naive T2D patients (median age 63 (IQR 54-66) years, 61% male, mean HbA1c 6.3±0.4 (%)) without cardiovascular disease were randomized (1:1) to linagliptin 5 mg or placebo for 26 weeks in a double-blind fashion. PWV, FT (corrected for total protein), nitrite, nitrate, and RXNO were assessed at baseline, and after 4 and 26 weeks of treatment and arterial inflammation at baseline and after 26 weeks. Arterial inflammation was quantified by FDG uptake as the pre-scan glucose corrected maximum standardized uptake value corrected for background (TBR) of the large vessels (meanTBR). For comparison, nitrite, nitrate, RXNO, and FT were also measured in young healthy controls (median age 24 (IQR 21-25) years, 80% female).
Results: RXNO levels (23.4 (12.1) vs 15.8 (5.9) nM; p=0.007) were significantly increased in patients vs controls, while nitrite (0.22 (0.15) vs 0.14 (0.08) μM; p=0.098) and nitrate (35.1(16.5) vs 27.6(12.6) μM ; p=0.18) were not significantly different. No differences in FT were observed (5.72(0.41) vs 5.82(0.44) vs μM/g protein; p=0.51). Nitrate (-3.27(15.0) vs 18.0(26.1); p=0.003) decreased from baseline to week 4 in linagliptin vs controls and a trend was observed for RXNO (-1.79(18.9) vs 8.8(18.7); p=0.078). No significant changes were observed from week 4 to 26 for both. No effects of linagliptin were seen on nitrite and FT levels. Only the change in nitrate (r=0.49;p=0.024) was positively associated with the decrease in meanTBR but not with PWV. Changes in reactive nitrogen oxide species were strongly interrelated.
Conclusion: Nitrosylation products are elevated and linagliptin reduces nitrate within 4 weeks of treatment in patients with early T2D. The decrease in nitrate is associated with reduction in FDG-PET assessed arterial inflammation. These data suggest that potential beneficial vascular effects of incretins and DPP4 inhibitors may be partly mediated by effects on the nitric oxide pathway.
Clinical Trial Registration Number: NCT02015299
Supported by: Boehringer Ingelheim BV, Alkmaar, the Netherlands
Disclosure: **J. Boersema:** None.

1132

Antiplatelet effect of aspirin during 24 hours in patients with type 2 diabetes without cardiovascular disease

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Background and aims: Primary prevention of cardiovascular disease (CVD) with aspirin in patients with type 2 diabetes (T2DM) is controversial. Patients with T2DM and a history of CVD have a reduced antiplatelet effect of aspirin, which has been associated with an increased platelet turnover. However, the antiplatelet effect of aspirin in patients with T2DM without CVD has not been explored. The primary aim of this study was to investigate if platelet aggregation increases during the standard 24-hour aspirin dosing interval in patients with T2DM and in healthy controls after 1 week of low-dose aspirin treatment. Furthermore, we evaluated platelet turnover and the acute effects of aspirin on platelet aggregation.
Materials and methods: We performed an open-label parallel group intervention study. We included 21 patients with T2DM and 21 age and sex-matched controls. Platelet aggregation was measured by impedance aggregometry (Multiplate® Analyzer) using arachidonic acid and thrombin-receptor-activating-peptide as agonists. Markers of platelet turnover were measured by flow cytometry (Sysmex® XE-5000). Blood samples were obtained at baseline and 1 hour after administration of 75 mg of aspirin. Participants were then treated for 6 days with once-daily aspirin, and blood samples were repeated 1 hour and 24 hours after aspirin intake.
Results: After 6 days of treatment, platelet aggregation levels increased during the 24-hour aspirin dosing interval in both patients and controls (T2DM: + 85 ± 101 U (aggregation units × minute), p<0.001, controls: + 80 ± 105 U, p<0.001, figure). At baseline, patients with diabetes had increased platelet aggregation compared to controls (949 ± 159 U vs. 835 ± 194 U, p=0.03). Platelet aggregation was reduced after the first dose of aspirin (T2DM: 731 ± 256 U, controls: 634 ± 218 U). Additionally, after six days of treatment, aggregation levels were further reduced (T2DM: 172 ± 101 U, p<0.001, controls: 215 ± 86 U, p<0.001). Patients with T2DM had a higher number of immature platelets compared to controls (8.0 ± 4.8 10^9/L vs. 5.9 ± 2.3 10^9/L), indicating an increased platelet turnover, although this finding was not statistically significant (p=0.09).
Conclusion: Aspirin-treated patients with T2DM without a history of CVD had increased platelet aggregation at the end of the standard 24-hour dosing interval, however this increase did not differ from the increase observed in healthy controls. Aspirin-naïve T2DM patients had increased platelet aggregation compared to healthy controls, confirming alterations in platelet aggregation in patients with T2DM. Our study indicates that patients with T2DM may achieve additional benefit from twice daily dosing of aspirin. Large-scale clinical outcome trials are needed to determine this.

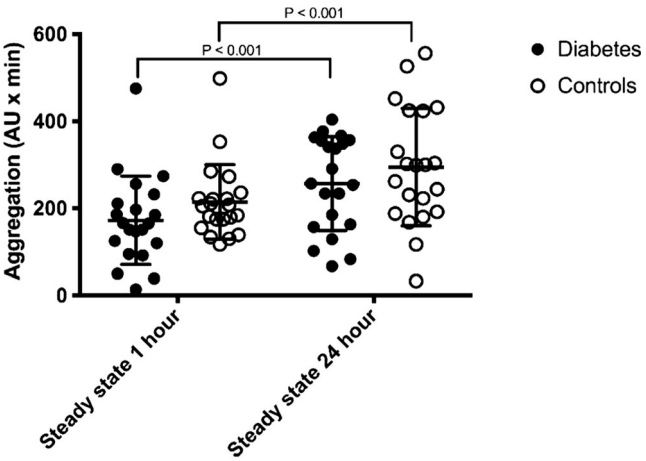

Figure: Steady state: Platelet aggregation 1 and 24 hours after intake of aspirin 75 mg using arachidonic acid as agonist in patients with type 2 diabetes and controls.
T2DM, type 2 diabetes mellitus

Clinical Trial Registration Number: 2016-000515-32
Supported by: The Danish Council for Independent Research, Aase og Ejnar Danielsens Fond
Disclosure: **L. Vernstroem:** None.

1133

Are the cardiovascular risk reductions seen with empagliflozin in the EMPA-REG OUTCOME trial explained by conventional cardiovascular risk factors?

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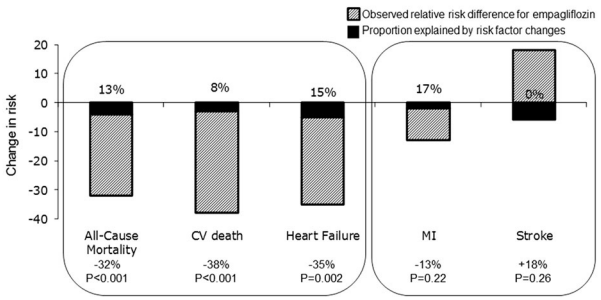
Background and aims: To estimate the degree to which cardiovascular risk reductions demonstrated with empagliflozin administration in the EMPA-REG OUTCOME trial might be explained by changes observed in conventional cardiovascular factors during the study.
Materials and methods: A T2D-specific computer simulation model was used to simulate three year cardiovascular event rates utilizing annual patient-level data from 7,020 EMPA-REG OUTCOME participants that included atrial fibrillation, smoking, albuminuria, HDL-cholesterol, LDL-cholesterol, systolic blood pressure, HbA_{1c}, heart rate, white cell count, haemoglobin and estimated glomerular filtration rate, as well as history of ischaemic heart disease, heart failure, amputation, blindness, renal failure, stroke, myocardial infarction or ulcer. Multiple simulations were performed for each participant to minimize first and second order uncertainty and to optimise the precision of the confidence intervals surrounding the cardiovascular risk point estimates. Estimated absolute event rates for empagliflozin and placebo assigned participants were used to calculate modelled cardiovascular relative risk reductions (RRRs).
Results: Compared to the observed RRRs, our simulated results suggest that empagliflozin could reduce absolute placebo rates for all-cause mortality by 4% (~13% of 32% RRR observed), cardiovascular death by 3% (~8% of 37% RRR observed), hospitalization for heart failure by 5% (~15% of 35% RRR observed), fatal and nonfatal myocardial infarction by 2% (compared with the non-significant 13% RRR observed), and fatal and nonfatal stroke by 6% (compared with the non-significant 18% relative risk increase observed).
Conclusion: Empagliflozin-associated changes in conventional cardiovascular risk factor values recorded in the EMPA-REG OUTCOME trial appear to explain only a small proportion of the actual cardiovascular risk reductions observed for key endpoints. Alternative risk-reduction mechanisms need to be explored to determine whether the observed changes in risk can be explained by other factors, or as a drug-specific effect.

Figure: Observed risk reductions and proportions attributable by simulation to conventional cardiovascular risk factors



Clinical Trial Registration Number: NCT01131676
Supported by: Boehringer Ingelheim and Eli Lilly and Company Diabetes Alliance
Disclosure: **R.L. Coleman:** None.

PS 100 Variable determinants of macrovascular disease

1134

Prognostic value of fasting plasma glucose, two hour postload glucose and HbA_{1c} in patients with coronary artery disease

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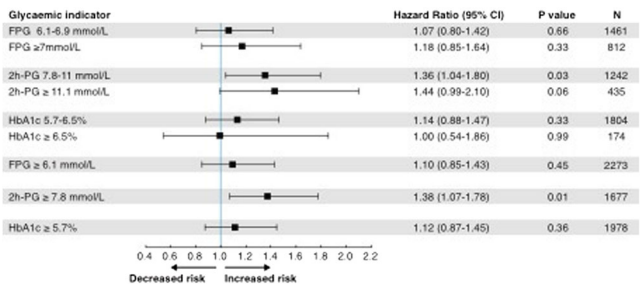
Background and aims: Three test are recommended for identifying dysglycaemia: fasting glucose (FPG), 2h-postload glucose(2h-PG) from an oral glucose tolerance test (OGTT) and glycated haemoglobin A1c (HbA1c). This study explores the prognostic value of these screening tests in patients with coronary artery disease (CAD).
Materials and methods: 4,004 CAD patients without a history of diabetes (age 18-80 years) were screened for dysglycaemia by means of FPG, 2h-PG and HbA1c. The prognostic value of these tests was studied after two years of follow up. The primary endpoint included cardiovascular mortality, non-fatal myocardial infarction, stroke or hospitalisation for heart failure and a secondary endpoint incident diabetes.
Results: Complete information including all three glycaemic parameters was available in 3,775 (94.3%) patients of who 246 (6.5%) experienced the primary endpoint. Neither FPG nor HbA1c predicted the primary outcome while the 2h-PG, dichotomised as <7.8 vs. ≥7.8 mmol/L, was a significant predictor (HR 1.38, 95% CI 1.07-1.78; p=0.01). During follow-up 78 (3.0%) of the 2,609 patients without diabetes at baseline developed diabetes. A FPG between 6.1-6.9 mmol/L did not predict incident diabetes while both HbA1c 5.7-6.5% and 2h-PG 7.8-11.0 mmol/L were significant independent predictors.
Conclusion: The 2h-PG, in contrast to FPG and HbA1c, provides significant prognostic information regarding cardiovascular events in patients with CAD. Furthermore, elevated 2h-PG and HbA1c are significant prognostic indicators of an increased risk of incident diabetes.



Supported by: ESC and Swedish Heart lung foundation
Disclosure: **B. Shahim:** None.

1135

The haemoglobin glycation index and risk for diabetes-related complications in the ADVANCE trial

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Background and aims: Previous studies have suggested that the hemoglobin glycation index (HGI) can be used as a predictor of diabetes-related complications in patients with type 1 or type 2 diabetes. We investigated whether HGI was better in predicting diabetes-related complications than HbA_{1c} in patients with type 2 diabetes, using data from the ADVANCE trial.

Materials and methods: We studied patients included in the ADVANCE trial with available baseline HbA_{1c} and fasting plasma glucose (FPG) (n=11,083). HGI is the difference between observed HbA_{1c} and HbA_{1c} predicted from a simple linear regression of HbA_{1c} on FPG. Using Cox regression, we investigated the association between HGI, both continuous and categorized, and adverse outcomes, considering treatment allocation (intensive or standard glucose control) and compared HGI and HbA_{1c} as a predictor of complications.

Results: Irrespective of treatment allocation, every standard deviation increase in HGI was associated with a significant risk increase of 14-17% for macrovascular and microvascular disease and mortality. However, adjusted for an identical set of standard covariates, HbA_{1c} was a stronger predictor for each of these outcomes than HGI. Intensive glucose control lowered mortality risk in high HGI patients only (HR 0.74, 95% CI 0.61-0.91, P=0.003), while there was no difference in the effect of intensive treatment on mortality in high HbA_{1c} patients.

Conclusion: HGI does predict risk for complications in ADVANCE patients, irrespective of treatment allocation, but no better than HbA_{1c}. Given the uncertain relevance beyond HbA_{1c}, clinical use of HGI in type 2 diabetes cannot be recommended.

Disclosure: **S.C.J. van Steen:** None.

1136

Glycaemic variability, but not HbA_{1c}, is an independent factor for the severity of coronary disease in patients with poorly controlled type 2 diabetes

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Background and aims: Some studies have shown an association between glycemic variability, assessed by the mean amplitude of Glycemic Excursions (MAGE) and the severity of coronary disease in patients with type 2 diabetes and have shown that this association was superior to that for HbA1c. However, we do not know whether this association is also observed in metabolically severe patients with type 2 diabetes in whom constant hyperglycemia may mask the effect of glycemic variability. This prompted us to analyze prospectively the relationship between MAGE and the severity of coronary disease in patients with type 2 diabetes hospitalized for an acute Myocardial Infarction (MI) in whom Continuous Intravenous Insulin Infusion (CIVII) was needed because of blood glucose at entry above 180 mg/dl.

Materials and methods: All consecutive diabetic patients admitted to our University Hospital for acute MI between January 2014 and March 2016 and who received CIVII were included. The mean amplitude of glycemic excursions (MAGE) was calculated within 2 days after the acute MI. The severity of the coronary disease was assessed by the angiographic SYNTAX score. The population was divided into tertiles according to MAGE, and bivariate and multivariate analyses were performed.

Results: Among the 431 patients with type 2 diabetes hospitalized for acute MI, 196 met the inclusion criteria. The mean age of the population was 71.3 ± 12.1 yrs with a mean duration of diabetes of 11.4 ± 10.2 yrs and 29% were treated with insulin prior the acute MI. Mean HbA1c was 7.5±1.6 % and mean MAGE was 0.72±0.41. In multivariate analysis, four parameters were significantly and independently associated with the highest tertile of MAGE: female sex (OR(95%CI): 5.67 (2.29-14.02) p<0.001), Hb1Ac (OR(95%CI): 1.77 (1.33-2.34) p<0.001), Systolic blood pressure (OR(95%CI): 0.99 (0.97-1), p= 0.049) and SYNTAX score (OR(95%CI): 1.05 (1.01-1.08), p=0.007). The SYNTAX score was significantly higher in patients in the highest MAGE tertile compared to those in the lowest tertile (19.8±13.7 vs. 14.9±11.5, p=0.01). In univariate analysis, the SYNTAX score was also associated positively with age (p=0.005), history of coronary heart disease (CHD) or stroke (p=0.005), triglycerides (p=0.05) and negatively with glomerular filtration rate (p=0.028). In multivariate analysis, age (OR(95%CI): 1.05 (1.02-1.09) p=0.001), history of stroke (OR(95%CI): 6.25 (1.65-23.5) p=0.007) and the highest tertile of MAGE (OR(95%CI): 2.60 (1.24-5.41) p=0.011) were independently associated with the SYNTAX score, whereas Hb1Ac was not.

Conclusion: Our study shows that, in patients with metabolically severe type 2 diabetes, glycemic variability is an independent factor associated with the severity of coronary disease when HbA1c is not. These data underline the association between glycemic variability and the severity of coronary disease even in patients with poorly controlled type 2 diabetes.

Disclosure: **B. Vergès:** None.

1137

Real world evidence for legacy effect of early glycaemic control in type 2 diabetes on risk of macrovascular disease

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Background and aims: Tight glycemic control right from the diagnosis of type 2 diabetes (T2D) may be effective in reducing long term incidence of cardiovascular disease. Clinical trials can have insufficient duration of follow-up to observe this effect. We evaluated whether the trajectory of HbA1c achieved within 12 months after diagnosis of T2D is independently associated with lowering the risk of new myocardial infarction (MI) or cerebrovascular accident (CVA) within 10 years.

Materials and methods: We performed a cohort analysis using a primary care data from the Royal College of General Practitioners Research and Surveillance Centre. The network of primary care practices covers 1.7% of population of England. The cohort of patients with a first diagnosis of T2D between 2006-16 were identified from the dataset. We examined risk for MI or CVA at anytime over 10 years of follow-up using logistic regression. Odds ratios (and 95% confidence intervals) for these macrovascular outcomes were calculated for nine separate glycaemic trajectories based on the following HbA1c thresholds at diagnosis and 1 year. Category A: HbA1c 48 - 57mmol/mol, category B: 58-74mmol/mol, category C: ≥75mmol/mol. Covariates including: gender, age, smoking (active; ever), blood pressure, BMI, ethnicity, chronic kidney disease (stage 3 to 5), atrial fibrillation, rheumatoid arthritis, LDL-cholesterol, insulin use, sulphonylurea, pioglitazone, rosiglitazone, metformin were included in the logistic model.

Results: 72,910 people with T2DM identified of whom 1962 had HbA1c data plus 10 years follow-up. Trajectories of improved control over 1-year were associated with less risk of later MI than those who stayed in upper categories of HbA1c (Table 1). Male gender 1.916 (1.177, 3.193; P<0.05), CKD 2.189 (1.285, 3.714; P<0.01), insulin use 2.247 (1.320, 3.819; P<0.01) were associated with greater risk of MI. Conversely, none of these independent variables were significantly associated with stroke.

Conclusion: In those with the highest HbA1c at diagnosis of T2D, achieving improvement in glycaemic control within 1-year reduced the likelihood of later myocardial infarction. Conversely, the level of glucose

control either at diagnosis or at 1-year had no impact on likelihood to have a CVA. Hypothetically the association of insulin use and later MI may relate to hypoglycaemia and MI risk. Further work is needed to determine whether trajectories of control after 12-months significantly impacts upon the risk of MI and at what timepoint the risk with insulin use emerges.

		10-year Odds Ratio for event	
		MI	CVA
HbA1c at diagnosis		0.980 (0.957, 1.001)	1.003 (0.982, 1.023)
HbA1c transition	Category A-> Category B	1.624 (0.505, 4.449)	0.983 (0.353, 2.342)
	Category A-> Category C	2.563 (0.127, 17.116)	4.49^{10-7} (0, 451)
	Category B-> Category A	2.691* (1.206, 6.008)	0.704 (0.324, 1.454)
	Category B-> Category B	3.481** (1.417, 8.418)	0.564 (0.180, 1.475)
	Category B-> Category C	5.499* (1.326, 19.048)	1.990 (0.522, 6.122)
	Category C-> Category A	2.459 (0.715, 8.395)	0.493 (0.148, 1.580)
	Category C-> Category B	3.318 (0.892, 12.064)	0.722 (0.200, 2.473)
	Category C-> Category C	6.975** (1.792, 26.837)	0.746 (0.170, 2.918)

*P<0.05 **P<0.01

Table 1. Odds ratios for MI and CVA based upon trajectory of control over first year after diagnosis of type 2 diabetes.

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Treatment with statins is independently associated with increased glycated haemoglobin levels in patients with type 1 diabetes

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Background and aims: Statin treatment has been associated with increased risk of incident type 2 diabetes (T2DM) and with impaired glycemic control in T2DM. So far, to the best of our knowledge, only one study reported an association between use of statins and glycated haemoglobin (HbA1c) levels in individuals with type 1 diabetes mellitus (T1DM).
Materials and methods: In this cross-sectional study, the association of statin treatment with HbA1c levels has been examined by uni- and multivariable models in 774 quite young and well treated T1DM belonging to a single centre (men/women 53/47%, 40.2±11.7 year-old with a mean duration of diabetes of 19.3±12.2 years and a mean HbA1c of 7.8±1.2%).
Results: Out of 774 individuals with T1DM, one hundred (12.9%) were on statin treatment. As expected, T1DM on statins were older, had longer diabetes duration, higher BMI, waist-to-hip ratio (WHR) and systolic blood pressure, higher total- and LDL-cholesterol and also higher triglycerides and non HDL-cholesterol, worse kidney function but similar urinary albumin to creatinine ratio (A/C ratio). Statins users were more likely on treatment with blood pressure-lowering drugs or RAS-blockers with no differences in gender distribution, family history of cardiovascular disease (CVD), A/C ratio strata. Advanced retinopathy (27.1% *vs.* 13.9%), eGFR stages ≥2b (17.0% *vs.* 5.2%) and any CVD (30.0% *vs.* 5.3%) were more frequent in statin users (p<0.0001 for all) than in non-users. Insulin dose (IU by kg/body weight) and HbA1c levels were similar in both groups. Upon stratification by HbA1c quartiles (thresholds 7.1%, 7.7% and 8.4%), rates of statin users increased from Q1 (8.9%) to Q2 and Q3 (15.9% and 17.7%, respectively) to drop again in Q4 (8.7%; Pearson Chi-square 11.252, 3df, p=0.010). After speculating that statins are unlikely to significantly contribute to the worst HbA1c levels (Q4), logistic regression with backward stepwise variables selection was applied to evaluate independent contributors to Q2&Q3 HbA1c levels (n. 399 T1DM) with the Q1 HbA1c strata as the reference group (n. 192 T1DM). By unadjusted regression, statin treatment doubled the risk of having Q2&Q3 HbA1c levels (Odds ratios (OR) = 2.077; 95%CI 1.183-3.647, p=0.011). By multiple regression, triglycerides (OR = 1.040 x 10 mg/dl, 95%CI 1.000-1.080, p=0.077) and eGFR stages ≥2b (OR = 0.670, 95%CI 0.469-0.959, p=0.029) other than statin treatment (OR 1.954, 95%CI 1.090-3.505, p=0.025) were independently related to HbA1c Q2&Q3 quartiles with no effects of gender, age, duration of diabetes, BMI and WHR, total cholesterol, A/C ratio (or A/C ratio strata), treatment with BP-lowering agents or RAS blockers, smoking habits or coexistence of any CVD.
Conclusion: In type 1 diabetes, use of statins is independently associated to impaired glycemic control. In particular, the probability to be at target for HbA1c is lower in individuals with type 1 diabetes on statin treatment (17%) than in T1DM not on statins (26%). Likely, in type 1 diabetes, insulin regimens should be revised at the time of starting treatment with statins.
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Disclosure: **G. Penno:** None.

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Visit-to-visit variability of blood pressure is independently associated with macrovascular and microvascular complications in patients with type 2 diabetes

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Background and aims: Recent evidence suggest that CVD risk is related on the consistency of blood pressure control between on-treatment visits. Visit-to-visit variability of systolic blood pressure is a biomarker of arterial stiffness, autonomic dysfunction, endothelial damage and systemic inflammation but it may also reflect poor patients' adherence to the pharmacologic therapy.
Materials and methods: We analyzed the relationships between macrovascular and microvascular complications of diabetes in 818 patients attending at least four times our secondary care diabetes clinic between 2013-15 with the coefficient of variation of the visit-to-visit measurements of the sitting systolic and diastolic blood pressure. We segregated these patients in tertiles of the coefficient of variation of systolic (cut-off: 7.7% and 11.8%) and diastolic (cut-off: 8.2% and 12.9%) blood pressure.
Results: Patients with the high visit-to-visit variability of systolic blood pressure were older (p<0.04), but no difference in the duration of diabetes (p=0.90), systolic (139±16, 139±16 and 139±15 mm Hg; p=0.87), diastolic (79±8, 78±9 and 77±9 mm Hg; p=0.35) blood pressure and heart rate (78±11, 79±10 and 77±10; p=0.19) were detected. Glycemic control was not different between tertiles of coefficient of variation (HbA1c 7.47 ±2.76%, 7.49±1.90% and 7.57±2.36%; p=0.97), meanwhile estimated GFR was lower in the tertile with high visit-to-visit coefficient of variation of blood pressure (p<0.03). Diabetic retinopathy and microalbuminuria, as a composite end-point, did not show a strong difference between tertiles (50%, 44% and 56%; p=0.09). In contrast, established CVD was more prevalent in patients with higher coefficient of variation of systolic blood pressure (39%, 48%, 52%; p<0.01). Treatments with statin (p=0.16), ACE-inhibitors (p=0.83) and ARB (p=0.98) were not different among tertiles but use of diuretics and Ca-antagonist was more frequent (p<0.05) in patients with higher variability. When the 10 year UKPDS Risk Engine was used as dependent variable, coefficient of variation of systolic and diastolic blood pressure were associated with the risks of coronary heart disease, fatal coronary artery

disease, and the risks of stroke and fatal stroke also when adjusted for drug therapy.

Conclusion: The results of this analysis support the hypothesis that in patient with type 2 diabetes a lack of stable control of ambulatory blood pressure is related to established and increased risk of macrovascular and, only to a lesser extent, microvascular complications.

Disclosure: **M.G. Radaelli:** None.

1140

Modelling incremental benefits on macro- and micro-vascular complications rates when targeting lower systolic blood pressure levels in type 2 diabetes

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Background and aims: Randomised controlled trials of systolic blood pressure (SBP) lowering demonstrate reductions in long-term complications to a level of 140mmHg in type 2 diabetes mellitus (T2DM) and 120mmHg in people without diabetes. Guidelines recommend individualisation of SBP targets; however, few data are available on the potential benefits that different targets might achieve. We have estimated 10-year risks for T2DM complications when targeting SBP levels between 160mmHg and 120mmHg to quantify the likely incremental benefits.

Materials and methods: We used a T2DM-specific computer simulation model (UKPDS Outcomes-Model version 2.0) to estimate 10-year event rates for T2DM complications: myocardial infarction (MI), stroke, blindness, amputation, and all-cause mortality using T2DM participant data from the Trial Evaluating Cardiovascular Outcomes with Sitagliptin Study. Complete baseline risk factor variables for age, sex, ethnicity, HbA1c, HDL, LDL, weight, smoking status, presence of albuminuria, atrial fibrillation and history of micro- and macrovascular events, were available for 5717 patients. Risk factor values were held constant over 10 years with 5 different simulations that allocated SBP levels to 160, 150, 140, 130 or 120mmHg. Cumulative relative risk reductions (cRRR) at each 10mmHg decrement were compared from 160mmHg using Kruskal-Wallis tests with $p<0.05$ as significant.

Results: Participants were mean (SD) age 66 (7.9) years, HbA1c 7.3 (0.6) %, LDL-cholesterol 2.3 (0.9) mmol/l, HDL-cholesterol 1.12 mmol/l (0.3), median (IQR) T2DM duration 9.8 years (5.1 to 15.6), with 28.3% women, 66.6% White ethnicity and 52.7% with history of smoking. For each 10mmHg SBP decrement from 160 to 120mmHg cRRRs differed significantly for all simulated outcomes; p for trend <0.001 (Table). When targeting a SBP of 140mmHg (current guideline target) compared to a baseline of 160mmHg cRRR estimates were 4.5%, 24.8%, 10.9%, 14.7% and 2.8% for MI, stroke, blindness, amputation and all-cause mortality respectively. When targeting a SBP of 120mmHg compared to 160mmHg the cRRR estimates for the same respective events were 10.0%, 44.9%, 20.9%, 27.4% and 5.2%.

Conclusion: These simulated complication rates could help inform the degree to which complications might be reduced by targeting particular SBP values in T2DM. Targeting a SBP of 140mmHg from 160mmHg, as recommended by guidelines, led to significant reductions in complication rates. Furthermore, there were continuous reductions in all modelled outcomes for complications including below 140mmHg.

10-year estimated absolute risks and 95% confidence intervals for individual macro- and micro-vascular events and mortality at different imposed SBP levels and corresponding relative risk reductions from an SBP of 160mmHg

SBP (mmHg)	160	150	140	130	120	p-value for trend
Myocardial Infarction (%)	21.1 (20.8-21.4)	20.7 (20.4-21.0)	20.4 (20.2-20.6)	20.1 (19.9-20.3)	19.8 (19.6-19.9)	<0.001
	Cumulative RRR (%)	2.2 (2.1-2.4)	4.5 (4.3-4.7)	7.0 (6.7-7.4)	10.0 (9.7-10.4)	<0.001
Stroke (%)	14.9 (14.6-15.2)	13.1 (12.8-13.4)	11.4 (11.1-11.6)	9.8 (9.6-10.1)	8.5 (8.3-8.7)	<0.001
	Cumulative RRR (%)	12.5 (12.3-12.7)	24.8 (24.6-25.0)	35.6 (35.4-35.8)	44.9 (44.8-45.1)	<0.001
Single eye blindness (%)	4.8 (4.8-4.9)	4.6 (4.5-4.6)	4.3 (4.2-4.4)	4.0 (4.0-4.1)	3.8 (3.8-3.9)	<0.001
	Cumulative RRR (%)	5.4 (5.1-5.7)	10.9 (10.6-11.2)	16.2 (16.0-16.4)	20.9 (20.7-21.2)	<0.001
Amputation (%)	2.4 (2.3-2.4)	2.2 (2.1-2.2)	2.0 (1.9-2.1)	1.8 (1.8-1.9)	1.7 (1.7-1.8)	<0.001
	Cumulative RRR (%)	7.4 (6.9-8.0)	14.7 (14.3-15.1)	21.6 (21.3-22.1)	27.4 (27.0-27.8)	<0.001
All-cause mortality (%)	52.6 (51.7-53.4)	51.7 (50.9-52.5)	50.8 (50.0-51.8)	50.1 (49.3-51.0)	49.3 (48.5-50.3)	<0.001
	Cumulative RRR (%)	1.4 (1.3-1.4)	2.8 (2.7-2.8)	4.1 (4.0-4.2)	5.2 (5.1-5.3)	<0.001

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High brown fat activity as a marker of low subclinical atherosclerosis at 5-year follow-up in nondiabetic adults

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Background and aims: Brown adipose tissue (BAT) activity correlates negatively with obesity and insulin resistance and BAT has been suggested to act as a protective factor against atherogenesis in animal models. We aimed to examine markers of subclinical atherosclerosis in a group of individuals who had 5 years earlier participated in positron-emission tomography (PET) studies with measurements of BAT activity.

Materials and methods: Study cohort (M/F=5/26, baseline age 41.4 ±7.9, baseline BMI 26.8±6.3kg/m^2) underwent PET imaging at baseline with [^{18}F]FDG (glucose uptake, GU) and [^{15}O]H_2O (perfusion) to measure BAT activity during cold exposure. At 5-year follow-up, ultrasound was performed to measure carotid intima-media thickness (IMT) in the common carotid (cIMT), carotid bulbus and internal carotid artery. Carotid distensibility (CDist) acts as marker of arterial elasticity and flow-mediated dilation (FMD) estimated endothelial function. Median values were used as cut-points for high cold-induced BAT activity (BAT GU>2.20 μmol/100g/min and perfusion>8.3 ml/100g/min).

Results: At follow-up, maximum IMT was 758±137 μm, Cdist was 93±48 μm/100 mmHg and maximum FMD was 6.0±6.2%. In high BAT activity group, bulbus IMT was lower (645±156 vs. 797±139 μm, P=0.017) and CDist higher (110±51 vs. 69±30 μm/100 mmHg, P=0.015) compared to low BAT activity groups. Baseline BAT perfusion associated with maximum cIMT at follow-up (β=-0.36, P=0.04) independently of 10-year Framingham cardiovascular disease risk score at baseline.

Conclusion: BAT activity at baseline correlated inversely with measurements of subclinical atherosclerosis at 5-year follow-up in asymptomatic

adults. Thus, cold-activated BAT glucose metabolism and perfusion may act as markers of low subclinical atherosclerosis in humans.
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Disclosure: **J.R.H. Raiko:** Grants; the South-Western Finland Cultural Foundation, the Finnish Medical Foundation, the Paulo Foundation, the Jalmari and Rauha Ahokas Foundation, the Research Foundation of Laboratory Medicine.

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Serum levels of angiogenic growth factors in subjects with type 2 diabetes with and without peripheral artery disease

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Background and aims: A growing body of evidence indicates abnormalities of angiogenesis in hyperglycemic conditions. However, the features of humoral regulations of angiogenesis in diabetic macrovascular complications remain to be clarified. The aim of our study was to assess the panel of circulating regulators of angiogenesis in type 2 diabetic subjects in relation to peripheral artery disease (PAD).
Materials and methods: We observed 196 patients with type 2 diabetes, 43 M/153 F, 43-70 years of age. The presence of PAD was verified by duplex ultrasound. The levels of vascular endothelial growth factor A, C and D (VEGF-A, VEGF-C, VEGF-D), placental growth factor (PLGF), angiopoietin-2, epidermal growth factor (EGF), heparin-bound EGF-like growth factor (HB-EGF), endoglin, soluble Fas ligand (sFASL), insulin-like growth factor-binding protein-1 (IGF-BP1), transforming growth factor α (TGF-α), and urokinase plasminogen activator (uPA) in blood serum were assessed by Multiplex assay and compared to control (25 healthy subjects matched by age and sex).
Results: The ultrasound sings of PAD were detected in 97 observed diabetic subjects, including 11 subjects with critical limb ischemia (CLI). Patients with PAD, as compared to those without, had increased serum levels of VEGF-A (p=0.004), VEGF-D (p=0.003), PLGF (p=0.0002), endoglin (p=0.03), sFASL (p=0.01), HB-EGF (p=0.01), TGF-α (p=0.0009) and uPA (p=0.02). The concentrations of EGF and angiopoetin-2 tended to be increased (p=0.1 and p=0.06 respectively). The levels of other regulators demonstrated no significant differences between diabetic groups. Among patients with PAD, the presence of CLI was associated with higher levels of VEGF-A (p=0.02) and endoglin (p=0.047). When compared to control, diabetic patients with non-affected low limb arteries demonstrated decreased concentrations of VEGF-A (p=0.04), VEGF-C (p=0.02), endoglin (p=0.03), PLGF (p=0.03), and uPA (p=0.01). Meantime, no differences were observed between diabetic subjects with PAD and healthy individuals. The concentrations of angiogenic factors were not related to age, body mass index, diabetes duration and glycaemic control.
Conclusion: The obtained results demonstrate the failure of humoral regulation of adaptive angiogenic response in type 2 diabetic subjects with PAD.
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Disclosure: **D.M. Bulumbaeva:** None.

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Fatty acid binding protein-4 predicts MACE events in PAD patients only in those without diabetes

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Background and aims: The adipokine fatty acid binding protein-4 (FABP4) is primarily produced by adipocytes and macrophages. FABP4 increases in type 2 diabetes mellitus (T2DM) and is associated with diabetic nephropathy. It promotes atherosclerosis development in the animal model and is associated with coronary artery disease plaque severity. This study evaluates a possible influence of FABP4 on cardiovascular outcome in patients suffering from peripheral artery disease (PAD) in relation to glucose metabolism.
Materials and methods: This study included 327 PAD patients (109 female, 218 male, Fontaine stage I-II, 124 T2DM). Patients were followed for the occurrence of cardiovascular events (myocardial infarction, stroke and death) for five years. Baseline fasting FABP4 was assessed using bead-based multiplex assay with a sensitivity of 95.7 pg/mL. Mann-Whitney U test, univariate and multivariable regression modeling were applied as appropriate.
Results: FABP4 serum levels were significantly lower in patients without diabetes (15.3 (9.1, 21.5) ng/mL) compared to PAD patients with T2DM (17.2 (10.9, 25.6) ng/mL, p=0.01). FABP4 was significantly linked to fasting insulin (R=0.202, p=0.002) and fasting C-peptide (R=0.405, p<0.001). Additionally, FABP4 was significantly connected to 2h-oGTT insulin (R=0.190, p=0.001), 2h-oGTT C-peptide (R=0.266, p<0.001), and 2h-oGTT glucose (R=0.120, p=0.033) of the oral glucose tolerance test. Serum FABP4 levels showed a significant association with the classical MACE endpoint (death, non-lethal myocardial infarction, non-lethal stroke) (p=0.038). In subgroup analysis revealed a more pronounced association of MACE and FABP4 in those without diabetes (p=0.002) resulting in a HR of 1.7 (95% CI: 1.22-2.42) per intra-quartile range increase. This association not significantly modified by adjustment for patient age, gender, LDL-cholesterol, systolic blood pressure, body mass index, c-reactive protein, kidney function (MDRD eGFR), and fasting insulin levels (HR 1.83 (95% CI 1.08-3.10, p=0.026). In the T2DM subgroup no significant link between FABP4 and MACE was found (p=0.873).
Conclusion: This is the first report on the impact of elevation of circulating FABP4 levels on cardiovascular events in peripheral artery disease. However, this association of FABP4 and MACE is only present in the absence of T2DM irrespective of other established risk factors.
Disclosure: **C. Hoebaus:** None.

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Empagliflozin reduces LV mass and improves diastolic function in an experimental model of heart failure with preserved ejection fraction

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Background and aims: Hypertension and volume overload are key factors that contribute to the diabetic patient's propensity to develop heart failure with preserved ejection fraction (HFpEF) wherein the expansion of extracellular fluid volume not only precipitates the congestive manifestations of heart failure, dyspnoea and oedema, but also leads to adverse cardiac remodelling that further exacerbate the extent of cardiac dysfunction. We hypothesised that by inducing a mild osmotic diuresis and thereby reducing volume overload, SGLT2 inhibition with empagliflozin would attenuate the adverse functional and structural manifestations in an experimental model of HFpEF, the uni-nephrectomised deoxycorticosterone-salt (DOCA Salt) rat model.

Materials and methods: Experiments were conducted in male Sprague-Dawley rats according to standard protocol. Rats underwent unilateral nephrectomy (UNX) followed one week later by the administration of DOCA with 1% saline as drinking water ad libitum. Animals were randomised to receive either empagliflozin or vehicle with administration to coincide with the DOCA implants and followed for a further 4 weeks. Cardiac function was assessed by echocardiography and conductance catheterisation just prior to termination.

Results: When compared to control UNX animals, DOCA salt rats developed hypertension, signs of volume overload and HFpEF as manifested by significant ($p<0.05$) (i) increased systolic blood pressure; (ii) increased heart weight; (iii) reduced haematocrit, a marker of volume overload; (iv) increased lung weight, an index of pulmonary interstitial ± alveolar oedema; and (v) prolonged Tau and reduced dp/dt min, markers of dysfunction in the early, energy-dependent phase of diastole. See Table 1. Without affecting blood pressure, empagliflozin significantly ($p<0.05$) reduced heart weight, increased haematocrit, lessened lung weight and attenuated the abnormalities in Tau and dp/dt min when compared with untreated DOCA rats. In addition, empagliflozin also increased ejection fraction.

Conclusion: DOCA salt UNX animals are a model of heart failure with preserved ejection fraction demonstrating evidence of diastolic dysfunction with pulmonary congestion. Empagliflozin therapy improved LV mass and reduced diastolic dysfunction, key markers of HFpEF. The role of SGLT2 inhibition as a strategy for treating HFpEF merits further investigation.

TABLE 1

Animal characteristic and PV-loop parameters

	UNX + control	UNX+ empa	DOCA + control	DOCA + empa
N	8	7	16	15
Body weight (g)	542.4 ± 25.2	489.7 ± 12.6*	423.1 ± 12.8*	448.0 ± 9.9*†
SBP (mmHg)	116.6 ± 1.0	111.7 ± 1.8	194.3 ± 10.5*	181.6 ± 5.5*
U Glu (mmol/d))	0.03 ± 0.02	2.91 ± 0.3*	0.15 ± 0.04	8.15 ± 0.75*†
HbA1c	4.92 ± 0.18	4.66 ± 0.07	4.02 ± 0.02*	4.12 ± 0.09*
HW/TL (mg/mm)	31.03 ± 1.77	27.85 ± 0.71	41.06 ± 1.59*	33.09 ± 0.78†
LW/TL (mg/mm)	38.85 ± 1.01	37.35 ± 0.69	44.07 ± 1.39*	37.88 ± 1.18†
Hct	42.13 ± 0.7	41.0 ± 0.6	32.13 ± 1.8*	36.5 ± 0.8*†
Tau (msec)	11.55 ± 0.24	12.7 ± 0.67	15.05 ± 0.42*	13.46 ± 0.64*†
EF (%, echo)	80.14 ± 2.13	80.28 ± 1.82	84.10 ± 1.93	88.39 ± 1.41*
dP/dt_{min} (mmHg/s)	-7596 ± 441	-7387 ± 317	-5580 ± 546*	-6504 ± 547

Legend: N = Number pre group; LV is left ventricular weight, TL is tibial length; SBP is systolic blood pressure., U Glu is urinary glucose; HW is heart weight; LW is lung weight, Hct is hematocrit, EF is ejection fraction; dpdtmin is rate of change of LV pressure. * $p < 0.05$ vs. UNX + control group; † $p < 0.05$ vs. DOCA + control group.

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Effects of saturated, mono- and poly-unsaturated fatty acids and effects of the GLP-1 analogue exendin-4 on survival of human cardiospheres and cardiac progenitor cells

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Background and aims: Mono- and polyunsaturated fatty acids exert beneficial effects on the cardiovascular system, whereas saturated fatty acids are associated with cardiovascular damage. The vitality of human cardiac progenitor cells (hCPC) and human cardiospheres (hCS) is essential for healthy myocardial tissue homeostasis. GLP-1 and its analogues exert prosurvival effects in cardiac cells. Thus, we investigated the effects of saturated, mono- and poly-unsaturated fatty acids and the protective effects of the GLP-1 analog exendin-4 on the viability of hCPC and hCS.

Materials and methods: hCPC and hCS were isolated from human right auricle biopsies and exposed to different concentrations of palmitate, oleate or eicosapentaenoic acid (EPA) up to 24 h. Apoptosis was assessed by caspase-3 cleavage and ELISA. Autophagy was evidenced by autophagosome labeling and immunoblotting of LC3-II and beclin1. Ceramide accumulation was evidenced by immunofluorescence. The expression of ceramide synthase-5 (CerS5), a key enzyme in *de novo* synthesis of ceramide, was studied by quantitative RT-PCR and immunoblotting.

Results: Palmitate, but not oleate or EPA, induced apoptosis of hCPC. Exposure to palmitate was also associated with impaired hCS isolation and increased hCPC autophagy, as well as it augmented CerS5 expression and ceramide accumulation in hCPC. However, both CerS5 inhibition with fumonisin-B1 and CerS5 knockdown with a specific siRNA reduced palmitate-induced apoptosis and autophagy. Noteworthy, pretreatment with exendin-4 preserved hCS isolation and reduced the palmitate-induced CerS5 expression, ceramide biosynthesis and hCPC apoptosis and autophagy. Co-incubation with the GLP-1 receptor (GLP-1R)

antagonist exendin(9-39), or inhibition of GLP-1R signaling with the PKA inhibitor H89 or by siRNA-mediated knockdown of the GLP-1R fully abolished the ability of exendin-4 to prevent apoptosis and autophagy.
Conclusion: Palmitate promotes apoptosis in hCPC, whereas oleate and EPA appear to have no impact on hCPC viability. Exendin-4 prevents the palmitate-induced abnormalities of hCPC and hCS by counteracting ceramide generation. Hence, GLP-1 and its analogues may limit the lipotoxic damage in the human heart by preserving the viability of myocardial progenitors.
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Disclosure: **R. D'Oria:** None.

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Anti-atherogenic effects of liraglutide independent of the AMPK pathway in diabetic apolipoprotein E-null mice

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Background and aims: Accumulating evidence supports the anti-atherogenic effect of glucagon like peptide (GLP)-1 in addition to its glucose lowering effect. Among various mechanisms proposed, AMP-activated protein kinase (AMPK) has been demonstrated as a central molecule mediating this effect of GLP-1. However, whether GLP-1 could suppress atherosclerosis independent of the AMPK pathway remains unclear.
Materials and methods: Male apolipoprotein E-null mice (BalBc-background) were intra-peritoneally injected with 100 mg kg^{-1} d^{-1} of streptozotocin for 5 consecutive days at 15 w/o, following which, they were again injected with streptozotocin at 50 mg kg^{-1} d^{-1} at 17 w/o. At 20 w/o, mice with blood glucose levels over 11 mmol/L were used for experiments. The diabetic mice were switched to a western diet (0.15% cholesterol and 30% fat), and were subcutaneously implanted with two osmotic pumps for agent delivery: one for saline or liraglutide 17 or 107 nmol kg^{-1} d^{-1} (low and high dose, respectively) and the other for saline or an AMPK inhibitor dorsomorphine hydrochloride (25 mg kg^{-1} d^{-1}). Thioglycolate-induced peritoneal macrophages and vessel samples were collected after 4 weeks.
Results: The diabetic mice showed severe hyperglycaemia (fasting blood glucose, 15±2 mmol/L; HbA1c, 8.9±0.4%) and dyslipidaemia (total cholesterol, 12.7±0.2 mmol/L). Although HbA1c levels tended to be lower in liraglutide-treated mice, there was no significant difference in physiological and biochemical parameters between the groups. Both doses of liraglutide reduced atherosclerotic plaque burden (oil red O staining) and intra-plaque macrophage accumulation (MOMA-2 staining) at the aortic sinus by approximately 50%. In addition, plaque area on the aortic surface was lower in liraglutide-treated mice than those in saline-treated mice. Treatment with the AMPK inhibitor enhanced atherosclerosis compared to that observed with saline treatment, without affecting physiological and biochemical parameters. In the mice co-treated with the AMPK inhibitor, anti-atherogenic effects of low-dose liraglutide were completely abolished, while those of high-dose liraglutide were preserved. In the right brachiocephalic artery, an atherosclerotic lesion-prone site, both doses of liraglutide reduced the expression of interleukin-6 and monocyte chemotactic protein-1 as assessed by real time PCR. High-dose liraglutide suppressed the expression of these molecules in the presence of the AMPK inhibitor, while low-dose liraglutide failed to do so. In the induced peritoneal macrophages, high-dose liraglutide also suppressed the expression of pro-inflammatory cytokines in the presence of the AMPK inhibitor.
Conclusion: We demonstrated that both AMPK-dependent and independent mechanisms are involved in the anti-atherogenic effects of liraglutide, and that a higher dose of liraglutide is required to exert anti-inflammatory effects independent of AMPK.
Disclosure: **M. Koshibu:** None.

1147

GLP-1 secretion after myocardial infarction is amplified by linagliptin and leads to improved left ventricular function and mitochondrial respiratory capacity

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Background and aims: The incretin hormone GLP-1 holds cardioprotective efficacy and was recently found to be increased by inflammatory stimuli. This study was performed to characterize the secretion of GLP-1 in response to myocardial infarction in mice and investigate its functional relevance.
Materials and methods: Myocardial infarction (MI) was induced by permanent LAD ligation in 6 week old, male C57BL/6J mice, the dipeptidylpeptidase-4 (DPP-4) inhibitor linagliptin (3 mg/kg p.o., bid) was given for 3 days before to LAD ligation, GLP-1 and exendin-9 (both 100 nM/kg i.p.) were given for 1 day before LAD ligation, experiments were performed in wild type and GLP-1 Receptor KO mice.
Results: Myocardial infarction (MI) led to a significant increase of circulating GLP-1 concentrations (from 7.9 pM to a maximum of 20.8 pM after 6 hours; n=6; $p<0.05$ in comparison to baseline and sham control). Prevention of GLP-1 degradation by pretreatment with linagliptin increased left ventricular contractility (10101± 1690 dp/dt by Millar catheter) relative to control (7830 ± 1445 dp/dt; $p<0.05$ n=8) 6h post MI, while antagonism of the GLP-1 receptor (exendin-9; 100 nM/kg i.p., 1 day pretreatment) worsened contractility (6469 ± 944 dp/dt; $p< 0.05$ n=7). Further, linagliptin failed to improve left ventricular function in GLP-1 receptor KO mice demonstrating a GLP-1 receptor-dependent effect. Mechanistically we found linagliptin or GLP-1 pretreatment to similarly increase myocardial AMPK-activation in non-infarcted tissue (1.6 fold induction by linagliptin; $p< 0.01$ n=6; 1.5 fold induction by GLP-1; $p<0.04$ n=4;), which was associated with improved respiratory capacity of isolated mitochondria from non-infarcted myocardial tissue (2 fold induction by GLP-1; $p<0.04$ n=6; 1.7 fold induction by DPP-4 inhibition; $p< 0.04$ n=7) detected by Clark electrode.
Conclusion: Myocardial infarction is a GLP-1 secreting stimulus, which improves left ventricular function in a GLP-1 receptor dependent manner. This is amplified by linagliptin dependent DPP-4 inhibition leading to AMPK-activation and improved mitochondrial respiration of cardiomyocytes in non-infarcted tissue.
Supported by: Boehringer Ingelheim
Disclosure: **M. Lehrke:** Grants; Boehringer Ingelheim.

1148

Effect of liraglutide on physical performance in type 2 diabetes (LIPER2): a randomised, double-blind, controlled trial

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Background and aims: Preclinical studies and small clinical trials suggest that glucagon-like peptide 1 (GLP1) may have a positive effect on ventricular function. A clinical trial showed reduced cardiovascular

events in patients with type 2 diabetes treated with the GLP1 analogue liraglutide. The aim of this study was to assess the effect of liraglutide on measures of cardiac function and physical performance in patients with type 2 diabetes.

Materials and methods: LIPER 2 is a phase IV, randomized, double-blind, placebo-controlled, parallel-design trial. Patients with type 2 diabetes and an HbA1c of 7-10% on oral agents (except DPP4 inhibitors) and/or intermediate/long-acting insulin were randomised (computer-generated sequence, ratio 1:1) to receive liraglutide 1.8mg/d vs placebo for 6 months. The primary end-point was the maximal oxygen consumption (VO2 max) during a cycle ergometry. Other end-points included distance covered during a 6-min walk test, left ventricular ejection fraction and other measures of ventricular systolic and diastolic functions assessed by echocardiography (following international guidelines), heart rate, blood pressure, pro-brain natriuretic peptide, C-reactive protein, HbA1c, lipids, apolipoprotein B, body weight and waist girth. Safety end-points were also monitored. Intention to treat analysis was performed (all randomised patients included). Last observation carry forward was performed for missing data.

Results: Twenty four patients (15 women), aged 52 (11.7) years, with 8.7 (5.8) years' diabetes duration, BMI 34.98 (6.2) Kg/m^2, HbA1c 8.2 (0.68)% were randomised to liraglutide (12) or placebo. There were no differences in VO_2máx (17.98 (4.8) vs 15.90 (4.96) ml/Kg/min, p>0.1), VE/VCO2 slope (30.18 (4.8) vs 32 (4.49)), left ventricular ejection fraction, measures of diastolic function such as E/E', or in the 6 min walk test (530.7 (86) vs 503.9 (84) metres) at 6 months. There was a trend towards lower maximal systolic blood pressure during the ergometry (171.7 (24.4) vs 192.5 (25.6) mmHg, p=0.052), as well as lower HbA1c (6.7 vs 7.7% p=0.005) at the end of the study in the liraglutide group. There were no severe adverse events. All the patients receiving liraglutide (1 drop-out) and 25% of those receiving placebo reported gastro-intestinal symptoms. Amylase and lipase were higher in the treated group.

Conclusion: In this small study, liraglutide improved glycaemic control in type 2 diabetes, but did not show significant effects on physical performance or myocardial function. Gastrointestinal symptoms were very common.

Clinical Trial Registration Number: UTN: U1111-1128-8762

Supported by: Investigator Initiated Study. Funded by Novo Nordisk

Disclosure: **A.M. Wägner:** Grants; Investigator-Initiated trial, Supported by NovoNordisk.

1149

Effect of cilostazol, a phosphodiesterase III inhibitor, on coronary artery stenosis and plaque characteristics in patients with type 2 diabetes

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Background and aims: Cilostazol is an antiplatelet agent that inhibits phosphodiesterase III, increases cAMP concentrations and consequently inhibits platelet aggregation. Cilostazol also has beneficial effect on vascular smooth muscle cell, endothelial cell and lipid metabolism. Therefore, it may be effective in treatment of macrovascular complication in diabetes. We performed a prospective interventional study to evaluate the effect of cilostazol compared with aspirin in Korean diabetic patients with subclinical coronary atherosclerosis.

Materials and methods: One hundred diabetic patients (64 men, ages 60.9 ± 9.1 years) who had mild to moderate atheroma evaluated with coronary multidetector-row CT (MDCT) were randomly assigned to either cilostazol 200 mg/day (CTZ group) or aspirin 100 mg/day (ASA group) (n = 50 each) for 12 months. Coronary artery calcium score (CACS) and coronary artery stenosis and plaque volume were investigated. Primary outcome was change of coronary artery disease assessed by coronary MDCT. Secondary outcomes included change in risk factors of atherosclerosis such as glucose and lipid metabolism and inflammatory parameter.

Results: The CACS was increased in both groups (316.6 ± 525.9 to 372.2 ± 575.2 in CTZ group, p <0.05 and 328.0 ± 481.7 to 388.5 ± 515.2, p <0.05 in ASA group). In CTZ group, there was significant decrease in maximal coronary stenosis (48.1 ± 17.9 to 38.8 ± 24.6%, p = 0.010), however, there was a small insignificant decrease in ASA group (41.7 ± 14.3 to 39.5 ± 13.3%, p = 0.118). The total plaque volume decreased from 75.2 ± 53.1 to 64.8 ± 52.2 mm^3 in CTZ group and slightly decreased from 74.9 ± 56.5 to 72.4 ± 55.3 mm^3 in the ASA group (p = 0.020 and p = 0.867, respectively). Furthermore, in CTZ group, non-calcified plaque volume decreased significantly (17.6 ± 9.5 to 11.5 ± 3.6 mm^3, p = 0.037) and calcified plaque volume also decreased but statistically insignificant (59.7 ± 48.8 to 52.9 ± 47.2 mm^3, p = 0.074). However, in ASA group, there were insignificant differences both in non-calcified and calcified plaque volumes (p >0.05 for all). Triglycerides and HDL-cholesterol improved significantly in CTZ group (135.5 ± 68.3 to 114.6 ± 46.5 mg/dL, p = 0.017 in triglycerides and 47.8 ± 10.1 to 51.2 ± 10.4 mg/dL, p = 0.011 in HDL-cholesterol).

Conclusion: The present study demonstrated that cilostazol treatment decreased coronary artery plaque, particularly in noncalcified portion and improved lipid profile. Cilostazol is a potential treatment option for preventing the progression of coronary atherosclerosis in patients with diabetes.

Clinical Trial Registration Number: NCT02266030

Disclosure: **D. Lee:** None.

1150

Higher one-year mortality in patients with diabetes and ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention

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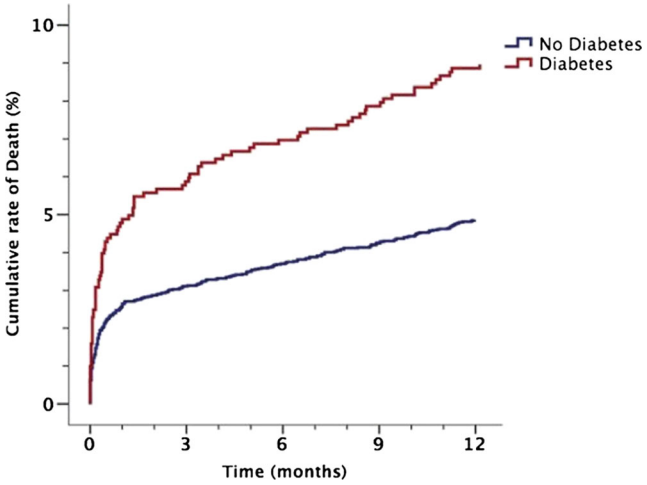
Background and aims: Patients with diabetes mellitus have a worse prognosis after acute coronary syndromes than patients without diabetes. Outcomes in patients with diabetes after ST-segment elevation myocardial infarction (STEMI) in the era of modern interventional treatment and antiplatelet therapy are less well studied. The aim is to characterise outcomes and complications in a contemporary population with diabetes and STEMI undergoing primary percutaneous coronary intervention (PCI).

Materials and methods: In the registry-based randomised Thrombus Aspiration in ST-Elevation myocardial infarction in Scandinavia (TASTE) trial, 7244 patients with STEMI were randomised to undergo manual thrombus aspiration followed by PCI or to undergo PCI alone. Thrombus aspiration did not affect mortality at one year in the 1005 patients (13.9%) with diabetes [Hazard ratio (HR) 1.04; CI 0.69-1.58, p=0.839]. Therefore, all patients with diabetes, irrespective of randomisation in TASTE, were studied as one cohort. All patients were followed for incidence of all-cause mortality, myocardial infarction or stent thrombosis until one year after index event. HRs were calculated using a Cox proportional hazard regression model adjusted for comorbidities.

Results: Patients with diabetes were older (mean age 67.6 vs 66.0 years, p<0.001), more often had a previous myocardial infarction (19.9 vs 10.3%, p<0.001) and undergone previous PCI (17.3 vs 8.4%, p<0.001).

Thrombus grade did not differ between patients with and without diabetes (Grade 0 to Grade 5, p=0.909) and neither did the type of affected coronary vessel. Pharmacological cardiovascular treatment did not differ between groups, but the use of drug eluting stents was higher in patients with diabetes (59.0 vs 48.4%, p<0.001). After adjustment for comorbidities, diabetes independently increased the risk for mortality (HR 1.57; CI 1.23-2.00, p<0.001), but was not an independent risk factor for future myocardial infarction or stent thrombosis.

Conclusion: Diabetes remained an adverse prognostic risk factor in this contemporary setting, resulting in increased one-year mortality in a large cohort of patients with STEMI treated with PCI. This was not influenced by thrombus aspiration and not explained by a higher thrombus burden or differences in cardiovascular medical therapy compared to patients without diabetes.



Clinical Trial Registration Number: NCT01093404
Disclosure: **P. Lundman:** None.

1151

Visit-to-visit blood pressure variability and cardiovascular outcomes in patients with type 2 diabetes following acute coronary syndromes in the EXAMINE trial

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Background and aims: Primary cardiovascular (CV) prevention studies have shown that high visit-to-visit systolic BP variability (VVV) may be associated with an increased risk of CV events. However, little is known about VVV of the BP and secondary CV outcomes in a very high CV risk population.

Materials and methods: We evaluated CV event rates in EXAMINE, a CV outcomes safety trial in 5380 patients with type 2 diabetes following acute coronary syndromes (ACS) randomized to the DPP-4 inhibitor alogliptin or placebo, according to VVV of the SBP calculated during the first 6 months post-randomization. The risks of major adverse CV events (MACE) and CV death or heart failure (HF) in all randomized patients were analyzed using a Cox proportional hazards model with adjustment for baseline covariates in quartiles of systolic BP VVV from 6 months to the end of the post-randomization period. The lowest (1st) quartile of VVV was the reference group. Events were prospectively adjudicated by an independent committee blinded to treatment assignment.

Results: Mean systolic BPs were higher in quartile 4 (133 mmHg) but quartiles 1, 2 and 3 were the same (128 mmHg). Baseline history of HF was higher in quartile 1 (34%) than in the quartiles 2, 3, and 4 (24-26%). Other clinical and demographic characteristics at baseline were comparable in the quartile groups of VVV of the BP. The rates of MACE were higher in quartiles 2 through 4 of VVV vs. quartile 1; comparable findings were found for CV death or HF compared to quartile 1 [Table]. Results for the event components of all-cause mortality, cardiovascular death, hospitalized heart failure, non-fatal myocardial infarction, stroke also had similar patterns to MACE.

Conclusion: In patients with type 2 diabetes and a recent ACS, systolic BP VVV was associated with worsened CV outcomes. Potential mechanisms of increased VVV of the BP include arterial stiffness, endothelial dysfunction/subclinical inflammation, and poor adherence to antihypertensive therapy. Studies designed to better understand the reasons for high VVV of BP may provide clinically useful information for reducing CV events in high CV risk patients.

SBP VVV group	Number of patients	Incident rate for MACE (%)	Adjusted MACE HR (95% CI) for quartiles of systolic BP VVV*	Incident rate for CV death or heart failure (%)	Adjusted CV death or HF HR (95% CI) for quartiles of systolic BP VVV*
Quartile 1	1200	7.4	**Reference**	4.7	**Reference**
Quartile 2	1191	12.0	1.60 [1.15, 2.23]	8.9	1.70 [1.12, 2.59]
Quartile 3	1222	15.7	1.43 [1.02, 1.99]	9.6	1.45 [0.95, 2.22]
Quartile 4	1203	16.8	1.85 [1.35, 2.55]	13.4	1.99 [1.33, 2.97]

Clinical Trial Registration Number: NCT00968708
Supported by: Takeda Development Center, North America
Disclosure: **W.B. White:** Employment/Consultancy; Chair, EXAMINE Steering Committee, Takeda Development Center North America.

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Total cardiovascular events analysis of the EXAMINE trial of patients with type 2 diabetes and recent acute coronary syndrome

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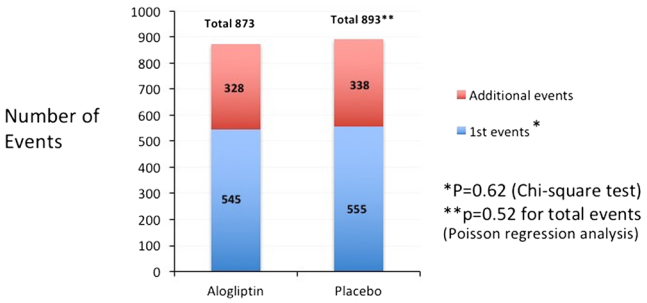
Background and aims: The Examination of Cardiovascular Outcomes with Alogliptin versus Standard of Care (EXAMINE) trial showed that alogliptin, an inhibitor of dipeptidyl peptidase 4, did not increase the rates of major adverse cardiovascular events versus placebo in patients with Type 2 Diabetes (T2DM) and recent acute coronary syndrome (ACS). Published analyses reported time to first event. We now report first, recurrent and total major adverse cardiovascular events (MACE) and components to provide a comprehensive picture of total cardiovascular outcomes.

Materials and methods: Patients with T2DM with a diagnosis of ACS within the previous 15 to 90 days were randomly assigned to alogliptin vs placebo and followed for a median of 18 months. We evaluated the composite

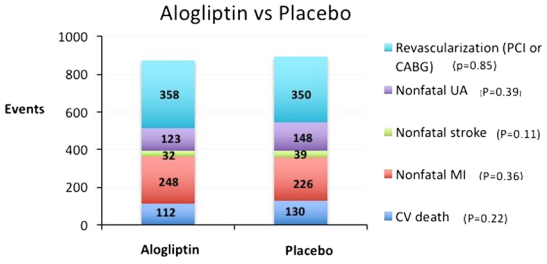
endpoint of CV death, nonfatal MI, nonfatal stroke, unstable angina or revascularization. We analyzed first, recurrent and total events during follow-up.
Results: There were 1100 first events and 666 additional events (328 in alogliptin and 338 in placebo). There was no significant difference in the number of first (Kruskal-Wallis test, p=0.54) or total events (Poisson regression, p=0.52) in the alogliptin vs placebo groups (Figure 1A). There were also no significant differences between treatments with respect to distribution of types.(Figure 1B).
Conclusion: Among a group of patients with T2DM and recent ACS, treatment with alogliptin did not increase the risk of 1st or total events as compared with placebo. These data support the cardiovascular safety of continued alogliptin therapy in a population with DM and CAD at high risk for recurrent MACE.

Figure 1A:
Number of First, Additional and Total events,
Alogliptin vs Placebo



There was no significant difference in the composite outcome between the two groups by the Finkelstein-Schoenfeld analysis (p=0.55)

Figure 1B
Total Number of Primary Endpoint Events by Type,
Alogliptin vs Placebo



There were no differences in types of events between the Alogliptin and Placebo groups
(p values per Poisson regression analysis)

Clinical Trial Registration Number: NCT00968708
Supported by: Takeda Pharmaceutical Company Limited
Disclosure: **C.P. Cannon:** Employment/Consultancy; Modest, CSL Behring, Essentials, Takeda. Grants; Takeda, Accumetrics, Arisaph, Astra Zeneca, Boehringer-Ingelheim, GlaxoSmithKline, Janssen, Merck, Regeneron, Sanofi.

PS 102 Cardiac complications, prediction and prognosis

1153

Exogenous H_2S improves heart function in streptozotocin-induced type 1 diabetes

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Background and aims: Hydrogen sulfide (H2S) is a gas transmitters, that has important role in the regulation of cardiovascular function.We have recently shown that exogenous H2S improves diastolic heart function in hypertension and aging. Diabetes is also accompanied by heart and endothelium dysfunction. The aim of work is to investigate the effect of hydrogen sulfide on heart function in streptozotocin-induced diabetic rats.
Materials and methods: Rats were divided into control and diabetic groups. Type-1 Diabetes mellitus was induced with a single intraperitoneally injection of streptozotocin (60 mg/kg). After 2 month animals with a random blood glucose level >15 mmol/l were considered to be diabetic and were included in the study. The functional cardiohemodynamic indicators registered via microcatheter and Pressure-Volume System. The donor of the hydrogen sulfide NaHS (15,8 mg/kg) was administered intraperitoneally.
Results: It was shown that exogenous donor of the hydrogen sulfide (NaHS) improves pumping function of diabetic heart. Stroke volume increases by 43,1% (p<0,05), cardiac output increases by 25,4% (p<0,05). Ejection Fraction increses by 48,6% (p<0,05). We found,that after NaHS end-systolic pressure decreases by 17,2% and index of contractility was decresed. The end-diastolic myocardial stiffness decreased by 20,1% (P<0,05) in streptozotocin - induced diabetic rats. After NaHS the arterial stiffness was decreased by 24%, that indicates an improvement the ventriculo-arterial coupling.
Conclusion: Thus, NaHS improves pumping heart function, end-diastolic myocardial stiffness and arterial stiffness reduced and end-systolic pressure decresed in streptozotocin - induced diabetic rats.
Disclosure: **N. Dorofeyeva:** None.

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Plasma copeptin, a surrogate of vasopressin, and risk for cardiovascular morbidity and mortality in people with type 2 diabetes

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Background and aims: Diabetic nephropathy is associated with increased risk of cardiovascular morbidity and mortality in people with diabetes. Experimental evidence supports a causal role for vasopressin (or antidiuretic hormone) in the development of chronic kidney disease and diabetic nephropathy through V2 receptor activation. Plasma copeptin, the COOH-terminal portion of pre-provasopressin and a surrogate marker of vasopressin, was shown to be positively associated with the development and progression of diabetic nephropathy, and with end stage renal disease in type 1 and type 2 diabetes. Here we assessed the association of plasma copeptin with the risk of cardiovascular events during follow-up in two prospective cohorts of French type 2 diabetic patients, and we examined if this association could be accounted for by deleterious effects of vasopressin on the kidney.

Materials and methods: We studied 3101 and 1461 unrelated type 2 diabetic patients from 2 French cohorts: DIABHYCAR and SURDIAGENE (microalbuminuria in 76% and 37%, and macroalbuminuria in 24% and 17% of participants at baseline, respectively). We considered the incidence during follow-up (median: 5 years) of a combined end point composed of CHD (myocardial infarction or coronary revascularisation), hospitalisation for congestive heart failure, and cardiovascular death. Copeptin concentration was measured in baseline plasma-EDTA samples by an automated immunoluminometric assay.
Results: The cumulative incidence of cardiovascular events was 18.7% (n=579) in DIABHYCAR and 30.5% (n=446) in SURDIAGENE. The incidence rate was 4.5 and 5.7 per 100 person-years, respectively. The cumulative incidence of cardiovascular events during follow-up by sex-specific tertiles of baseline plasma copeptin was 15.7% (T1), 18.7% (T2) and 21.7% (T3) in DIABHYCAR (p=0.002), and 20.7% (T1), 28.4% (T2) and 42.5% (T3) in SURDIAGENE (p<0.0001). Cox proportional hazards survival regression analyses confirmed the association of copeptin with cardiovascular events in both cohorts: adjusted HR with 95% CI for T3 vs. T1 was 1.39 (1.13 - 1.71), p=0.002 (DIABHYCAR), and 2.44 (1.91 - 3.13), p<0.0001 (SURDIAGENE), adjusted for sex, age, BMI, duration of diabetes, systolic and diastolic blood pressure, HbA1c, total cholesterol, and previous history of myocardial infarction at baseline. Associations remained significant when further adjusted for the estimated GFR and urinary albumin concentration (UAC) at baseline: adjusted HR 1.28 (1.03 - 1.58), p=0.03 (DIABHYCAR), and 1.66 (1.27 - 2.19), p=0.0003 (SURDIAGENE). No interaction was observed between plasma copeptin and eGFR (p=0.36) or UAC (p=0.53) categories on the risk of cardiovascular events in analyses of pooled cohorts.
Conclusion: Plasma copeptin is positively associated with severe cardiovascular events in people with type 2 diabetes. This association cannot be solely accounted for by the association of copeptin with kidney-related traits.
Disclosure: **G. Velho:** None.

1155

Hypoglycaemia increases platelet reactivity and promotes formation of proatherogenic platelet-leucocyte aggregates

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Background and aims: Hypoglycaemia is emerging as a trigger for cardiovascular (CV) events observed in intensive glycaemic control of type 2 diabetes. Potential mechanisms include inflammation-induced thrombosis. High levels of circulating monocyte and neutrophil platelet aggregates (MPA & NPA) strongly increase CV risk. We hypothesised that hypoglycaemia increases platelet reactivity and MPA & NPA levels.
Materials and methods: Sixteen healthy volunteers underwent a hyperinsulinaemic hypoglycaemic (2.5 mmol/L) or euglycaemic (6.0 mmol/L) clamp. Blood was sampled at baseline and 60 minutes. Platelet aggregation induced by ADP 6.45 μM was measured using Multiplate impedance aggregometry. MPA and NPA were phenotyped and enumerated using multi-colour flow cytometry.
Results: Platelet aggregation increased following hypoglycaemia vs euglycaemia (AUC mean ± SEM: 74 ± 7 vs 53 ± 4; P<0.05). Total MPA (median [IQR] cells/μL: 106 [41-135] vs 51 [27-71]; P<0.05) and NPA (626 [471-820] vs 237 [71-786]; P<0.05) increased following hypoglycaemia vs euglycaemia. Hypoglycaemia increased intermediate monocyte specific MPA [11 cells/μL [5-15] vs 3 [2-9]; P<0.01) and non-classical monocyte MPA (P<0.01, Fig 1) with no significant change in classical monocyte MPA.
Conclusion: Hypoglycaemia increases platelet reactivity, MPA and NPA levels and leads to increased interaction between pro-inflammatory monocyte subsets and platelets. These novel data reveal mechanisms that may contribute to CV risk associated with diabetes and its therapy.

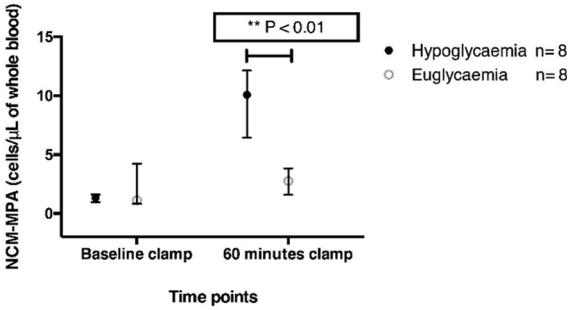

Figure 1: Effect of hypoglycaemia on the absolute number of circulating non-classical monocyte specific monocyte platelet aggregates (NCM-MPA). Data are presented as median with interquartile range.

Supported by: MRC Clinical Research Training Fellowship Award to Dr Ahmed Iqbal
Disclosure: **A. Iqbal:** Grants; Medical Research Council Clinical Research Training Fellowship.

1156

Cardiovascular disease risk prediction in type 1 diabetes

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Background and aims: The prevalence of type 1 diabetes (T1DM) continues to increase rapidly all over the world. T1DM is associated with an increased risk of cardiovascular disease (CVD). However, there are few comprehensive cohorts of subjects with T1DM and these often have small sample sizes, making it difficult to calculate event rates and build risk prediction models. We use recent data (2004-2014), on patients with T1DM, from a Scottish National dataset to calculate standardised incident CVD event rates and to build risk prediction tools.
Materials and methods: The study included 26680 adults from the Scottish population. Patient entry is defined as the latest of: 1/1/2004, date of diabetes diagnosis, date they turn 18 years of age, or date first evaluable for CVD events. Exit is defined as earliest of: 31/12/2014, death, last evaluable for events, or the date of first event. Follow-up time was split into one year intervals, where current age was time updated. Poisson regression was performed with backward selection, multivariable fractional polynomials and random forests to select the best model. Discrimination is measured using AUROC and calibration with Hosemer-Lemeshow tests.
Results: Incidence rates of CVD are falling with time, especially in the higher age bands. The overall incidence rates for males and females (standardised to the European Standard Population 2013) were 3858 and 3074 per 100,000 person years respectively. After removing patients with a prior CVD, 2449 people experienced a CVD event during follow-up, of which 1469 were male. Patients that develop CVD were older at diagnosis (and entry into the study), more likely to be ever-smokers, had higher systolic BP (SBP) and triglycerides, lower eGFR, and were more likely to be on anti-hypertensive treatment and statin therapy. A Poisson model fit using backwards elimination retained HbA_{1c}, weight, total cholesterol, SIMD, eGFR, HDL, diabetes duration, diagnosis year, current age, smoking status, gender, antihypertensive treatment, statin therapy, age^2 and age^3. This model yielded an AUC of 0.805. A forward selection model yielded an AUC of 0.804 with fewer variables. Random Forest

analysis dropped weight, height, SBP, gender, age at diabetes diagnosis, and diastolic BP as not being significant contributors to the outcome, giving an AUC of 0.802. Multivariable Fractional Polynomials gave the highest AUC at 0.809. Finally, we fit a model with age and gender interactions with all variables, and used a LASSO penalty to make the model more tractable. This gave an AUC of 0.806.

Conclusion: We show incidence rates using contemporary data on the national Scottish Type 1 diabetes population. We built models with good calibration and discrimination that can be used to calculate the individual risk estimates for patients, and this can be used for enabling clinical decisions such as initiating/stopping drug therapy.

Supported by: CH&SS

Disclosure: **B. Farran:** None.

1157

Comparison of measures of aortic stiffness in asymptomatic patients with type 2 diabetes and association with outcomes

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Background and aims: With aging there is progressive stiffening of the aorta that appears to be accelerated by risk factors including hypertension and diabetes. Cardiovascular magnetic resonance (CMR) can be used to reproducibly measure aortic stiffness without exposure to contrast agent or ionising radiation. The aim of this study was to establish if aortic stiffness measured by CMR is associated with adverse outcomes in asymptomatic patients with type 2 diabetes and to establish if it can be improved on treatment with renin-angiotensin-aldosterone (RAAS) inhibition.

Materials and methods: 94 asymptomatic patients with type 2 diabetes and no history of cardiac, renal or established microvascular disease underwent assessment of cardiovascular risk and CMR assessment of ascending aortic distensibilty (AAD), descending aortic distensibilty (DAD) and aortic pulse wave velocity (PWV). 25 of these patients with recent onset microalbuminuria were treated with RAAS inhibition and imaging repeated after one year. All 94 patients were followed up for 2.4 years for major adverse cardiovascular disease (CVD) events including silent myocardial infarction detected on late gadolinium enhancement CMR.

Results: 19 (20%) patients had at least one CVD event on follow up including silent MI on baseline scan 15 (16%), stroke 3 (3%), cardiovascular death 2 (2%), ST elevation MI 2 (2%), silent MI on follow up scan 1 (1%), percutaneous coronary intervention 2 (2%), heart failure 1 (1%) arrhythmia 1 (1%). On logistic regression only AAD had a significant association with CVD events; hazard ratio (HR) 0.49, 95% confidence interval 0.25-0.95, P=0.01, Table. The associations of DAD and PWV did not reach significance (P=0.19 and 0.45 respectively). AAD, DAD and PWV all had a significant association with age and 24 hour systolic blood pressure but only AAD had a significant association with glycaemic control, measured as HbA1c (Beta -0.016, P=0.04). The association between HbA1c and AAD persisted even after correction for age and hypertension. On treatment with RAAS inhibition, AAD, but not DAD or PWV, showed significant improvement from 1.51±1.15 to 1.97±1.07 10^{-3}mmHg^{-1}, P=0.007.

Conclusion: Ascending aortic distensibility measured by CMR is independently associated with poor glycaemic control and adverse cardiovascular events and appears to be reversible on treatment with RAAS inhibition. AAD is a promising surrogate marker for future longer term outcome studies. Table Logistic regression of the association between aortic stiffness and clinical factors with CVD events.

	Hazard Ratio	95% CI	P Value
AAD	**0.49**	**(0.25; 0.95)**	**0.01**
DAD	0.70	(0.41; 1.20)	0.19
PWV	1.07	(0.90; 1.26)	0.45
Age	1.05	(1.00; 1.10)	0.07
Gender	0.19	(0.02; 1.52)	0.12
Body mass index	0.97	(0.86; 1.09)	0.62
Duration of diabetes	0.99	(0.88; 1.10)	0.80
HbA1c	0.98	(0.95; 1.02)	0.35
Median HbA1c since diagnosis	1.01	(0.97; 1.04)	0.75
Maximum HbA1c since diagnosis	1.00	(0.97; 1.02)	0.74
Microalbuminuria	2.19	(0.77; 6.16)	0.13
24 hour Systolic BP	1.02	(0.99; 1.06)	0.18
24 hour Diastolic BP	1.01	(0.95; 1.06)	0.80
Total cholesterol	0.89	(0.56; 1.43)	0.64
Smoking	**3.38**	**(1.03; 11.15)**	**0.05**
Serum Aldosterone	1.00	(0.99; 1.00)	0.33
High sensitivity C reactive protein	0.99	(0.90; 1.09)	0.87

Supported by: British Heart Foundation fellowships FS/12/88/29901 to PPS

Disclosure: **P.P. Swoboda:** None.

1158

Short-term progression of coronary artery calcification in type 1 diabetes compared to matched controls

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Background and aims: Type 1 diabetes is associated with increased cardiovascular (CV) morbidity and mortality by mechanisms not fully understood. Coronary artery calcium (CAC) is associated with CV disease and progression of CAC is an independent predictor of mortality. The aim of this study was to examine whether short-term progression of CAC is increased in patients with type 1 diabetes compared with matched controls.

Materials and methods: Fifty-three normoalbuminuric patients with long-term (>10 years) type 1 diabetes were matched in a 1:2 ratio with 106 controls from the general population according to age, gender, and baseline CAC score and was examined through cardiac computed tomography scans at a mean (±SD) of 25 (3) months and 29 (5) months, respectively, for re-measurement of CAC score. Progression of CAC was determined according to the square root method, where progression was defined as a change ≥2.5 between the square root transformed values of follow-up and baseline CAC volume score.

Results: Forty of the 159 individuals had progression of CAC. Among patients with type 1 diabetes, 18 (34%) had progression of CAC compared to 22 (21%) of the controls (p=0.08). Due to adherence to treatment guidelines, patients with diabetes were more often on treatment with blood pressure-lowering medication and statins. In type 1 diabetes compared to controls blood pressure was mean (±SD) of 123/74 (13/7) mmHg vs. 142/88 (21/10) mmHg (p<0.001), LDL cholesterol was 2.4 (0.6) mmol/l vs. 3.3 (0.9) mmol/l (p<0.001), and BMI was 24.5 (3.0) kg/m^2 vs. 25.8 (3.7) kg/m^2 (p=0.03). There was no difference in smoking status. In multivariable logistic regression, adjusted for these risk factors of CV disease and risk factors of progression, including scan interval, type 1 diabetes was associated with an odds ratio of 4.0 (95 % CI 1.5 - 10.9, p<0.01) for progression of CAC. In patients with type 1 diabetes, HbA_{1C} was 63 (11) mmol/mol and diabetes duration was 33 (12) years.

Conclusion: To our knowledge, this is the first study to examine short-term progression of coronary artery calcium in patients with type 1 diabetes compared with matched controls from the general population. We found that even in well-treated, normoalbuminuric patients with type 1 diabetes, there was an odds ratio of 4.0 for short-term progression of CAC. This might explain some of the increased CV morbidity and mortality in patients with type 1 diabetes.

Supported by: Arvid Nilssons Foundation and AP Moeller Foundation

Disclosure: **H.Ø. Hjortkjær:** None.

1159

Long term prognostic value of coronary computed tomography angiography in asymptomatic elderly population including diabetic patients

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Background and aims: There is still controversy about the long-term prognostic value of coronary computed tomography angiography (CCTA) for coronary heart disease (CHD) in asymptomatic individuals including diabetic patients. Also, CCTA's role among elderly population has not been established. We investigated the long-term prognostic value of CCTA in asymptomatic elderly patients with or without diabetes.

Materials and methods: A community based-cohort study of 470 asymptomatic elderly individuals (mean age 75.1 ± 7.3 years, 156 [33.2%] diabetic patients) who underwent CCTA and coronary calcium scoring (CACS) was conducted. Major adverse cardiac events (MACE) defined as composite of cardiovascular death, nonfatal myocardial infarction, unstable angina requiring hospitalization and late coronary revascularization were measured. CCTA findings were categorized by various means including the presence of proximal LAD or left main disease (pLAD/LM). CACS and conventional CHD risk factors related to Framingham risk score (Age, sex, systolic blood pressure, treatment of hypertension, diabetes, total cholesterol, HDL-cholesterol and current smoking status) were adjusted for analysis.

Results: There were 57 individuals (12.1%) with pLAD/LM findings for overall population and 18 (11.5%) for diabetic patients. During median follow up of 7.2 years (interquartile range: 6.8-7.3), MACE were occurred in 28 (6%) subjects among overall population and 10 (6.4%) among diabetic patients. Compared to individuals with negative pLAD/LM findings, positive group were significantly associated with MACE for overall population (4.4% [18 events] vs 17.5% [10 events]; hazard ratio (HR), 3.211 [95%CI 1.226-8.409], p = 0.018). Among diabetic patients, there were more significant association between pLAD/LM findings and MACE (2.9% [4 events] vs 33.3% [6 events]; HR, 14.050 [95%CI 1.406-140.388], p = 0.024).

Conclusion: Among asymptomatic elderly population, especially for diabetic patients, CCTA showed considerable prognostic value additive to conventional CAD risk factors and CACS over more than 6 years.

Disclosure: **S. Moon:** None.

1160

Risk of cardiac arrhythmias and electrophysiological responses during spontaneous hyperglycaemia in young people with type 1 diabetes

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Background and aims: Little is known about the effects of hyperglycaemia on cardiac repolarisation and risk of cardiac arrhythmias. To our knowledge, frequencies of cardiac arrhythmias during hyperglycaemia have never been studied and data reporting the relation of hyperglycaemia and changes in QT_c-intervals are conflicting. Recently published animal and tissue culture studies suggest pro-arrhythmogenic effects of hyperglycaemia. We therefore examined the effects of spontaneous hyperglycaemia in young people with type 1 diabetes (T1D) on electrophysiological responses and risk of cardiac arrhythmias.

Materials and methods: Thirty-seven individuals with T1D (age <50 years, duration of diabetes >4 years) underwent 96 hours of simultaneous ambulatory ECG and blinded continuous interstitial glucose (IG) monitoring (CGM). Relative frequencies of arrhythmias were expressed as incident rate ratios (IRR). Heart rate, heart rate variability (HRV) (high frequency and normalised low frequency power) and cardiac repolarisation parameters (QT_c, T_pT_{end}) were established during hyperglycaemia (IG ≥ 15 mmol/l, duration ≥ 20 min) and compared to time-matched euglycaemia (IG 5 - 10 mmol/l) divided into night (23.00 - 7.00) and day.

Results: A total of 2395 hours of simultaneous ECG and CGM recordings were obtained. During hyperglycaemia (Fig.1), the risk of ventricular premature beats was higher at night (IRR 7.025, p=0.001), but lower during daytime (IRR 0.343, p=0.018) compared to euglycemia. Bradycardia was less frequent during both nocturnal and daytime hyperglycaemia (IRR 0.062, p<0.001 and IRR 0.010, p=0.001, respectively). No differences in heart rate, HRV and QT_c duration were detected during nocturnal or daytime hyperglycaemia in comparison to time-matched euglycaemia. T_pT_{end} interval was shortened during nocturnal hyperglycaemia (p=0.003).

Conclusion: We found increased risk of ventricular premature beats during nocturnal hyperglycaemia, but this risk was low during daytime hyperglycaemia. Bradycardia was less common during hyperglycaemia during both night and day. No significant differences in heart rate, HRV or QTc duration were detected during hyperglycaemia which suggests different pro-arrhythmogenic mechanisms compared to hypoglycaemia. These findings are novel but require further studies to etablish their clinical significance.

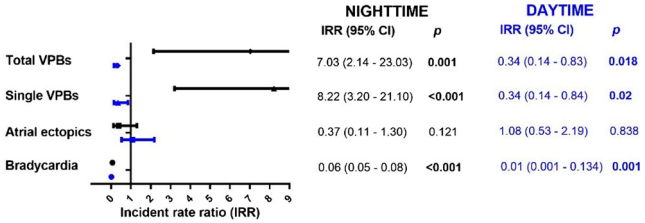

Figure 1. Relative frequencies of cardiac arrhthmias during hyperglycaemia (IG ≥ 15 mmol/l) at night and day in comparison to euglycaemia (IG 5 -10 mmol/l) expressed as incident rate ratios (IRR). VPBs - ventricular premature beats. Total VPBs - sum of single VPBs, couplets, triplets and short runs.

Supported by: partially funded by NIHR

Disclosure: **P. Novodvorsky:** Lecture/other fees; SRH reports personal fees from Sanofi Aventis, Eli Lilly, Takeda, NovoNordisk and Astra Zeneca..

1161

Prognostic differences in men and women with atrial fibrillation and diabetes: a nationwide report

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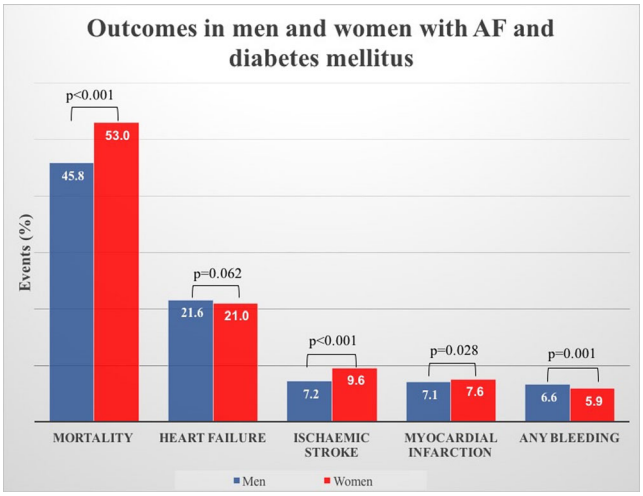
Background and aims: To the best of our knowledge, the sex-specific prognosis in patients with atrial fibrillation and diabetes mellitus is less well studied in a contemporary population with an extensive national diabetes control program. The aim was to describe nationwide complication patterns in men and women with atrial fibrillation and diabetes.
Materials and methods: All men (n= 180 861) and women (n= 145 971) in Sweden with non-valvular atrial fibrillation during 2006 to 2012 were identified, and information on events, comorbidities and pharmacological therapy was extracted using nationwide mandatory registers. Patients were followed until 31 December 2013 and the mean follow-up time was 3.7 years (0.9 to 8 years). Hazard ratios (HR) were calculated using a Cox proportional hazard regression model adjusting stepwise for age, comorbidities and medication.
Results: Diabetes was present in 18.6% of men and 16.7% of women. The most frequent events in men with atrial fibrillation and diabetes compared to women were mortality (45.8% vs. 53.0%; p<0.001), heart failure (21.6% vs. 21.0%;p=0.062), ischaemic stroke (7.2 vs. 9.6%; p<0.001), myocardial infarction (7.1 vs. 7.6%; p=0.028) and any bleeding (6.6 vs. 5.9%; p=0.001) respectively. When adjusted for age, comorbidities and medication, female sex was associated with lower risk of mortality (HR; 95% confidence interval [CI], 0.77; 0.75-0.79), combined event (first of mortality, heart failure, ischaemic stroke or myocardial infarction; 0.82;0.80-0.84), but higher risk for ischaemic stroke (1.08;1.01-1.16). The standardized mortality ratio for men with atrial fibrillation and diabetes compared to the general population was 1.85 (CI; 1.78-1.92) and for women was 2.04 (CI; 1.96-2.13). Anticoagulants were used in 50.9% vs. 42.1% of men and women respectively.
Conclusion: Women with atrial fibrillation and diabetes have higher event rates for mortality, ischaemic stroke, myocardial infarction compared to men, lower rates for any bleeding and lower rates, that did not reach statistical significance, for heart failure. However, after adjustments, female sex was associated with lower risk of all types of cardiovascular complications, after diagnosis of atrial fibrillation, except for higher risk of ischaemic stroke in women.



Supported by: Swedish Association of Diabetology and AstraZeneca
Disclosure: **S. Karayiannides:** None.

PS 103 Lipids: lowering lipids and lipodystrophy

1162

S447X polymorphism of lipoprotein lipase and its association with the distribution of triglyceride values after an oral fat tolerance test in diabetes patients

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Background and aims: Postprandial hypertriglyceridemia is an independent risk factor for coronary heart disease, as it is responsible for an atherogenic profile for at least eight hours .Postprandial hypertriglyceridemia is a multifactorial phenomenon as it is influenced by genetic and environmental factors. Lipoprotein Lipase (LPL) plays a key role in lipoprotein metabolism and influences the interaction between atherogenic lipoproteins and the receptors on the vascular wall. S447X is one of the most frequent polymorphisms of LPL in Caucasians. Its effect on LPL activity is controversial. The aim of the current study is to assess the effect of S447X polymorphism of LPL on triglyceride response after fat loading in patients with type 2 diabetes (DM2).
Materials and methods: The study population consisted of 51 males with DM2 (inclusion criteria BMI< 30 Kg/ m^2 and normal fasting plasma triglycerides [TG≤ 150 mg/dl]) who were treated with oral hypoglycemic agents with good glycemic control ($HbA_{1c} \leq 7\%$). Exclusion criteria were history of coronary heart disease and therapy with lipid lowering agents. The study group: (1) underwent an oral fat tolerance test (OFTT) with TG measured before and 2, 4, 6 and 8 hours after the test meal, and (2) was genotyped and S447X polymorphism of LPL was examined. Statistical analysis was performed with Mann-Whitney U test for comparison of numerical values between two groups (SS vs SX) . Areas under the curve (AUC) for serial measurements of TG at baseline and after OFTT were calculated using trapezoid rule. To assess the influence of of alleles S and X on TG levels we performed multiple regression analysis adjusting for age and BMI.
Results: The frequency of LPL S447X genotypes was 65,3% for SS and 34,7% for SX. The results of multiple regression analysis with TG -AUC as dependent variable showed that both alleles S and X were not related with TG-AUC in statistical significant level in total sample (S vs X , coefficient: -0.018, standard error: 154.640, p-value:0.907). In the group with positive OFTT (postprandial hypertriglyceridemia) there was a trend of association of S allele with the TG AUC (S vs X , coefficient : 0.338, standard error : 172,165, p value : 0.07)
Conclusion: S447X polymorphism of LPL is not associated with the distribution of TG values after OFTT in diabetic subjects. There was a trend of association of S allele with triglyceride distribution after fat loading only in the group with positive OFTT, but did not reach statistical significance.
Disclosure: **P. Gavra:** None.

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Anti-apolipoprotein A-I antibodies in patients with type 2 diabetes: epitope mapping

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Background and aims: In previous work, we reported the existence of anti-HDL (aHDL) antibodies and more particularly anti-apolipoprotein A-I (aApoA-I) antibodies (the main apolipoprotein contained in HDL) and anti-paraoxonase 1 (PON1, the main antioxidant enzyme in HDL) in patients with type 2 diabetes. Furthermore these antibodies may account for the HDL quantitative and/or qualitative fault as a protective factor. The identification of the antigen-binding site of the antibodies is essential for manipulate these pathways by defining new therapeutic targets. Herein we intend to determinate the main epitopes (molecule sections or peptides) recognized by the aApoA-I antibodies in patients with type 2 diabetes.
Materials and methods: Human ApoA-I was submitted to limited proteolysis using different endoproteases The proteolytic products were analyzed by SDS-PAGE and mass spectrometry. Different recombinant ApoA-I peptides:_C1 (residues D25-K231), C2 peptide (residues D25-K251), and ApoA-I full-length were produced in E. coli and purified by affinity and size exclusion chromatography. Since these three peptides only allow distinguish epitopes in the C-terminal region of Apo A-I a series of overlapping peptides were designed and constructed to span the entire sequence of Apo A-I. Immunoreactivity against these peptides was tested by ELISA in a cohort of 55 patients with type 2 diabetes.
Results: The expression and purification of ApoA-I peptides was achieved successfully. The preliminary data regarding the scan of immunoreactivities against these peptides suggest that patients with type 2 diabetes presented a significant decrease in immunoreactivity against the C1 peptide which suggests that the majority of the anti-ApoA-I antibodies present in these patients recognize an epitope in the C-terminal region of protein (Lys^{206}-Gln^{243}).
Conclusion: The identification of specific epitope(s) recognised by the anti-Apo A-I antibodies, may lead to a better understanding of their pathogenic role and potentially highlight novel targets for therapeutic intervention.
Supported by: FCT - SFRH/BPD/112411/2015 and iNOVA4Health - UID/Multi/04462/2013
Disclosure: **B.R. Joana:** None.

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Increase in apolipoprotein A-II levels is associated with lower cardiovascular risk in the ACCORD Lipid Trial

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Background and aims: Despite the disappointing results of fibrates, CETP-inhibitors, and niacin cardiovascular disease (CVD) trials, recent genetic and epidemiological studies have reinvigorated the possible beneficial effect of improving lipid profile beyond LDL-cholesterol reduction. The aim of our study was to assess the relationship between temporal changes in lipids and apolipoproteins levels and CVD incidence in the Action to Control Cardiovascular Risk in Diabetes (ACCORD) Lipid Trial.
Materials and methods: 5518 subjects with type 2 diabetes were randomized to fenofibrate + simvastatin or simvastatin alone. Hazard Ratios of MACE occurrence were estimated for 1-S.D. change from baseline to the average on-trial value of triglycerides, HDL-cholesterol, LDL-cholesterol, nonHDL-cholesterol, and 1-S.D. change from baseline to 12-months apolipoproteins (Apo A-I, Apo A-II, Apo B and Apo C-III) plasma levels. Covariates included gender, age, study treatment arms, CVD history, clinical center, and baseline levels of the lipid biomarker. Apolipoproteins levels at baseline and 12 months after randomization were available for 1683 of these subjects.
Results: Associations with lower CVD risk were observed for decreases in LDL-c (HR 0.86; 95% CI 0.76-0.98), nonHDL-c (HR 0.84; 95% CI 0.75-0.94), and triglycerides (HR 0.90; 95% CI 0.81-0.996), and an increase in HDL-c (HR 0.79; 95% CI 0.72-0.86). The LDL-c and nonHDL-c effects were larger in the simvastatin-alone than in the fenofibrate + simvastatin group (interaction P less than 0.05), whereas HDL-cholesterol and triglycerides had similar effects in the two groups. Among apolipoproteins, the increase in Apo A-II levels was associated with lower CVD (HR 0.82; 95% CI 0.73-0.93). This association, independent from changes in HDL-c and triglycerides levels, was statistically similar in the simvastatin-alone (HR 0.90; 95% CI 0.71-1.14) and fenofibrate + simvastatin group (HR 0.78; 95% CI 0.67-0.90, interaction P = 0.4).
Conclusion: These findings, while confirming known associations between lipid changes and CVD, point to an as yet unknown atheroprotective effect of increasing Apolipoprotein A-II levels in type 2 diabetes. Further studies are needed to test the causality of this association.
Clinical Trial Registration Number: NCT00000620
Supported by: NIH, NHLBI
Disclosure: **M.L. Morieri:** Grants; NIH, NHLBI.

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Atorvastatin increases FADS1, FADS2, and ELOVL5 gene expression via GGPP-dependent RHO kinase pathway in 3T3-L1 cells

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Background and aims: The health benefits of omega-3 long-chain polyunsaturated fatty acids (LCPUFAs), mainly consisting of eicosapentaenoic acid (EPA, 22:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3), have been extensively researched to demonstrate the significant relative cardiovascular risk reduction by intake of omega-3 LCPUFAs. Therefore, the balance between EPA or DHA and arachidonic acid (AA, 20:4 n-6), a 20-carbon, 4-double-bond LCPUFA of the omega-6 type, in the human body is likely to be important for regulating the production of mediators and subsequently vascular function. Indeed, serum EPA to AA ratio (EPA/AA) has been found to be a good biomarker for the risk of cardiovascular disease. Although the efficacy of statin for both primary and secondary prevention of cardiovascular disease has been established, it has been reported that increase in plasma AA concentration and decrease in plasma omega-3 fatty acid concentration and/or plasma omega-3/AA ratio have been observed in patients treated with statin, which might be involved in the residual risk after initiation of statin treatment, These data indicate that statin affects the endogenous synthesis of LCPUFAs, which is regulated by fatty acid desaturases (FADSs) and elongation of very long-chain fatty acids proteins (ELOVLs). We investigated the statin-induced regulation of these desaturases and elongases as well as cell viability using mouse 3T3-L1 cells.
Materials and methods: Mouse 3T3-L1 preadipocytes were purchased from Health Science Research Resources Bank (Osaka, Japan). Cell viability was assessed by measuring mitochondrial activity that reduces 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt (WST-8) to formazan, using the Cell Counting Kit-8 (Dojindo, Kumamoto, Japan). Gene expression was analyzed by quantitative real-time PCR.
Results: Atorvastatin increased Fads1, Fads2 and Elovl5 mRNA expression to 105.4±3.5, 109.5±7.9 and 106.0±4.1 % at 10 uM, and 168.1±1.5,

235.6±5.5 and 147.1±1.0 % at 30 uM, respectively, in a dose-dependent manner at 48 h. Mevalonate and geranylgeranyl-pyrophosphate (GGPP), but not cholesterol, fully reversed the atorvastatin-induced upregulation of gene expression. The Rho-associated protein kinase inhibitor Y-27632 inhibited the mevalonate- and GGPP-effected reversal of atorvastatin-induced upregulation of gene expression.

Conclusion: These findings demonstrate that statin may affect the endogenous synthesis of LCPUFAs by regulating Fads1, Fads2 and Elovl5 gene expression via the GGPP-dependent Rho kinase pathway.

Disclosure: **I. Tatsuno:** Lecture/other fees; IT received lecture fees from Takeda Pharmaceutical Co., Ltd., and received research grants from Takeda Pharmaceutical Co., Ltd. and Mochida Pharmaceutical Pharma Co., Ltd.

1166

High refill adherence to lipid-lowering therapy associated with lower risk for cardiovascular disease in type 1 diabetes

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Background and aims: Cardiovascular disease (CVD) is the leading cause of shorter life expectancy in type 1 diabetes (T1D). The aim of this study was to assess risk of non-fatal and fatal CVD in relation to refill adherence to lipid-lowering therapy (LLT) in T1D patients.

Materials and methods: We included 6192 T1D patients, aged 18 years or older, from the Swedish National Diabetes who were also registered in the Swedish Prescription Drug Register initiating use of LLT between 1 July 2006 and 31 Dec 2010. Socioeconomic characteristics were collected from Statistics Sweden and comorbidities and outcome events from the National inpatient- and cause of death registers. First, patients were followed for 18 months estimating refill adherence to LLT and thereafter followed for CVD events, death or end of follow-up 31 Dec 2013. Adherence was estimated during the first 18 months of observation, by calculating Medication Possession ratio (MPR), i.e the proportion of days with medicines at hand during this period. Discontinuation was defined as being without medication at hand for more than or equal to 180 days. Cox regression analysis was performed to analyze MPR as predictor of non-fatal and fatal CVD, comparing the group with MPR above 80% to the group with MPR lower than or equal to 80%. We also analyzed the patients discontinuing LLT to the ones continuing. Analyses were adjusted for traditional risk factors, co-morbidities and socioeconomic status.

Results: In all, mean age was 45±12 years, diabetes duration 30±13 years, 58% were male, 13% were smokers, mean HbA1c was 66 ±14 mmol/mol, LDL 3.3±0.8 mmol/L and 24% had albuminuria. 93% were born in Sweden, 16% had insulin pump therapy, 43% were on antihypertensives and 9 % had a previous CVD. The mean MPR over 18 months was 72±28%. 52% had an MPR above 80%. Discontinuation of LLT was more common in patients without previous CVD and in those with no concurrent medication. Smokers were less adherent than non-smokers. There was a total of 767 non-fatal CVD and 58 fatal CVD events during mean follow-up of 5.3 years. Patients with an MPR above 80% had a 19% lower risk of non-fatal CVD compared to patients with MPR lower than or equal to 80%, HR 0.81 (95% CI 0.69-0.95) p=0.008. Patients discontinuing LLT had a 30% higher risk of non-fatal CVD, HR 1.30 (1.10-1.54) p = 0.002. In this study adherence did not affect risk of fatal CVD.

Conclusion: In T1D, high refill adherence to LLT was associated with lower risk for non-fatal CVD. Patients discontinuing LLT had a 30% higher risk of CVD. It is important to evaluate and emphasize adherence to prescribed LLT at clinical visits in order to reduce the risk of CVD in patients with T1D.

Disclosure: **C. Hero:** None.

1167

Changes in lipid-lowering therapy following a cardiovascular event in patients with diabetes

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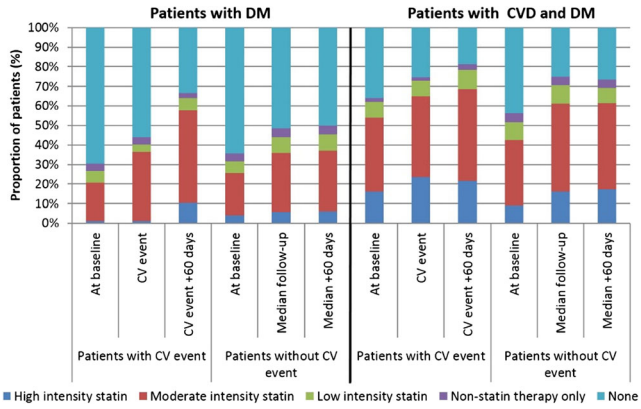
Background and aims: Patients with diabetes mellitus (DM) are at increased risk of cardiovascular (CV) events, yet many do not receive sufficient guideline-recommended lipid lowering treatment (LLT). We evaluated LLT patterns before and after a CV event subsequent to a DM diagnosis.

Materials and methods: Residents of Olmsted County, Minnesota, USA with DM from 2005-2012 were identified from electronic medical records and divided into 2 groups: 1) those with incident DM and 2) those with incident CVD (MI, unstable angina, revascularization, or ischemic stroke/TIA) with concomitant DM. Patients were followed from the incident DM or CVD diagnosis for 18 months for a subsequent CV event. LLT was characterized before and after the CV event, or for those without an event, before and after the median follow-up time of patients with an event.

Results: 4503 patients had incident DM (mean age 57.2 years, 52.2% male) and 568 had incident CVD with concomitant DM (mean age 70.4 years, 50.5% male). The mean baseline (±90 days) low density lipoprotein cholesterol (LDL-C) was higher among patients with incident DM (2.87 ± 0.92 mmol/L) than incident CVD and concomitant DM (2.22 ± 0.80 mmol/L; p-value <0.01). At baseline, 878 (19.5%) of the incident DM patients were prescribed moderate intensity statins, with only 1456 (32.3%) prescribed any LLT. Among the incident DM patients, 82 experienced a CV event (rate per 100 person-years: 1.5 (1.2-1.8)). Median time to CV event was 208 days, with 35.4% and 1.2% prescribed moderate and high intensity statins, respectively, at the time of the event. Moderate and high intensity statin prescriptions increased to 47.4% and 10.3% sixty days post-event (Figure). Mean LDL-C decreased from 3.07 ± 1.08 at baseline to 2.20 ± 0.81 within 2-6 months post-CV event. Patients without a CV event had a similar LLT pattern until the median follow-up time, with no change thereafter. Among patients with CVD and DM, 60% were prescribed LLT at baseline, nearly double the proportion in the incident DM group. Subsequent CV events were observed in 111 patients (rate per 100 person-years: 18.8 (15.8-22.2); median time to CV event: 144 days). Among patients with CVD and DM who had a subsequent CV event, 41.4% and 23.4% were prescribed moderate and high intensity statins, respectively, at the time of the event, which increased to 47.1% and 21.6% 60 days post-event. Mean LDL-C decreased from 2.31 ± 0.75 at baseline to 1.96 ± 0.84 within 2-6 months post-CV event, which was similar to the post-CV event LDL-C in patients with DM alone (p=0.17). Fewer prescriptions for high intensity statins were observed among patients without a subsequent CV event.

Conclusion: Despite conclusive evidence of statin benefit in prevention of CV events, a large proportion of patients with DM were not on an appropriate intensity of statin or under the LDL-C goal, even after a CV event subsequent to their DM diagnosis. More aggressive efforts are necessary to reduce CV events in this high-risk group.



Disclosure: **T. Okerson:** Employment/Consultancy; Amgen, Inc. Stock/Shareholding; Amgen, Inc.

1168

The early detection of lipodystrophy in insulin treated diabetic patients with the use of the thermal imaging camera

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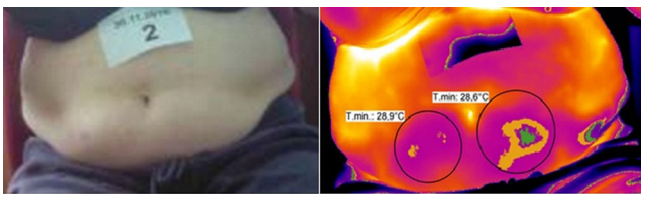
Background and aims: The number of diabetic patients treated with insulin have substantially increased in recent years, partly due to steadily growing incidence of type 1 diabetes and partly because of a common practice of the early start of using insulin in the treatment of patients with type 2 diabetes. Long term use of insulin is associated with skin related local complications like subcutaneous fat tissue lesions (lipodystrophy). It occurs in diabetic patients with a prevalence of 20-50%. Lipohypertrophy is the most common cutaneous complication of insulin therapy, characterized by swelling of the fatty tissue around insulin injection sites. The aim of the study was to evaluate the incidence of lipodystrophy in diabetic patients treated with insulin and to verify the effectiveness of the thermal imaging camera to detect these pathological symptoms.
Materials and methods: The analysis was conducted on the data collected from patients hospitalized at Department of Internal Diseases, Diabetology and Endocrinology of our Medical University from November 2016 to March 2017. Patients with BMI ≥ 25 kg/m2 and over 6 months insulin therapy were included in the study. We assessed HbA1c, biochemical profile, body mass index (BMI). All patients had QuickDash and Mini Mental State Examination. Insulin administration was observed and estimated. Manual evaluation of adipose tissue and imaging with the thermal camera FLIR T 660 2.0 was performed.
Results: The study included 60 patients with type 1 and 2 diabetes. 53% was male and 47% female. The duration of insulin therapy in patients ranged from 1 to 26 years. The BMI was 25 to 39 kg/m2. Average HbA1c level was 8,02%. Almost every third diabetic patient did not receive insulin properly. It was associated with greater traumatism of the tissues and worse control of diabetes (HbA1c > 8%) (p< 0,05). Subcutaneous tissue lesions were detected in 15% of patients in palpation. Using thermal imaging camera, the changes (an example picture) were apparent in 51 % of the subjects (p<0,05).
Conclusion: Lipodystrophy is an important and frequent complication of insulin therapy but is often underestimated. Subcutaneous adverse effects of insulin injections can lead to poor glycemic control. We often forget about it and look for other causes of uncontrolled glycemia. The main risk factors of lipodystrophy are: using the same skin region for insulin injections, poor education and long duration of diabetes. The thermal imaging camera is a safe, inexpensive and non-invasive way to detect early changes in adipose tissue. It should be available in every Diabetology Department. Our study shows the need for repetitive training of diabetic patients for insulin therapy even if they have been taking insulin for a long time.



Disclosure: **A. Maksymiuk-Klos:** None.

PS 104 Factors affecting endothelial function in vitro

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Prep1 deficiency improves vascular function by increasing endothelial nitric oxide production

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Background and aims: Endothelial dysfunction is associated with several pathophysiological conditions, including cardiovascular disease, insulin-resistance and type 2 diabetes. The hallmark of endothelial dysfunction is the reduced Nitric Oxide (NO) bioavailability which leads to an increase of vasoconstriction and induces smooth muscle cell proliferation and migration. NO production is induced by endothelial nitric oxide synthase (eNOS), which, in turn, is regulated by several protein kinases on different specific sites. Moreover, phosphorylation on Threonine495 residue inhibits eNOS function while phosphorylation on Serine1177 residue activates NO production. We have previously identified Prep1 as a homeodomain transcription factor which plays an important role in the metabolic response. In particular, Prep1 hypomorphic heterozygous mice ($Prep1^{i/+}$) expressing low levels of protein, are protected from streptozotocin-induced diabetes and show improved insulin sensitivity both in muscle and in liver. In this study, we have evaluated the role of the transcription factor Prep1 in the regulation of vascular function.
Materials and methods: Blood Pressure of both systolic and diastolic measurements have been performed in WT and $Prep1^{i/+}$ mice using a pressure transducer catheter. NO production has been measured by a nitrate/nitrite colorimetric assay, while aortas have been characterized by histological analysis and Western blot. Mouse aortic endothelial cells (MAEC), transfected with Prep1 cDNA, were co-cultured with vascular smooth muscle cells (VSMC) and proliferation and migration were investigated by using the transwell permeable supports.
Results: $Prep1^{i/+}$mice feature a 30% and 20% decrease of systolic and diastolic blood pressure, respectively, and a 40% decrease of residual vasoconstriction after phenylephrine stimulation compared to WT littermates. Immunohistochemistry analysis from aorta sections indicate that Prep1 is more expressed in the endothelium than the other layers and is lower in $Prep1^{i/+}$ mice than the WT animals. $Prep1^{i/+}$ mice show a 20% increase of serum NO release, paralleled by a significant decrease of eNOSThr495 and a 25% increase of eNOSSer1177 phosphorylation, compared to the WT mice. Experiments performed in MAEC cells overexpressing Prep1 have, in part, confirmed the data obtained in vivo, as NO production is strongly reduced. In addition, a co-culture system of Prep1 overexpressing MAEC cells and VSMC, show a significant increase of proliferation and migration of VSMC cells.
Conclusion: Our data indicate that Prep1 deficiency improves endothelial function by increasing NO production and suggest Prep1 as a novel possible candidate in the vascular complication of type 2 diabetes.
Disclosure: **I. Cimmino:** None.

1170

Disturbance of the cytosolic proteome and dominant methylglyoxal-modified proteins in human aortal endothelial cells in high glucose concentration in vitro

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Background and aims: Hyperglycemia-induced dysfunction of vascular endothelial cells is a driver of vascular complications of diabetes. Increased glucose metabolism by endothelial cells produces abnormal secretion and processing of extracellular matrix proteins, inflammation, oxidative stress, increased formation of advanced glycation endproducts (AGEs) and other processes. Insights into pathways driving these processes may be gained by quantitative cellular proteomics. The aim of this investigation was to incubate human aortal endothelial cells in primary culture in low and high glucose concentration (5 mM and 20 mM glucose, respectively), perform deep high resolution mass spectrometry proteomics of the cytosolic proteome and explore cell dysfunction through bioinformatics pathways analysis. We also investigated proteins modified by the AGE precursor, methylglyoxal (MG), which accumulates in endothelial cells in hyperglycemia.
Materials and methods: Human aortal endothelial cells (HAECs) were incubated in primary culture with 5 mM or 20 mM glucose for 3 days (n = 3). Cytosolic protein extracts were then preapared, reduced, alkylated and digested with trypsin. Tryptic peptides were analysed by nanoflow liquid chromatography-Orbitrap Fusion™ (Thermo) mass spectrometry and a relative quantification of protein concentration was performed using non-conflicting peptides in Progenesis QI™ software (Nonlinear Dynamics, Newcastle upon Tyne, U.K.). Protein ontology was evaluated using literature and Web-based tools (http://www.reactome.org/) to identify functional annotation to characterise molecular functions and biologic processes. Protein identification was based on at least 2 unique peptides and protein sequence coverage range was 5 - 40%.
Results: The mean number of proteins identified in cytosolic extracts of HAECs in low and high glucose concentration was 1893; 25 proteins were unique to low glucose concentration incubations and 12 proteins were unique to high glucose concentration incubations. There was 403 proteins upregulated in high glucose conditions. Reactome analysis indicated up-regulation of glycolysis and glucose metabolism ($P<0.001$). In these, the following proteins were up-regulated (fold increase): serine/threonine-protein phosphatase 2A (1.7 fold); pyruvate kinase (1.4 fold), phosphoglycerate kinase 1 (1.8 fold); Phosphoglycerate mutase 1 (1.4 fold); glyceraldehyde-3-phosphate dehydrogenase (1.2 fold); triosephosphate isomerase (1.5 fold); Fructose-bisphosphate aldolase C (1.6-fold); Fructose-bisphosphate aldolase A (1.3-fold); ATP-dependent 6-phosphofructokinase (1.2 fold); malate dehydrogenase, mitochondrial (1.6 fold); phosphoglucomutase-2 (1.6 fold); aspartate aminotransferase, cytoplasmic (2.9 fold). There were 3 proteins were found modified by MG: far upstream element-binding protein 2 FUBP2 (KH-type splicing regulatory protein) - an important regulator of multiple inherently instable mRNAs mostly coding for pro-inflammatory mediators; rho GDP-dissociation inhibitor 2; and isoform 2 of ADP-ribose pyrophosphatase, mitochondrial (NUDT9).
Conclusion: Quantitative proteomics revealed upregulation of enzymes of glucose metabolism and glycolysis in endothelial cells. A prominent MG-modified protein, FUBP2, is involved in mRNA processing of inflammatory mediators and may contribute to vascular inflammation.
Disclosure: **P.J. Thornalley:** None.

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High glucose exposure induces cellular dysfunction and metabolic changes in endothelial colony forming cells

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Background and aims: Endothelial dysfunction is a common problem in diabetes resulting in diminished vascular regeneration and progression of vascular complications. Recent clinical trials demonstrate that Endothelial Progenitor Cells (EPCs) play important roles in vascular repair and therefore represent an ideal candidate for therapeutic revascularisation. However, it has recently been shown that EPCs may

be impaired in diabetes. It has also been demonstrated that the number and function of circulating EPCs are reduced in patients with cardiovascular risk factors such as hyperglycaemia. The purpose of this study was to examine the effect of a diabetic milieu on a subset of well-defined EPCs called Endothelial Colony Forming Cells (ECFCs), by exposing them to high glucose.

Materials and methods: ECFCs were exposed to short-term (3-5 days) or long term high D-Glucose (DG) (25mM), control medium (5mM DG) or osmotic control (5mM DG+20mM L-Glucose (LG). Changes in ECFC functionality were measured using a scratch wound migration assay and a 3D Matrigel model of angiogenesis. The Seahorse Bioscience XFe96 analyser was used to compare the Oxygen Consumption Rate (OCR) and Extracellular Acidification Rate (ECAR) of short and long term high DG treatment.

Results: Short-term (3-5 days) exposure to high D-Glucose (DG) (25mM) resulted in a significant increase in ECFC tubule formation when compared to control medium (5mM DG) or osmotic control (5mM DG+ 20mM LG). On the contrary, long-term (4 week) exposure to high DG resulted in a significant decrease in ECFC tubulogenic capacity ($p \leq 0.001$). Long-term high DG also negatively affected ECFC function, as shown by a significant reduction in ECFC migratory capacity using an in vitro scratch wound assay ($p \leq 0.001$). Interestingly, long-term exposure to high DG resulted in premature senescence of ECFCs, which was characterised by a significant increase in senescence-associated β-galactosidase activity ($p \leq 0.001$); and a significant increase in 53BP1 foci ($p \leq 0.05$). The glycolytic reserve, measured using the Seahorse bioanalyser was exclusively diminished by long term exposure to high DG as cells failed to increase ECAR after ATP synthase inhibition by Oligomycin, which was not seen with chronic LG treatment. In addition, basal OCR and ECAR values were reduced by chronic treatment with either LG or DG indicating that metabolism is partially altered by the osmotic effect of glucose.

Conclusion: Our results demonstrate the deleterious effect of high DG on ECFC functionality and indicate that this effect may be due to the induction of premature senescence mechanisms. This finding is important as it indicates that ECFCs isolated from diabetic patients may be dysfunctional and therefore, will require functional evaluation, and consecutive repair if found to be dysfunctional, before using them as an autologous cell therapy.

Supported by: JDRF, NAT EYE RC

Disclosure: **C.L. O'Neill:** None.

1172

A diabetic microenvironment renders monocytes and myeloid angiogenic cells less pro-angiogenic

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Background and aims: Myeloid angiogenic cells (MACs) promote revascularisation of ischaemic tissues in pre-clinical models by the paracrine release of angiogenic factors such as MMP-9, IL-8 and MCP-1. IL-8 is key to this process by stimulating angiogenesis in a concentration-dependent manner. These pro-angiogenic properties of MACs have been harnessed to develop a novel cell therapy for ischaemic diseases. However, MACs were suggested to be dysfunctional in diabetes. An investigation was therefore led to observe how a diabetic milieu can affect the immunophenotype and function of MACs.

Materials and methods: MACs were isolated from peripheral blood and exposed to either 25mM D-Glucose (DG), control media (5mM DG) or an osmotic control (5mM DG + 20mM L-Glucose) for 4 days. RNA was extracted using Qiazol and converted to cDNA for use in RT-PCR. Flow cytometry was performed by staining mononuclear cells from patients and volunteers with anti-CD14 and anti-CD16 antibodies before running on Attune NxT flow cytometer. Gating was set by using appropriate isotype controls for each flurophore.

Results: Our evidence demonstrates MACs are a distinct subtype of macrophage that shares M2 anti-inflammatory and pro-angiogenic characteristics, but can be distinguished from M2 macrophages based on their significantly high expression of CD163, both in terms of RNA and cell surface protein expression. Results indicate that both MACs and MACs conditioned medium (MACs-CM) significantly reduced their *in vitro* pro-angiogenic capacity following a 4-day high DG treatment. IL1β mRNA and protein were significantly increased in MACs under high DG conditions. By blocking IL1β, the pro-angiogenic function associated with MACS-CM was rescued. Most importantly, IL1β was also significantly up-regulated in MACs isolated from type 1 diabetic patients with microvascular complications when compared to MACs from patients without microvascular complications or non-diabetic volunteers. Evaluating the immunophenotype of macrophage precursors (monocytes) from circulating blood suggested phenotypic changes in the mononuclear cell fraction in patients with type 1 diabetes without complications, with an increase in classical (CD14++/CD16-) and non-classical (CD14+/CD16+) monocytes observed compared to age and sex matched healthy donors. This is indicative of an inflammatory response in diabetic patients.

Conclusion: IL1β inhibition presents an attractive therapeutic target in restoring MACs vasoreparative function in diabetic conditions. As monocytes are the precursors to macrophages upon entering localised tissues, these data suggest that monocytes may be "primed" by the diabetic microenvironment to shift towards an M1 pro-inflammatory phenotype. This could potentially account for the loss of MACs pro-angiogenic function when isolated and exposed to high DG conditions.

Supported by: Leverhulme Trust

Disclosure: **K.J. Mcloughlin:** None.

1173

O-GlcNAcylation of FoxO1 is crucial for the overexpression of Angiopoietin 2 in NDPK B depleted endothelial cells

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Background and aims: Protein O-GlcNAc modification is involved in plenty of protein functions in various diseases such as diabetes mellitus, cancer, neurodegenerative and cardiovascular disease. Elevated protein O-GlcNAc modification has been found *in vivo* in the NDPK B-deficient mouse retinas which demonstrated vasoregression mimicking diabetic retinopathy, and *in vitro* in NDPK B depleted endothelial cells (ECs). Angiopoietin-2 (Ang-2), the crucial factor for initiation of retinal vasoregression, was found to be concomitantly upregulated. In this study, we investigated the role of FoxO1, an upstream regulator of Ang-2 upon NDPK B depletion in ECs.

Materials and methods: Human umbilical endothelial cells were used. Depletion of NDPK B was achieved by gene knockdown with NDPK B siRNA using Lipofectamine. Suitable non-specific siRNAs served as controls. The expression of NDPK B, Ang-2, FoxO1, protein phosphorylation and protein GlcNAc modification was assessed by Western blot and/or immunofluorescence using specific antibodies. Subcellular fractions were used to analyze FoxO1 translocation. FoxO1 GlcNAc modification was assessed by co-immunoprecipitation. O-GlcNAc inhibitors were used to evaluate the role of FOXO GlcNacylation.

Results: Phosphorylation of FoxO1 was significantly increased whereas total FoxO1 increased concomitantly in NDPK B depleted ECs. Translocation of FoxO1 was not altered upon NDPK B depletion. Neither the phosphorylation of AKT/SGK, nor the phosphorylation of JNK, p38 and ERK was changed in NDPK B depleted ECs compared with controls. Similar to the FoxO1 expression pattern, O-GlcNAc was detected mainly in the nucleus and few in the cytoplasm. Unexpectedly,

O-GlcNAc and FoxO1 were partially colocalized. Immunoprecipitation experiments demonstrated that FoxO1 was O-GlcNacylated in NDPK B depletion conditions. Moreover, inhibition of protein O-GlcNAcylation with O-GlcNAc inhibitors eliminated upregulation of FoxO1 and Ang-2 induced by NDPK B depletion.
Conclusion: We demonstrate in this study that upregulated Ang-2 in NDPK B depleted ECs is associated with O-GlcNAcylation of FoxO1. Our data supply evidence for the role of protein O- GlcNAcylation in NDPK B associated endothelial damage, indicating protein O-GlcNAcylation as a novel target in retinal vasoregression.
Supported by: EFSD
Disclosure: **Y. Feng:** None.

1174

Circulating Endothelial Cells (EPC) and Endothelium-derived Microparticles (EdMPs) time-course throughout the natural history of type 2 diabetes

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Background and aims: Vessel health status may result from the balance between endothelial insult and injury repair. The balance between Endothelium derived Microparticles (EdMPs) and Endothelial Progenitor Cells (EPCs) has been proposed to reflect the concomitant processes of endothelial injury and vascular repair capacity. A deeper understanding of the mechanisms involved in the regulation of such a balance is crucial to improve the quantification of the cardiovascular risk. In this study we describe the changes in circulating EPCs, circulating EdMPs and the EdMPs/EPCs ratio across the progression of Type 2 diabetes mellitus (T2DM).
Materials and methods: Circulating EdMPs ($CD42^-CD31^+$) and circulating EPCs ($CD34^+CD133^+KDR^+$) were quantified by flow cytometric (FACS) analysis (M±SD) in the following four subjects categories: 196 individuals with T2DM (age 61.7±8.8 years, diabetes duration 28.1±11.8 years, BMI 30.8±6.1 kg/m^2, HbA1c 7.3±1.2%); 30 newly-diagnosed T2DM (nT2D; 54.8±9.5 years, BMI 29.5±5.2 kg/m^2, HbA1c 11.1 ±2.3%); 20 individuals with pre-diabetes (pT2D; 53.8±8.8 years, BMI 29.7±7.7 kg/m^2, HbA1c 5.9±0.2%) and 20 healthy controls (C, age 52.1 ±8.9 yrs, BMI 24.1±1.8 kg/m^2, HbA1c 5.5±0.3%).
Results: Compared to control subjects (Krukal-Wallis non-parametric test), stem cells ($CD34^+$ and $CD34^+CD133^+$: 2193±707 and 967±380 cells/ml, respectively) were marginally reduced in pT2D (1742±517 and 841±488 cells/ml, respectively) and nT2D (1686±979 and 796 ±496 cells/ml; p=0.028 and p=0.070 vs T2DM, respectively), but not in T2DM (2212±1262 and 1067±797 cells/ml, respectively). On the contrary, EPCs ($CD34^+KDR^+$ and $CD34^+CD133^+KDR^+$) progressively dropped from C (879±354 and 435±227 cells/ml) to pT2D (590±277 and 302±191 cells/ml), nT2D (496±487 and 263±282 cells/ml) and T2DM (408±338 and 222±207; p<0.001 and p=0.003, respectively). EdMPs increased, though in a not significant manner, from C (7903 ±2501 n/ml), to pT2D (11664±8050 n/ml) and T2DM (11261±15979 n/ml) to nT2D (14843±27238 n/ml). Therefore, EdMPs/EPCs ratio progressively increased from C (21.6±13.2) to pT2D, nT2D (55.0±46.9 and 56.9±50.5, respectively) and T2DM individuals (85.2±148.5; p=0.016 vs. C). By logistic regression analysis, crude odds ratios (ORs) for CD34+CD133+KDR+ cells below the median were 13.0 for nT2D (p=0.012) and 15.2 for T2DM (p=0.009) with ORs, covariated for gender, age, BMI, blood pressure, lipids, blood glucose, HbA1c, urinary albumin to creatinine ratio and CKD-EPI eGFR of 9.8 (p=0.048) and 9.0 (p=0.040), respectively; crude OR for EdMPs/EPCs above the median were 8.7 for nT2D (p=0.014) and 6.3 for T2D (p=0.019) with covariated ORs of 3.1 (ns) and 6.9 (p=0.029), respectively. Covariates showed no independent effects even when subjects category was removed from the regression.
Conclusion: Alteration in circulating endothelial progenitor cells (EPCs) and endothelium-derived microparticles (EdMPs) as also reflected by the EdMPs/EPCs ratio get progressively worse across the different stages of the disease. Stages of the disease were the only factor affecting changes in EPCs and EdMPs/EPCs with no significant effects of the conventional cardiovascular risk factors.
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Disclosure: **D. Lucchesi:** None.

1175

CD133 antigen expression in dermal microvascular endothelial cells in adults with type 1 diabetes

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Background and aims: CD133 (CD-cluster of differentiation) is a membrane glycoprotein responsible for regulating cell growth and cell differentiation. CD133 antigen expression is high in early endothelial cells and decreases as they mature and differentiate. In the presented study, CD133 antigen expression was used as a marker of skin angiogenesis in adult patients with type 1 diabetes (DM1). The aim of the study was to assess microvascular density (MVD) and maturity in skin biopsy of patients with DM1 and correlate these data with clinical features.
Materials and methods: The study included 78 patients [42 men, 36 women, median age 43 (35-54) years, diabetes duration 24 (18-30) years] with DM1. Inclusion criteria were: age> 18 years, duration of diabetes> 5 years, consent to participate in the study. Exclusion criteria were: coagulopathy (APTT> 37s, INR> 1.1, PLT <100G/ mm3 blood), anticoagulant or antiplatelet medication, skin lesions in the biopsy area. Skin biopsy specimen was taken at the distal end of the leg. All samples were exposed to monoclonal mouse anti-human antibody anti-CD133. MVD - averaged number of blood vessels per 1 mm² - was calculated using "hot spots" technique.
Results: Median HbA1c was 7.8 (7.1-9.2)%. The prevalence of chronic diabetic complications was assessed by standard methods. Retinopathy was diagnosed in 40 (51.3%) patients, diabetic kidney disease in 10 (13%), peripheral neuropathy in 35 (45%), autonomic neuropathy in 11 (14.1%). 36 (46.2%) individuals were diagnosed with hypertension and 20 (25.6%) smoked cigarettes. MVD for anti-CD133 for all tested specimens was 83.3/mm 2 (66.6-95.8). The exspression of endothelial cells CD 133 antigen was inversely associated with body mass index (Pearson R = -0.35 p = 0.001). In the multivariate linear regression model, the association was independent of sex, age, diabetes duration, smoking, HbA1c, LDL cholesterol, C-reactive protein and peripheral neuropathy (beta = -0.39, p = 0.007, R2 = 0.19).
Conclusion: Presented results suggest angiogenesis suppression in individuals with higher BMI. Presented study is in line with results from animal models. It is not yet clear whether this findings could play a role in pathogenesis of diabetic microangiopathy.
Supported by: Poznan Unversity of Medical Sciences
Disclosure: **A. Adamska:** Grants; Poznan Unversity of Medical Sciences.

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Proof-of-concept for an enhanced surrogate marker of endothelial function in diabetes

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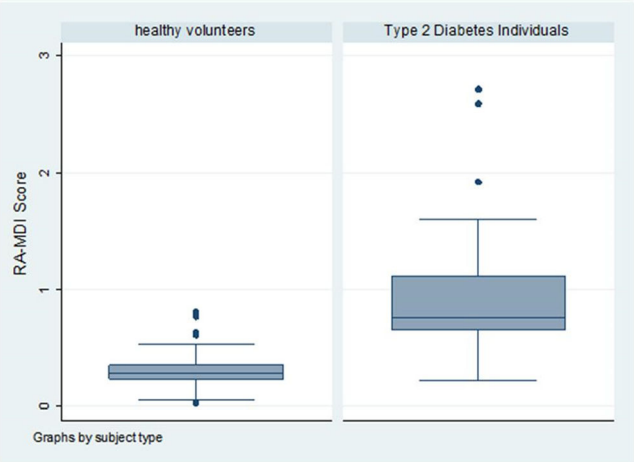
Background and aims: Diabetes affects distal small vessels earlier and to a larger extent than proximal vessels. Vascular disease starts from activation of the endothelial cells which if prolonged may lead to decrease in the distensibility of the blood vessel when maximally stimulated. Hence a device which measures distensibility of a distal vessel should be a good biomarker for subclinical disease. We developed a device with the capability to measure maximum possible pulse-wave-amplitude of the radial artery after a period of occlusion with the aim to see if the measurements correlate with traditional surrogate markers of cardiovascular risk in type 2 diabetes mellitus (T2DM)
Materials and methods: This device is based on the measurement of magnetic flux disturbance as blood flows through a uniformly applied magnetic field. Hemodynamic modulation is used to develop a mathematical model to quantify maximum possible dilatation using arterial form waveforms. The baseline waveforms from both hands is compared to waveforms obtained after a period of occlusion for 5 minutes in the occlusion arm to determine the radial-artery maximum distensibility index (RA-MDI). We recruited 96 subjects (46 patients with T2DM and 50 healthy volunteers) to study correlations of RA-MDI with cardiovascular risk factors, scoring systems, and carotid-artery-intima-media thickness (CIMT).
Results: The RA-MDI scores were significantly higher in T2DM (Median 0.76 (IQR:0.46)) compared to healthy individuals (Median: 0.28(IQR:0.13)); p<0.001. Linear regression analysis showed RA-MDI correlated with the risk scoring systems : a. Framingham Heart Study (lipids full CVD 30 years) : β=0.01 (95% CI: 0.01-0.01), p<0.001, b. UKPDS: β=1.6 (95% CI: 0.8-2.4), p<0.001, c. ADVANCE: β=9.67 (95% CI: 4.66-14.67), p<0.001 and CIMT: β=0.97 (95% CI: 0.61-1.32), p<0.001. Correlations with cardiovascular risk factors : HbA1c β=0.10 (95% CI: 0.06-0.15), p<0.001, Age β=0.02 (95% CI: 0.01-0.03), p<0.001, systolic BP β=0.01 (95% CI: 0.01-0.01), p<0.001, diastolic BP β=0.01 (95% CI: 0.01-0.02), p<0.001, and BMI β=0.03 (95% CI: 0.01-0.04), p=0.01.
Conclusion: We found that the RA-MDI correlated significantly with traditional cardiovascular risk factors, scoring systems and CIMT. Further large scale prospective studies need to be conducted to ascertain the correlations with hard cardiovascular outcomes.



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Disclosure: **R. Dalan:** Grants; National Health Innovation Centre.

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Sulforaphane and pyridoxamine supplementation normalise endothelial dysfunction associated with type 2 diabetes

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Background and aims: Nuclear factor erythroid 2-related factor 2 (Nrf2), a basic leucine zipper transcription factor, plays a critical role in the cellular defense system by mediating the coordinated upregulation of antioxidant responsive element-driven detoxification and antioxidant genes. To determine whether activators of Nrf2 can be used in addition with inhibitors of advanced glycation end products formation to attenuate oxidative stress and improve endothelial dysfunction in type 2 diabetes we investigate pyridoxamine (PM) and/or sulforaphane (SFN) as therapeutic interventions in endothelial dysfunction associated with type 2 diabetes in both aorta and mesenteric arteries.
Materials and methods: Goto-kakizaki (GK) rats, an animal model of non-obese type 2 diabetes and age-matched control Wistar rats were treated with or without PM (100 mg/kg/day) and /or SFN (1 mg/kg/day) during 8 weeks. At the end of the treatment, nitric oxide (NO)-dependent and independent vasorelaxation in isolated aorta and mesenteric arteries were evaluated. Metabolic profile, NO bioavailability and vascular oxidative stress and Nrf2 levels were also assessed.
Results: Diabetic GK rats presented significantly lower levels of Nrf2 and concomitantly exhibited higher levels of oxidative stress and endothelial dysfunction (associated with decrease NO bioavailability). PM and SFN as monotherapy were capable of significantly improving endothelial dysfunction (by 20 and 25 %, respectively) in aorta and mesenteric arteries decreasing vascular oxidative damage (accumulation of anion superoxide and 3-nitrotyrosine in aorta and mesentery arteries, respectively) and HbA1c levels. Furthermore, SFN+PM proved more effective reducing systemic free fatty acids levels, normalizing endothelial function and NO bioavailability in GK rats.
Conclusion: Activators of Nrf2 can be used therapeutically in association with inhibitors of AGE formation to normalize endothelial dysfunction in type 2 diabetes and prevent atherosclerosis progression.
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Disclosure: **C.M. Sena:** None.

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Vascular protective effects of glucose-dependent insulinotropic polypeptide via the endothelial PKA/AMPK/eNOS pathway

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Background and aims: Restenosis is a complication that arises after percutaneous transluminal angioplasty, which is a widely used revascularization procedure. The main pathophysiology of restenosis is neointimal hyperplasia, comprising exaggerated regeneration of the damaged intima. Recent technological advances such as drug-eluting stents have drastically reduced the incidence of restenosis; however, diabetic patients remain at high risk for the condition. In the present study, we investigated the protective effects of glucose-dependent insulinotropic polypeptide (GIP) against restenosis, using a mouse model of restenosis.
Materials and methods: Eight-week-old male wild-type C57BL6 (control) and db/db mice were implanted with osmotic pumps for agent delivery, before undergoing left femoral artery wire injury. Arteries were collected for analysis 25 days after injury.
Results: In the wild-type C57BL6 mice, GIP treatment (25 and 50 nmol kg^{-1} day^{-1}) induced a dose-dependent reduction (approximately 25 and 50%, respectively) in the neointimal area without affecting physiological and biochemical parameters, and an associated reduction in cell proliferation in the intima and media ($r = 0.85$ and 0.53, respectively; $p < 0.01$). In contrast, treatment with inactive GIP (3-42) had no observed effect on either the neointimal area or cell proliferation. The protective effects of GIP were completely abrogated by co-administration of *N*-omega-nitro-l-arginine methyl ester, a general nitric oxide synthase (NOS) inhibitor. The db/db mice all developed obesity and diabetes (fasting blood glucose level >20 mmol/L) at the initiation of GIP treatment (50 nmol kg^{-1} day^{-1}), and neither glucose tolerance nor weight gain was affected by the treatment. Similar to the wild-type mice, GIP-treated db/db mice exhibited a significant reduction (approximately 50%) in the neointimal area and cell proliferation. *In vitro*, GIP treatment dose-dependently increased NO production in human umbilical vein endothelial cells, and this effect was inhibited by a protein kinase A (PKA) or AMP-activated protein kinase (AMPK) inhibitor, but not by an exchange protein directly activated by cAMP or Akt inhibitor. Similarly, GIP treatment also induced phosphorylation of AMPK and endothelial NOS, and this effect was blocked by a PKA inhibitor. Notably, the effect of GIP treatment on NO production was not impaired under hyperglycaemic culture conditions (25 mmol/L). In human aortic vascular smooth muscle cells, GIP treatment did not suppress platelet derived growth factor-induced cell proliferation. Finally, we investigated the effect of internal GIP enhanced by a dipeptidyl dipeptidase-4 inhibitor by using the mouse model. We found that vildagliptin treatment (3mg kg^{-1} day^{-1})suppressed neointimal hyperplasia. This effect was preserved by approximately 50% in the presence of a glucagon like peptide-1 (GLP-1) receptor antagonist alone, with the complete abrogation by co-administration of antagonists of GLP-1 and GIP receptors.
Conclusion: GIP treatment modulated endothelial NO via the PKA/AMPK/eNOS pathway, and thereby suppressed neointimal hyperplasia to exert a protective effect against restenosis. This vasoprotective effect was maintained under hyperglycaemic conditions.
Disclosure: **Y. Mori:** None.

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The effect of methylglyoxal accumulation on angiogenesis in endothelial cells

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Background and aims: Diabetes mellitus (DM) is a metabolic disorder representing a leading health problem worldwide. The high mortality associated to DM is mainly caused by vascular defects. DM impairs physiological angiogenesis, leading to long-term complications, by molecular mechanisms that are not fully understood. Generation of Advanced Glycation End-products (AGEs) has an important role in the development of hyperglycemia-induced endothelial damage. It was demonstrated that exposure of endothelial cells to hyperglycemia induces sustained activation of the transcription factor nuclear-κB (NFκB), at least in part by the AGEs/RAGE pathways, leading to accelerated vascular disease. The major precursor of AGEs is Methylglyoxal (MG), a highly reactive dycarbonyl produced as a byproduct of glycolysis and detoxified by the Glyoxalase System of which Glyoxalase 1 (Glo1) is the rate limiting enzyme. MG concentration is increased 2 to 5-fold in diabetic patients. Several studies suggest that MG contributes to endothelial dysfunction and vascular complications, but the underlying mechanisms remain to be clarified. This work aims at evaluating MG effect on angiogenic process in endothelial cells and the molecular mechanisms involved.
Materials and methods: Proliferation, migration and invasion were evaluated by cell-growth curves and transwell assays in mouse aortic endothelial cells isolated from Glo1 knockdown mice (GloKD MAEC) and their wild type littermates (WT MAEC). Glo1, HoxA5 and NFκB mRNA levels were measured by Real Time PCR, while MG intracellular concentrations by HPLC. HoxA5 expression was silenced by the use of specific siRNAs. HoxA5 and VEGFR2 protein levels were evaluated by western blot. NFκB protein levels and activation were evaluated by western blot on protein lysates from cytosol/nuclear fractionation. Binding of NFκB to HoxA5 promoter was then analyzed by chromatin immunoprecipitation assay. NFκB activation was inhibited by the chemical inhibitor JSH-23.
Results: GloKD MAEC show a 50% reduction of Glo1 mRNA levels and a 5-fold increase of MG intracellular concentrations compared to WT MAEC *($p=0.001$)*. GloKD MAEC show a slower cell growth (*$p=0.01$*), a reduced migration (*$p=0.0002$*) and invasion ability (*$p=0.04$*) compared to WT MAEC. Both mRNA (*$p=0.001$*) and protein levels (*$p=0.04$*) of the anti-angiogenic gene HoxA5 are 2-fold increased in GloKD MAEC compared to WT MAEC, and its silencing improves both migration (*$p=0.03$*) and invasion (*$p=0.004$*) ability of GloKD MAEC. Moreover, GloKD MAEC show a reduced protein levels of HoxA5 target VEGFR2 (*$p=0.03$*) compared to WT MAEC. NFκB mRNA (*$p=0.001$*) and protein levels in both the cytosol and the nucleus of GloKD MAEC are ~2-fold increased compared to WT MAEC (*$p=0.04$*) and the binding of NFκB to HoxA5 promoter is 1.4-fold higher in GloKD MAEC compared to WT MAEC (*$p=0.02$*). Interestingly, NFκB inhibition reduces HoxA5 expression in GloKD MAEC (*$p=0.009$*).
Conclusion: This study demonstrates that high levels of MG impair angiogenic ability of MAECs via a mechanism involving NFκB and the antiangiogenic factor HoxA5.
Supported by: EFSD/Novo Nordisk
Disclosure: **A. Leone:** None.

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Vascular actions of vitamin D with implications for endothelial dysfunction

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Background and aims: Vitamin D deficiency has been associated with obesity, cardiovascular disease and type 2 diabetes. The present study was designed to investigate the effects of vitamin D deficiency on the metabolic profile, oxidative stress and endothelial function in normal Wistar (W) and type 2 diabetic Goto-Kakizaki (GK) rats.
Materials and methods: Normal W and diabetic GK rats were divided in eight experimental groups at 2 months of age and subjected to specific diets for four months (normal or atherogenic diets (AD) with normal or low levels of vitamin D). The effects of chronic vitamin D deficiency and AD diets were investigated on NO-dependent vasorelaxation in isolated rat aortic arteries from the different groups. NO bioavailability, vascular oxidative stress, vitamin D receptor (VDR) and systemic levels of 25-hydroxyvitamin D (25(OH)D) were also evaluated.
Results: AD induced endothelial dysfunction in normal W rats inhibiting maximal endothelium-mediated relaxation of phenylephrine-precontracted rings in response to ACh declined by ~22 % ($p<0.001$). AD also aggravated the endothelial dysfunction present in GK rats by 10% ($p<0.05$). Chronic vitamin D deficiency significantly deteriorated NO-dependent vasorelaxation in W and GK rats (by 20 and 14%, respectively) treated with normal diet but had no additional effect on rats treated with AD diet. Endothelial dysfunction was accompanied by a decrement in NO bioavailability and by a significant increment in vascular oxidative stress (as determined by superoxide anion immunofluorescence) in both AD treated (30 % increment) and vitamin D deficient groups of rats (45 % increment). In addition, diabetic and atherogenic diet groups with low vitamin D content presented significantly decreased systemic 25(OH)D levels and lower aorta VDR expression levels subsequently promoting oxidative stress.
Conclusion: These results indicate that vitamin D deficiency in the vasculature is an essential nutrient and its absence leads to endothelial dysfunction in normal rats and to a severe deterioration of NO-dependent vasorelaxation in type 2 diabetes mellitus thereby enhancing morbidity due to cardiovascular disease.
Supported by: COMPETE: POCI-01-0145-FEDER-007440; COMPETE: POCI-01-0145-FEDER-016784
Disclosure: **F. Carrilho:** None.

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Effect of acute hyperinsulinaemia and postprandial state on the vascular reactivity in type 2 diabetes patients and metabolically healthy volunteers

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Background and aims: Endothelial dysfunction has been linked to the pathogenesis of cardiovascular disease. Mechanisms involved in the regulation of vascular reactivity are not fully understood. The aim of the study was to test the effects of isolated acute hyperinsulinemia and postprandial state on the vascular reactivity and oxidative stress.
Materials and methods: We enrolled 30 patients with type two 2 diabetes (T2D) on metformin treatment and 30 volunteers (C) with overweight or obesity and with normal glucose metabolism. All subjects underwent the hyperinsulinemic euglycemic clamp combined with indirect calorimetry and meal test using standard breakfast. Parameters of the endothelial function - Augmentation Index (AI) and Reactive Hyperemia Index (RHI) were measured by finger pulse plethysmography before and during the clamp and before and after the meal test. Repeated-measures ANOVA models, multivariate regression using the method of orthogonal projections to latent structure (OPLS) and Pearson's correlation coefficient were used for for statistical analysis.
Results: AI did not change in response to hyperinsulinemia in either group. RHI tended to decrease in C (ΔRHI_C: -0.112±0.71, $p>0.05$), while there was a trend toward increase in T2D (ΔRHI_{T2D}: 0.084±0.062, $p>0.05$). There was a statistically significant difference between the groups over time (Gxt $p<0.05$). AI decreased in both groups in response to meal test (ΔAI_{T2D}: -10.5±2.781, $p<0.001$; ΔAI_C: -7.631±2.03, $p<0.01$), whereas RHI did not change. Activity of superoxid dismutase (SOD) increased in both groups during the meal test (ΔSOD_{T2D}: 0.566 ±0.383, $p<0.05$; ΔSOD_C: 0.295±0.344; $p<0.01$). RHI was one of the independent predictors of type 2 diabetes ($p<0.01$) along with glycated hemoglobin ($p<0.01$), fasting glycemia ($p<0.01$), rest energy expenditure ($p<0.01$) and fasting oxidation of carbohydrates($p<0.01$). See the figure.
Conclusion: Postprandial state changes decreases arterial stiffnes during meal test. Oxidative stress is possible mediator of this changes. RHI is strong predictor of type 2 diabetes.

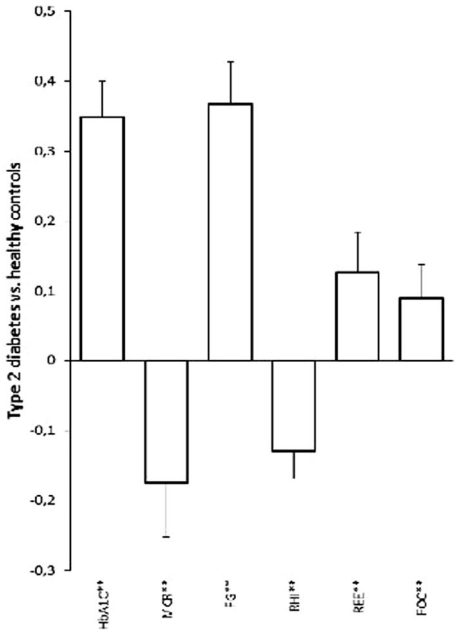

Fig. 2. The relationship between dependent metabolic variables and type 2 diabetes. HbA1c...glycated haemoglobin, MCR...metabolic clearance rate of glucose, FG...fasting plasma glucose, RHI... Reactive hyperaemia index, REE...resting energy expenditure, FOC...fasting oxidation of carbohydrates. Significance of the correlation is indicated by * (in $p<0.05$, ** (in $p<0.01$.

Clinical Trial Registration Number: NCT01946347
Supported by: the project AZV15-27431A from the Ministry of Health, Czech Republic
Disclosure: **J. Veleba:** None.

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Exenatide LAR improves cardio-metabolic parameters independently of the presence of endothelial dysfunction in type 2 diabetic patients: an 8 month prospective study

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Background and aims: Exenatide has been shown to augment endothelial function, few studies have examined the effects of exenatide once-

weekly (long-acting release, LAR) on endothelial function nor the dependence of these responses on baseline endothelial function. The aim of this study was to investigate: 1) the effect of exenatide LAR on cardio-metabolic parameters and endothelial function in patients with type 2 diabetes (T2DM) and 2) if such effects differ in the presence of endothelial dysfunction.

Materials and methods: Sixty subjects with T2DM (41 men and 19 women; 60±10 yrs) naïve to incretin-based therapies, and treated with exenatide LAR as add-on to metformin (from 1500 up to 3000 mg/day) for 8 months, were included in this prospective study. Exclusion criteria included the presence of a previous major cardiovascular (CV) event, as well as moderate and severe renal and liver function. Fasting blood samples were collected at baseline and after 8 months for routine biochemical analysis. The cohort of patients was subdivided in those with endothelial dysfunction (flow mediated dilation (FMD) ≤7%, n=30) and those without endothelial dysfunction (FDM >7%, n=30). Carotid intima-media thickness (cIMT) was assessed by B-mode real-time ultrasound.

Results: Statistical analysis was performed by paired t-test and ANOVA. After 8 months of exenatide therapy, glycemic and cardio-metabolic parameters improved in both groups, independently of the presence of endothelial dysfunction (Table).

Conclusion: It seems that exenatide LAR provides additional glycemic and cardio-metabolic control when added to metformin in diabetic patients with or without endothelial dysfunction. More studies of a longer duration are needed to confirm this encouraging finding.

	Baseline FMD ≤7 %	p=	After 8 months FMD ≤7 %	Baseline FMD >7 %	p=	After 8 months FMD >7 %	p= between groups
Weight (kg)	87±16	*0.0581*	85±16	92±20	***0.0011***	88±18	*0.1794*
BMI (kg/m^2)	31±5	*0.0819*	31±5	32±7	***0.0008***	31±7	*0.1099*
Waist circumference (cm)	109±11	*0.5697*	109±12	108±15	***0.0077***	103±12	***0.0289***
Fasting glycaemia (mmol/l)	9.3±2.8	***0.0012***	7.6±2.1	8.4±2.8	***0.0052***	7.0±2.2	*0.8614*
HbA1c (%)	8.1±0.3	***0.0018***	7.3±1.2	8.0±0.4	***<0.0001***	6.5±0.7	***0.0052***
Total cholesterol (mmol/l)	4.3±1.0	***0.0151***	4.1±1.0	4.5±0.9	***0.0331***	4.3±0.9	*0.9065*
Triglycerides (mmol/l)	1.4±0.6	*0.9729*	1.4±0.5	1.5±0.7	*0.9110*	1.5±0.6	*0.7408*
HDL-cholesterol (mmol/l)	1.3±0.3	*0.2007*	1.3±0.2	1.2±0.3	*0.0994*	1.2±0.3	*0.7941*
LDL-cholesterol (mmol/l)	2.4±0.9	***0.0036***	2.1±0.9	2.7±0.8	***0.0120***	2.4±0.9	*0.9047*
Flow mediated dilation (%)	4.7±0.4	***<0.0001***	5.6±1.0	6.9±1.0	***<0.0001***	7.9±1.2	*0.1945*
Carotid IMT (mm)	0.99±0.14	***0.0005***	0.90±0.15	1.0±0.1	***<0.0001***	0.8±0.1	*0.1373*

Clinical Trial Registration Number: NCT02380521

Supported by: AstraZeneca

Disclosure: **C. Mannina:** Other; I have participated in clinical trials sponsored by AstraZeneca and Novo Nordisk.

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Effects of vildagliptin combined with metformin on vascular endothelial function and systemic metabolism in patients with type 2 diabetes

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Background and aims: We investigated the impact of vildagliptin and metformin combination (Vilda-add) compared with high-dose metformin (high-Met) on vascular endothelial function in patients with type 2 diabetes mellitus.

Materials and methods: This was multicenter, open-labeled prospective randomized, parallel-group comparison study. We enrolled 97 subjects with type 2 diabetes mellitus, HbA1c levels between 7.0% and 8.5% in the absence of a history of arteriosclerotic diseases. Patients were administered metformin (500 to 750 mg/day) in addition to diet and exercise therapy. Sulfonylureas and/or glinides use was allowed throughout the study in any patient. After acquiring consent, patients were assigned to the Vilda-add or high-Met group according to age, body mass index (BMI), HbA1c and flow-mediated dilation (FMD) at the assignment agency. In the Vilda-add group, vildagliptin (100 mg/day) was administered to patients taking metformin (500 to 750 mg/day). In the high-Met group, the metformin dose used before the study was doubled (1000-1500 mg/day). Strict blood glucose control was performed for 12 weeks. The primary endpoint, FMD, was analyzed at our hospital before and after the trial by the same technician who was blinded to the treatment groups. In addition, assessments of glycemic control and metabolic parameters were conducted.

Results: Ninety-seven subjects (58.7 ± 11.0 years of age; BMI, 25.9 ± 4.4 kg/m^2; HbA1c, 7.3 ± 0.5%; FMD, 5.8 ± 2.6%) were enrolled and randomized. Eight subjects dropped out by the end of the study. After 3 months, HbA1c significantly improved in the Vilda-add group compared with that in the high-Met (−0.87 ± 0.38% vs. −0.40 ± 0.47%, respectively; p <0.01). However, there were no significant differences in FMD between those two groups(−0.93 ± 2.18% vs. −0.86 ± 2.75%; p = 0.89). The apoB/apoA1 ratio was significantly improved in the Vilda-add compared with that at baseline (0.676±0.175 vs. 0.635±0.182; p<0.01), but the change did not significantly differ between the two groups (−0.041 ± 0.084 vs. −0.026 ± 0.079; p=0.38). Adiponectin levels were significantly increased in the Vilda-add compared with those of the high-Met (0.750 ± 1.072 μg/ml vs. 0.012 ± 0.872 μg/ml; p <0.01). There were no significant differences in other lipid profile or oxidative stress parameters.

Conclusion: Combination therapy of vildagliptin and metformin did not further improve vascular endothelial function compared with that of high dose metformin administration.

Clinical Trial Registration Number: UMIN000011063

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Comparison of acute and chronic effects of sitagliptin vs glimepiride on endothelial dysfunction during an oral glucose loading in drug naive patients with type 2 diabetes

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Background and aims: Recently, we have reported that administration of sitagliptin, a DPP-4 inhibitor, for 12 weeks significantly increases the number of endothelial progenitor cells (EPCs) in people with type 2 diabetes, compared with glimepiride, a sulfonylurea. However, no reports have examined acute and chronic effects of DPP-4 inhibitors on endothelial function. The aims of our randomized, prospective, parallel design study were to investigate whether a single administration of sitagliptin (50 mg) can improve acute endothelial dysfunction evaluated by both flow-mediated dilatation (FMD) of the brachial artery and reactive hyperemia index (RHI) and increased the number of circulating EPCs during oral glucose loading (OGTT) in drug naïve patients with type 2 diabetes, and

to evaluate endothelial function after 12 weeks treatment with sitagliptin (50 mg/day) and glimepiride (1.0 mg/day).
Materials and methods: Fourteen drug-naïve diabetic patients were randomized into a sitagliptin 50 mg (N=9) or a glimepiride 1 mg (N=5) treatment group. At baseline and day 1 (acute dosing) after each treatment, endothelial function (FMD and RHI) and the number of EPCs (CD34+CXCR4+ cells measured by flow cytometry) were measured at 0, 30, 60,120, and 180 min during 75g OGTT. We also measured FMD and RHI after 12-week treatments.
Results: During the OGTT, in the sitagliptin group, both FMD and RHI trended to be increased at 60 min (P=0.286, 0.398 , respectively), while in the glimepiride group they tended to be decreased (P= 0.730, 0.287 , respectively). In the sitagliptin group, the number of EPCs tended to be decreased during the OGTT, while in the glimepiride group, it showed no significant changes. After 12 weeks of each treatment, both FMD and RHI showed an increasing tendency in the two groups.
Conclusion: Acute dosing of sitagliptin, but not glimepiride, improved endothelial function evaluated by FMD and RHI during the OGTT, while it tended to reduce the number of EPCs, suggesting acute effects of sitagliptin on endothelial function may be not associated with the number of circulating EPCs. Acute and chronic administration of sitagliptin may improve endothelial dysfunction in drug-naïve people with type 2 diabetes.
Clinical Trial Registration Number: NCT02301806
Supported by: MSD K.K.
Disclosure: **S. Sakurai:** None.

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Dapagliflozin acutely restores endothelial dysfunction, reduces aortic stiffness and renal resistive index in type 2 diabetic patients: a pilot study

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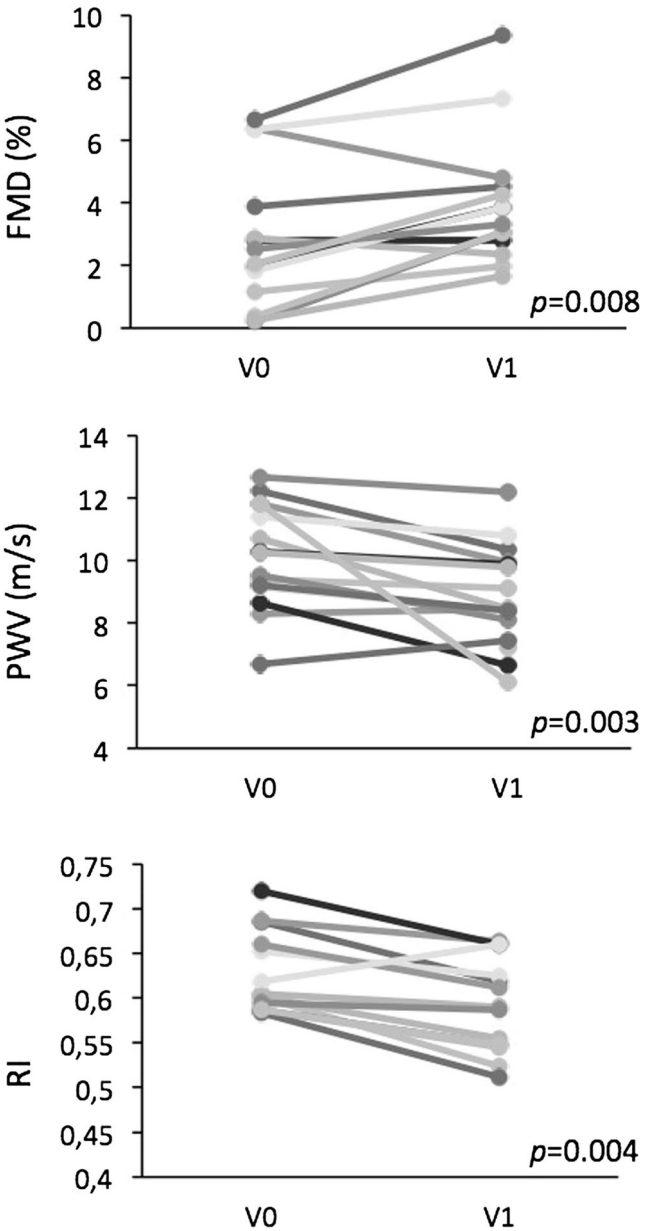
Background and aims: Sodium-glucose co-transporter-2 inhibitors reduce blood pressure (BP) and renal and cardiovascular events in patients with type 2 diabetes (T2DM) through not fully elucidated mechanisms. Aim of this study was to investigate whether dapagliflozin (Dapa) is able to acutely modify systemic and renal vascular function and improve endothelial function, whose amelioration by pharmacologic intervention may represent an ancillary goal in the treatment of T2DM.
Materials and methods: Neuro-hormonal and vascular variables, together with 24h-diuresis, urinary electrolytes, glucose, 8-isoprostanes and free-water clearance were assessed before (Visit 0) and after (Visit 1) a 2-day treatment with Dapa 10 mg QD in sixteen T2DM patients. Brachial artery endothelium-dependent and independent vasodilation (by flow-mediated dilation) and pulse wave velocity were assessed. Renal resistive index was obtained at baseline and after glyceril trinitrate administration.
Results: As expected by its mode of action, Dapa decreased systolic BP (from 130.6±12.8 to 125.4±11.2 mmHg, p=0.02) and induced an increase in 24h-diuresis (from 1400[750] to 2000[750] ml/24h, p=0.004) and 24h-urinary glucose output (406[3465] to 66239[43780] mg/24h, p<0.0001). Reduction in fasting plasma glucose did not reach the significance; interestingly, serum magnesium rose after Dapa treatment (from 1.92±0.19 to 2.04±0.17 mg/dl, p<0.0003), while no changes in the urinary electrolytes excretion, including sodium, were observed. Dapa administration resulted in a significant decline of plasma renin activity (p=0.03), but did not influence aldosterone levels, as well as the neuro-hormonal pattern, given that noradrenaline and adrenaline concentrations were unmodified. Oxidative stress was reduced, as by a decline in urinary isoprostanes (from 1659±1029 to 1157±663 pg/ml, p=0.04). Among the vascular variables, flow-mediated dilation was significantly increased (from 2.8±2.2 to 4.0±2.1%, p<0.05), while pulse-wave-velocity was reduced (from 10.1±1.6 to 8.9±1.6 m/s, p<0.05), even after correction for mean BP (Figure 1). Renal resistive index decreased (0.62±0.04 to 0.59±0.05, p<0.05), as well as its response to nitrates.
Conclusion: An acute treatment with Dapa significantly improves systemic endothelial function, arterial stiffness and renal resistive index; this effect is independent of changes in BP and occurs in the presence of stable natriuresis and plasma glucose levels, indicating a fast, direct beneficial effect on the vasculature, possibly mediated by a reduced oxidative stress. A minor contribute of serum magnesium increase to vascular protection might be suggested.



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Supported by: AZ
Diclosure: **M. Seghieri:** None.

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Association of the atherosclerosis candidate-genes polymorphism with endothelial function and atorvastatin response in type 2 diabetes

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Background and aims: To estimate the association of the endothelial dysfunction (ED) and the lipid-lowering response to atorvastatin therapy in T2D patients with genetic markers of atherosclerosis.
Materials and methods: We include 122 T2D statin-naïve patients: M/F 23/77; mean age 64 yr. Drug: atorvastatin, dose: 10-20 mg, treatment period: 12 month. 97 patients are completed clinical protocol. Before and after of statin therapy, patients had fasting lipid profiles and ED parameters performed. Assessment of endothelial function was performed by non-invasive peripheral arterial tonometry. The endothelial improvement was estimate as the postocclusive wave amplitude (Apw) increase. The genotypes for a complex of polymorphic markers were identified by PCR in real time with the TaqMan probes. Statistic analysis was evaluated using the Mann-Whitney, Wilcoxon tests, p <0,05.
Results: With statin therapy, PPARG2Pro/Pro had significantly TC, LDL-C lowering compared with PPARG2Pro/Ala, PPARG2 Ala/Ala patients (for TC: 20.74% vs. 4.6% and 5.61%; p = 0.04, respectively; for LDL-C: 26.00% vs. 6.11% and 7.32 %; p = 0.029, respectively). The % of Apw increase on atorvastatin was greater in GG vs GA carries of TNFα G(238)A: +8,16% vs. -0,93%, p=0,04; and GA vs. GG of TNFα G(308)A: +44% vs. -4.4%, p=0,004. The % of Apw and TC, LDL-C improvement didn't depend on age, diabetes duration, basal lipids levels and HbA1c. There were statistically significant differences in the genotypes distribution in the maximum and minimum lipid-lowering effect groups: in the group with the maximum decrease in lipids: all patients (100%) were carries of the two genotypes associated with the best lipid-lowering effect (*E4E4, E3E3, ProPro* - named as genotypes responders), among them 75% of patients were carries of the two genotypes associated with the Apw improvement (*GA* carries of *TNF-α G(-308)A* and *GG* carries of *TNF-α G (-238)A*), other 25% of patients were carries of the genotypes associated with the Apw decrease (*GG* carries of *TNF-α G(-308)A* and *GA* carries of *TNF-α G (-238)A*). Conversely, in the group of resistant patients who did not respond to statin therapy with lipid reduction: there didn't carries of the two genotypes responders, there were 75% of one genotype responder carries and 25% of genotypes non-responders carries (*E2E4, E3E4, E2E3, ProAla, AlaAla*), among them only 22,2% of patients were carries of the two genotypes associated with the Apw improvement, other 77,8% of patients were carries of the genotypes associated with the Apw decrease.
Conclusion: The result of statin therapy, both clinical - lipid-lowering effect, and pathogenetic - improvement of endothelial function, has genetic determinants and associated with *PPARG2 Pro12Ala*, *APOE E2/E3/E4* and *TNF-α* polymorphisms, that might be used for the personalized evaluation of cardiovascular prognosis improvement on statin therapy in type 2 diabetic patients.
Disclosure: **N.O. Lebedeva:** None.

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Modification of cardiac morphology was associated with impaired myocardial sensitivity to ischaemia-reperfusion injury in a diet-induced metabolic syndrome model

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Background and aims: Metabolic Syndrome (MetS) is defined by multiple risk factors that predict type 2 diabetes and cardiovascular complications, such as myocardial infarction, especially in women. Consequently the aim of this study was to investigate in vivo and ex vivo the effects of a high-fat-high-sucrose diet (HFHSD) on the development of metabolic syndrome (MetS), cardiac morphology and sensitivity to ischemia-reperfusion injury of female Wistar rat.
Materials and methods: Female Wistar rats, subjected to HFHSD (FHFD) or Normal Diet (FND) during 5 months, were explored in vivo every month with multimodal cardiovascular magnetic resonance (CMR). Cine-MRI (Magnetic Resonance Imaging) and arterial spin labeling (ASL-FAIR) techniques were used to determine cardiac morphology, function and perfusion. Triglyceride (TG) content in heart and liver was also evaluated with ^{1}H Magnetic Resonance Spectroscopy (MRS). Sub-cutaneous and visceral adipose tissues were measured with ^{1}H MRI. Then, rats underwent an intraperitoneal glucose tolerance test (IPGTT) to determine glycemic status. Finally, isolated heart were perfused with a physiological buffer containing 0.4 mM palmitate for 24 minutes before switching to 1.2 mM palmitate during 32 minutes low-flow (0.5 mL/min/g wet wt) ischemia. Next, flow was restored with 0.4 mM palmitate buffer for 32 minutes. High-energy phosphates and intracellular pH were measured during the experimental course by ^{31}P magnetic resonance spectroscopy with simultaneous measurement of contractile function. Coronary flow was measured before and after ischemia. At the end of experiments, hearts were freeze-clamped for biochemical assays.
Results: In FHFD vs. FND, CMR showed an increase of systolic wall thickness over time ($p<0.05$) and diastolic wall thickness at 3 and 5 months ($p<0.01$); ^{1}H MRS showed that hepatic TG content was increased ($p<0.01$) at 5 month but myocardial TG content was not different. IPGTT showed a significant glucose intolerance ($p<0.001$) and plasma free fatty acids were increased ($p<0.05$) in FHFD vs. FND. At 5 months, weight was not different between groups but FHFD exhibited an abdominal obesity with increased visceral adipose tissue ($p<0.05$), % fat ($p<0.05$) and % visceral fat ($p<0.05$) compared with FND. Ex vivo myocardial function was impaired in FHFD vs. FND before ($p<0.01$) and after ischemia ($p<0.05$).
Conclusion: HFHSD-induced MetS was characterized by glucose intolerance, abdominal obesity, hepatic fat deposit which were associated with modification of cardiac morphology and higher myocardial sensitivity to ischemia-reperfusion injury. These results may be related to higher risk of cardiovascular complications among type 2 diabetic obese women.
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Disclosure: **N. Fourny:** None.

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Riluzole rescues the high-fat diet-induced depression in mice: the role of hippocampal glial glutamate transporters

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Background and aims: Epidemiological studies indicate an association between metabolic disorders and depression. We recently found that diet-induced obesity mice exhibit depression-like behaviors. Maladaptation of reward circuit is a major cause of depression. An over-activated glutamatergic output projecting from the hippocampus is known to cause depression. The hippocampus is also a region vulnerable to metabolism disturbances. Here, we characterized the role of hippocampal glutamatergic transmission in the pathogenesis of high-fat diet (HFD)-induced depression. We also investigated the therapeutic effect of riluzole, a glutamatergic transmission normalizer, on HFD-induced depression.
Materials and methods: Eight-week-old male B6 mice were divided into Chow and HFD groups which were respectively fed with normal diet and HFD for 12 weeks. Forced swimming test (FST) and sucrose preference test (SPT) were used to assay the depression-like behaviors. The hippocampal expression of glutamatergic transmission-related proteins was measured. In testing the therapeutic effect of riluzole on depression, the mice were treated with daily injection of riluzole (4 mg/kg body weight, i.p.) for 21 days starting from the 10^{th} week of the 12-week feeding period.
Results: HFD increased the body weights (g, 28.7 ± 0.8 vs. 44.2 ± 1.1, $p < 0.0001$) and induced systemic insulin resistance (fasting plasma glucose: mg/dL, 128.6 ± 7.4 vs. 99.2 ± 7.8, $p < 0.05$; fasting plasma insulin: ng/mL, 1.5 ± 0.1 vs. 0.4 ± 0.0, $p < 0.0001$; HOMA-IR, 3.6 ± 04 vs. 0.7 ± 0.0, $p < 0.0001$) in mice. HFD also induced depression-like behaviors (FST: immobility %, 72.8 ± 4.0 vs. 42.1 ± 10.3, $p < 0.05$; SPT: sucrose preference %, 58.9 ± 1.8 vs. 68.8 ± 2.4, $p < 0.05$) in mice. Moreover, HFD decreased the expression of glial glutamate transporters, i.e. GLAST (relative expression, 0.4 ± 0.0 vs. 1.0 ± 0.1, $p < 0.001$) and GLT-1 (relative expression, 0.6 ± 0.1 vs. 1.0 ± 0.1, $p < 0.05$), in the hippocampus without obvious morphological changes in astrocytes and microglia. The 21-day riluzole treatment restored the hippocampal expression of GLAST (relative expression, 0.7 ± 0.1 vs. 0.2 ± 0.1, $p < 0.05$) and GLT-1 (relative expression, 0.8 ± 0.1 vs. 0.3 ± 0.1, $p < 0.05$), and rescued the HFD-induced depression-like behaviors (FST: immobility %, 28.7 ± 5.4 vs. 63.5 ± 3.3, $p < 0.05$; SPT: sucrose preference %, 63.0 ± 1.1 vs. 55.5 ± 2.4, $p < 0.05$).
Conclusion: HFD concurrently reduces the hippocampal levels of GLAST and GLT-1 and induces depression-like behaviors in mice. Riluzole restores the expression levels of GLAST and GLT-1 in the hippocampus and rescues the HFD-induced depression in mice. These results suggest that the decreased expression of the hippocampal GLAST and GLT-1 may be involved in the development of depression-like behaviors in animals with metabolic disorders.
Supported by: MOST General Research Project
Disclosure: **T. Wang:** None.

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Hyperglycaemia renders nigrostriatal dopaminergic neurons more vulnerable to damage in a mouse model of Parkinson's disease

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Background and aims: Increasing evidence suggests that diabetic patients present a higher risk of developing neurodegenerative diseases as they age. For instance, it is well documented that diabetes can be associated to the development of cognitive impairment and Alzheimer's disease. In the case of Parkinson's disease, one of the most common neurodegenerative diseases characterized by the loss of dopaminergic cells in the substantia nigra that project to the striatum, this association is less well documented, and the underlying mechanisms by which hyperglycemia may result in increased neuronal damage are unknown. In the present study, we sought to investigate whether diabetes increases the vulnerability of neurons of the nigrostriatal dopaminergic system to neurodegeneration in mice.
Materials and methods: We used C57BL/6 mice in which diabetes was induced by repeated administration of streptozotocin. Mice were unilaterally injected with a reduced dose of the neurotoxin 6-hydroxydopamine (6-OHDA) into the striatum two weeks or one month after the onset of diabetes. A sub-threshold dose of 6-OHDA insufficient to cause motor impairment in normal non-diabetic animals was used. Motor function was measured with well-established tests widely used in animal models of Parkinson's disease. Neurodegeneration was monitored histologically. Molecular markers of neuronal function were determined by RT-qPCR.
Results: Motor performance was unaffected in control non-diabetic animals injected with 6-OHDA. In contrast, diabetic mice developed significant motor impairments. Immunohistochemical analysis of 6-OHDA-injected striata showed that the content of tyrosine hydroxylase, a molecular marker of nigrostriatal innervation, was significantly reduced in diabetic as compared with non-diabetic animals. The number of tyrosine hydroxylase positive cells in the substantia nigra was determined stereologically in 6-OHDA injected mice, and a significant decrease was only observed in diabetic but not in non-diabetic animals. Two week-diabetic mice showed an intermediate phenotype between non-diabetic and one month-diabetic mice, suggesting a time-dependent effect of hyperglycemia. Expression of phenotypic markers of substantia nigra neurons was not affected by diabetes, but expression of neurotransmission-related genes was reduced. Furthermore, we observed a marked deregulation of genes encoding free-radical scavenging enzymes, suggesting increased oxidative stress in these neurons as a consequence of diabetes.
Conclusion: Our data indicate that hyperglycemia increases the vulnerability of dopaminergic neurons to neurodegenerative damage leading to motor impairment, thus providing new insights into the pathophysiology of a possible relationship between diabetes and Parkinson's disease.
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Disclosure: **I. Pérez-Taboada:** None.

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Cardiac effects of combined SGLT1/2 inhibition following myocardial infarction

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Background and aims: Dual SGLT1/2 inhibition offers the potential to not only increase glucosuria beyond that seen with selective SGLT2 inhibition alone but to reduce glucose absorption from that gut as well and to thereby also stimulate glucagon-like peptide 1 (GLP-1) secretion. However, beyond the kidney and gut, SGLT1 is expressed in a range of other organs including the heart where it potentially assists GLUT-mediated glucose transport. Since cardiac myocytes become more reliant on glucose as a fuel source in the setting of stress, the present study sought to compare the effects of dual SGLT1/2 inhibition with selective SGLT2 inhibition in the normal and diseased heart.
Materials and methods: Hyperglycaemic (plasma glucose 10-15 mmol/l) Fischer F344 rats were randomized to receive either vehicle, the dual SGLT1/2 inhibitor, T-1095 (150 mg/kg/d p.o.), or the selective SGLT2 inhibitor, dapagliflozin (1 mg/kg/d p.o.). Animals were then further randomized to undergo sham surgery or ligation of the left anterior descending (LAD) coronary artery to induce infarction of the left ventricle (LV). Cardiac function was assessed by echocardiography and conductance catheterisation just prior to termination 4 weeks later that was followed by assessment of cardiac structure.
Results: Dapagliflozin and T-1095 induced glucosuria to a similar extent in both the control and myocardial infarction settings with similar effects on glycaemia as assessed by serum fructosamine. Neither dapagliflozin nor T-1095 had any demonstrable effect on cardiac function or structure in the control setting. Following myocardial infarction, however, significant ($p<0.05$) differences were noted. When compared with vehicle or dapagliflozin-treated animals, rats that received T-1095 displayed worse cardiac function in both systole and diastole as evidenced by (i) lower

ejection fraction, (ii) reduced maximal rate of LV pressure rise in early systole (dP/dt max), (iii) impaired isovolumic relaxation in diastole (dP/dt min), (iv) prolongation of the early, energy-dependent phase of relaxation in diastole (Tau), and (v) reduced passive LV compliance as measured by an increase in the end-diastolic pressure volume relationship (EDPVR). While vehicle and dapagliflozin-treated rats underwent hypertrophic changes following myocardial infarction this did not occur in animals that had received T-1095 where LV mass and cardiac myocyte cross-sectional area were similar to those of control, uninfarcted rats. Following myocardial infarction, lung weight, a marker of interstitial/alveolar oedema was also higher in T-1095 treated rats than in those animals that had received either vehicle of dapagliflozin.

Conclusion: Dual SGLT1/2 inhibition with T-1095 prevented the hypertrophic response following experimental myocardial infarction in the rat with adverse consequences on both systolic and diastolic function. These findings suggest that the heart may be more reliant on SGLT1-mediated glucose transport in the disease setting. The gap between animal and human studies notwithstanding, these findings further suggest the need for caution with dual SGLT1/2 inhibition in patients with diabetes at high cardiovascular risk.

Supported by: AZ

Disclosure: **R.E. Gilbert:** Grants; Astra Zeneca. Honorarium; Astra Zeneca. Lecture/other fees; Astra Zeneca.

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Effect of insulin or metformin on the wound healing process in rats with streptozotocin-induced diabetes

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Background and aims: Good glycemic control is crucial for the proper wound healing in patients with diabetes. We conducted a study aiming at assessing the effect of insulin and metformin administration on the wound healing wounds in an experimental model in rats with streptozotocin induced diabetes.

Materials and methods: The study was performed on a group of 80 male Wistar rats with streptozotocin-induced diabetes. In the first step, 45 rats were divided into 3 groups: group 1 received human NPH insulin intraperitoneally (5 j.m./kg m.c.) once daily, group 2 received metformin intragastrically (500 mg/kg m.c.) once daily, and group 3 (controls) were given saline intraperitoneally. The treatment goal was to achieve blood glucose levels of 350-450 mg/dl in order to reflect hyperglycemic conditions often associated with wound development. After 30 days of treatment the rats had the thin layer of the skin cut out from the dorsal skin 2x2 cm and sutured with a silicone disc with a hole in the centre in order to stabilize the skin and standardize the process of healing. The wounds were assessed every 3 days: digital photography was performed and a wound surface biopsy was conducted. The wounds healing was followed up for 9 days. Biopsy samples were subject to H+E staining and immunohistochemical asssays were conducted to evaluate the expression of the proliferation marker - Ki67 antigen. Metformin or insulin were administered during the wound healing follow-up time.

Results: Analysis of variance revealed significant influence of treatment type (insulin, control or metformin) on the relative wound area ($p < 0.001$). Mean changes of relative wound area for 3 types of treatments at 3 moments are presented in Table 1. Healing in the rats treated with insulin was more advanced than in the control group after 3 days, and it was more advanced than metformin after 6 and 9 days. There were no differences between the metformin and control groups at any stage of the study. Wound tissue samples taken from the insulin treated animals presented with significantly lower level of inflammatory infiltratio than those obtained from the rats treated with metformin. Immunohistochemical assessment showed the greatest density of centers of profileration in insulin treated animals.

Conclusion: The results of our experimental study suggest that insulin treatment is more beneficial than metformin for accelerating wound healing process in animal model. As the observations were made at a similar level of hyperglycemia in all groups, it might be speculated that there is an additional effect of insulin treatment on wound healing beyond glucose lowering action.

Table 1. Mean decrease of wound area in three studied groups during follow-up.

Follow-up	Insulin	Control	Metformin
3 days	-28.2 ± 6.5%	-26.2 ± 9.4%	-22.2 ± 3.9%
6 days	-39.0 ± 11.5%	-33.8 ± 6.6%	-26.5 ± 6.3%
9 days	-66.8 ± 9.7%	-48.0 ± 9.8%	-40.4 ± 15.8%

data are given as mean ± SD

Disclosure: **B. Mrozikiewicz-Rakowska:** None.

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Beneficial effects of combination therapy with pioglitazone and dapagliflozin on diabetic nephropathy in db/db mice

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Background and aims: Although dapagliflozin and pioglitazone have glucose lowering and anti-inflammatory effects, the therapeutic efficacy of combination therapy on diabetic nephropathy has not been investigated. The aim of this study was to investigate the therapeutic effect of combination therapy in an animal model to support the experimental rationale for the combination therapy of dapagliflozin and pioglitazone.

Materials and methods: 9-week-old male db/db mice were randomly assigned to four groups with (1) vehicle, (2) dapagliflozin-, (3) pioglitazone-, (4) dapagliflozin and pioglitazone combination-treated groups. Dapagliflozin (2 mg/kg/day), and pioglitazone (30 mg/kg/day), and combination (dapagliflozin 2 mg/kg/day + pioglitazone 30 mg/kg/day) were administrated by oral gavage for 9 weeks. To further understand cellular mechanisms underlying the protective effect of combination treatment, protein expressions and cell survival of HK-2 cells were studied.

Results: The mean random blood glucose and urine albumin-creatinine ratio were lowered in all treatment groups ($P<0.05$). Glomerular tuft area and mesangial expansion of kidney were the most suppressed in the combination group. Podocyte foot process width and glomerular basement membrane thickness were decreased regardless of the treatment, and the combination group showed the lowest renal hypertrophy progression ($P<0.05$). The combination treatment decreased the expression of TGF-beta, type IV collagen, and type 1 collagen in renal cortex. High glucose and palmitic acid upregulated TGF-beta and IL-6, and stimulated angiotensinogen and renin expressions. Treated HK-2 cell with those 3 medications inhibited TGF-beta expression (all $P<0.05$), only combination treatment decreased angiotensinogen expression ($P=0.033$). Furthermore, combination treatment enhanced cell survival in the presence of high glucose and palmitic acid ($P=0.001$).

Conclusion: Dapagliflozin and pioglitazone treatment preserved renal function in db/db mice and the combination therapy showed the best beneficial effects. These findings suggest that the combination therapy might prevent the progression of diabetic nephropathy in patients with type 2 diabetes.

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Topical linagliptin is on par to systemically acting linagliptin in improving diabetic wound healing in db/db mice

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Background and aims: Linagliptin is a potent dipeptidyl peptidase (DPP)-4 inhibitor, approved as an oral treatment for patients with type 2 diabetes mellitus. We recently reported that linagliptin improved diabetic wound healing disorders in obese (*ob/ob*) mice. The observed improved re-epithelialization of the wounded skin was associated with an improved glucose tolerance in the animals and also with a reduction in inflammatory polymorphonuclear neutrophil infiltration. In order to discriminate the anti-hyperglycaemic effect from potential other effects, we tested different topical formulations of linagliptin which caused either relevant systemic exposure of linagliptin in the plasma, or not. The aim of this study was to evaluate whether linagliptin exerts direct effects on the skin which are not mediated via improved glucose control.
Materials and methods: 8 to 12 week old female diabetic *db/db* mice or CD-1 mice (Charles River) were wounded with 4 full excisional wounds on the back of each animal. Oil/water emulsions of Linagliptin (0.5%, 0.01%, and 0.001%) were prepared and administered (~1 mg every second day) with a sterile applicator to each wound. Wound closure and histology were monitored after 10 days. For drug monitoring in plasma, blood was taken 3 hours after last application. DPP-4 activity, glucose, and active glucagon-like peptide-1 (GLP-1) were also monitored.
Results: In the pilot study in CD-1 mice, we aimed to define topical linagliptin formulations which did and did not show systemic DPP-4 inhibition. DPP-4 activity (relative fluorescence unit [RFU]) in plasma was 1284±43.8 RFU with vehicle and significantly ($p<0.01$) reduced with the 0.5% (272.5±6.3 RFU) and the 0.01% (500.5±13.4 RFU) linagliptin formulations, but there was no reduction with the 0.001% formulation (1206±42 RFU). Topical formulations of 0.5% and 0.001% were subsequently used in *db/db* mice. Wound closure in vehicle-treated *db/db* mice was 18.7±2.8% after 10 days, which was significantly ($p<0.01$) improved in animals receiving the 0.5% (74±6.6%) and 0.001% (64.7±15.9%) formulations. In contrast, active GLP-1 was only increased with the 0.5% formulation (0.73±0.19 ng/ml, $p<0.001$) compared with controls (0.067 ±0.02 ng/ml) and the 0.001% formulation (0.17±0.12 ng/ml, n.s). In line with this observation, there was no reduction of DPP-4 activity in vehicle- (1857±142 RFU) and 0.001%-treated mice (1882±272.1 RFU) and a significant reduction in 0.5%-treated mice (528.8±68.2 RFU, $p<0.0001$). Fed glucose levels were only significantly ($p<0.05$) reduced compared to vehicle in the 0.5%-treated group by 31 ± 2.6%.
Conclusion: Topical linagliptin at doses that were shown not to affect systemic DPP-4 activity nor active GLP-1 levels was equipotent to systemically acting linagliptin at improving diabetic wound healing. These findings suggest a mechanism that involves local DPP-4 substrates rather than systemic DPP-4 inhibition and glucose control.
Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance
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Linagliptin alleviates podocyte injury and enhances glomerular nephrin expression in a model of type 2 diabetes

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Background and aims: A growing body of evidences indicates the potency of dipeptidyl peptidase-4 (DPP-4) inhibitors for reducing of albuminuria in diabetes. It have been demonstrated that the disturbances in podocyte function can be responsible for elevation of albumin excretion rate at initial stages of diabetic nephropathy. Expression of nephrin and other key podocyte-specific proteins are downregulated in diabetes. Therefore, the aim of our study was to assess the effect of DPP-4 inhibitor linagliptin on podocyte structure and glomerular nephrin expression in db/db diabetic mice.
Materials and methods: Eight-week-old male diabetic db/db mice (BKS.Cg-Dock7^{m}+/+Lepr^{db}/J) were treated with linagliptin (10 mg/kg per day by gavage) or vehicle for 8 weeks. Non-diabetic heterozygous db/+ mice were acted as control. The concentrations of insulin, glucagon, leptin and resistin in blood plasma were determined by Multiplex analysis, and body composition was assessed by MRI at week 0 and 8 of experiment. Renal structural changes were analyzed quantitatively from the light and electron microscopic images. Nephrin staining in glomeruli was assessed by immunohistochemistry.
Results: Severe hyperglycemia and obesity developed prior to the beginning of experiment in db/db mice. These mice demonstrated substantially elevated serum levels of leptin and insulin and increased fat percentage at week 0 and week 8 (all $p<0.00001$). The blood glucose levels remained markedly elevated throughout the experiment in both linagliptin and vehicle groups. Nevertheless, linagliptin-treated mice, as compared to vehicle-treated animals, demonstrated attenuated mesangial expansion estimated by fractional mesangial volume ($p=0.03$). The width of glomerular and tubular basement membrane was diminished by linagliptin significantly ($p=0.03$ and $p=0.007$ respectively). Podocytopathy in diabetic mice was manifested by the foot process effacement. In linagliptin group, as compared to placebo, the mean width of podocyte foot process was reduced and the number of podocyte foot processes was increased significantly (both $p<0.01$). Vehicle-treated diabetic mice had weak staining for nephrin in glomeruli. Meantime, the volumetric density of nephrin-expressing glomeruli was increased markedly in linagliptin group ($p=0.02$).
Conclusion: The data from the current study demonstrate that DPP-4 inhibitor linagliptin ameliorates podocyte injury and enhances glomerular expression of nephrin in a model of type 2 diabetic nephropathy. The data provide further explanation for the mechanism of antialbuminuric effect of DPP4 inhibitors in diabetes.
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Differential effects of experimental diabetes versus chronic polyuria on the rat bladder transcriptome

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Background and aims: Polyuria occurs in both clinical and experimental diabetes. Early symptoms of diabetic bladder dysfunction manifest clinically as overactive bladder (e.g. urgency, increased frequency of urination). Whilst at later stages diabetic cystopathy is associated with decreased sensation, overflow incontinence and micturition problems. A high post-void residual urine volume can predispose patients to lower urinary tract infections and increase risk of kidney damage. Late stage changes may reflect more chronic effects of hyperglycaemia on the bladder. The aim of this study was to compare the impact of long-term (16 weeks) experimental diabetes (with associated polyuria) to polyuria alone on the transcriptome of the rat urinary bladder.
Materials and methods: Adult male Wister rats (starting weight 289-425g) were either left untreated, treated with streptozotocin (STZ; 55mg/kg, i.p.) or with 5% sucrose in their drinking water for 16 weeks in accordance with the UK Animals Scientific Procedure Act (1986) and institutional regulations. Water consumption was monitored for the

duration of the study. After 16 weeks, the rats were culled, bladders were removed, weighed and processed for either histology or RNA analysis by Affymetrix GeneChip® rat genome 230 2.0 array. dChip (V2005) was used to perform the outlier analysis and check technical quality. Quintile normalisation and background correction were conducted using RMA. GeneVene and Ingenuity Pathway Analysis (IPA) software were used to analyse data and identify over-represented pathways with differentially expressed genes ($p<0.05$; with fold change>1.3). Statistical Analysis was performed using Graphpad Prism.

Results: Both STZ-diabetic and sucrose-treated rats rapidly developed polydipsia, drinking significantly more than age-matched non-diabetic control rats, and this was associated with a significant increase in the weight of the bladders in both groups compared to non-diabetic controls ($p<0.05$). GeneChip analysis revealed that STZ-induced diabetes caused significant dysregulation of 1467 transcripts in the bladder compared with non-diabetic control rats (56.9% upregulated; 43.1% downregulated). The 5 most upregulated transcripts were *Grem1* (+27.9), *Sgcg* (+10.9), *Ildr2* (+7.7), *Bdnf* (+7.4) and *Adprhl1* (+4.7). Sucrose-treatment caused fewer (366) transcripts to significantly change compared to control rats (34.7% upregulated; 65.3% downregulated). The 5 most upregulated transcripts were *Grem1* (+4.2), *Ildr2* (+3.2), *Cyr61* (+3.0), *Ctgf* (+2.5) and *Acta2///Actc1* (+2.5). GeneVenn analysis revealed 1298 differentially expressed transcripts were exclusively affected by experimental diabetes, while only 197 transcripts were exclusively related to sucrose-treatment. Interestingly, IPA analysis revealed significant dysregulation of 45 pathways in the bladders of STZ-diabetic rats compared with non-diabetic controls, including 'Axonal Growth-Related' and 'Extracellular Matrix-Related' pathways.

Conclusion: Late-stage pathogenic changes have not been thoroughly characterised in the STZ-rat urinary bladder to date, nor compared to diuresis-induced remodelling. Our data provide a detailed comparison of the distinct effects of diabetes and of polyuria on the urinary bladder that reveal specific transcriptomic changes that may contribute to diabetic bladders dysfunction.

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Disclosure: **E.A. Hindi:** Grants; King Abdulaziz University, Saudi Arabia.

PS 107 A complicated story: complications of diabetes

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An under-recognised complication: the presence of cheiroarthropathy in the type 1 diabetes exchange

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Background and aims: Diabetic cheiroarthropathy is a long-term complication of diabetes that often causes significant morbidity with impaired ability to complete activities of daily life. This complication has not been well studied in individuals with type 1 diabetes (T1D). To quantify the frequency of cheiroarthropathy in adults (≥18 yrs old) with T1D, T1D Exchange registry participants were invited to complete a survey to address this question as well as to assess participant and clinical characteristics associated with the diagnosis.

Materials and methods: 6,200 adults in the T1D Exchange clinic registry were invited to complete an internet-based survey inquiring about the diagnosis of cheiroathropathy- specifically asking about the diagnosis and treatment of frozen shoulder, carpal tunnel syndrome, trigger finger, Dupuytren's contracture, and limited joint mobility. 1,912 adults (62% female, 90% non-Hispanic White, mean age 40 yrs, median diabetes duration 20 yrs, mean HbA1c 7.8%) responded (response rate 32%).

Results: 586 (31%) adults indicated diagnosis of 1 or more joint problem; 293 (50%) were diagnosed with frozen shoulder, 293 (50%) with trigger finger, and 261 (45%) with carpal tunnel, thus showing the concomitant presence of multiple joints involvement in some participants. Only 92 (16%) and 66 (11%) were diagnosed with Dupuytren's contracture and limited joint mobility, respectively. Adults diagnosed with joint disease were more likely to be older (mean 53 yrs vs. 34 yrs; $P<0.001$) and had longer duration of diabetes (median 35 yrs vs. 16 yrs; $P<0.001$). 333 (57%) participants were treated through physical therapy, 293 (50%) through surgery, and 234 (40%) with steroids, highlighting the fact that multiple therapy modalities have been used for some survey participants. HbA1c levels at the time of survey completion were 7.6% in participants with cheiroarthropathy vs 7.9% in participants who did not report cheiroarthropathy.

Conclusion: With one-third of survey respondents noting a history of the condition, cheiroarthropathy is common in adults with type 1 diabetes. Development of standards of care for early recognition and treatment of diabetic cheiroarthropathy is needed, particularly for older adults and individuals with long-term diabetes. Counseling on the risk of cheiroarthropathy is warranted in clinical practice so patients are aware that they should seek medical attention should symptoms arise.

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Lower limb amputation in diabetes: incidence estimates and patients characterisation

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Background and aims: Diabetes Mellitus (DM) is the leading cause of lower limb amputation (LLA) in adults in the US. It is not clear how the incidence of LLA varies between type 1 (T1DM) and type 2 DM (T2DM). Using data from the Truven Health MarketScan databases, we evaluated the incidence rate of LLA in patients with and without T1DM or T2DM and assessed the presence of comorbidities at the time of the LLA.

Materials and methods: Using the Aetion evidence platform (Aetion, Inc., New York, New York, USA), we analysed data from 2010-2014. Four cohorts of patients were defined based on the presence of DM claims: no-diabetes, any DM, T1DM and T2DM. Cases of non-traumatic LLA were identified using diagnosis and procedure codes. All patients had at least one year of enrollment prior to cohort entry. Analyses were restricted to patients with no history of lower limb amputation. We evaluated the incidence rate per 1000 person-year (PY), and 95% confidence interval (CI), by DM cohort and sex. We also assessed the presence of comorbidities in the 4 weeks preceding the LLA.
Results: Mean age (SD) was 42.8 years (17.1) in the no-diabetes, 56.8 years (14.0) in the any DM, 50.6 years (18.2) in the T1DM and 57.8 years (12.3) in the T2DM cohorts. Proportion of men was between 47.2% in the no-diabetes and 53.5% in the T1DM cohorts. Crude overall incidence rates and confidence interval of LLA were 0.08 (0.08, 0.09) in the no-diabetes, 1.50 (1.48, 1.52) in the any diabetes, 5.79 (5.56, 6.03) in the T1DM and 1.62 (1.59, 1.65) in the T2DM cohorts. In all three diabetes cohorts, LLA incidence was about 1.5 to 2 times higher in men than women. In the four weeks preceding the LLA, compared to non-diabetics, patients with DM more often had a claim for Charcot foot (1.8% vs 0.2%), foot and leg ulcers (73.3% vs 39.7%), and cellulites (56.5% vs 29.3%) or osteomyelitis (65.0% vs 34.8%). Foot deformities were in contrast more often reported in non-diabetics (16.7% vs 5.4%). Claims for end-stage renal disease were most frequently reported in association with LLA in T1DM patients (29.4%).
Conclusion: We observed a higher incidence rate of LLA in T1DM than T2DM and in men than women when measured over the same period in a US-based insurance claims database. This could be due to differences in stage of the disease or imbalances in risk factors between groups, such as smoking status which could not be assessed.

	Number of cases of LLA	Person-years (PY) follow up	Incidence Rate in 1000 PY
No Diabetes			
Overall	6,322	75,464,818	0.08 (0.08,0.09)
Male	3,396	35,305,643	0.1 (0.09,0.1)
Female	2,926	40,159,175	0.07 (0.07,0.08)
Any Diabetes			
Overall	17,798	11,862,292	1.5 (1.48,1.52)
Male	12,261	6,096,320	2.01 (1.98,2.05)
Female	5,537	5,765,972	0.96 (0.93,0.99)
Type 1 diabetes			
Overall	2,366	408,328	5.79 (5.56,6.03)
Male	1,606	218,959	7.33 (6.98,7.69)
Female	760	189,370	4.01 (3.73,4.3)
Type 2 Diabetes			
Overall	9,222	5,691,794	1.62 (1.59,1.65)
Male	6,483	3,041,407	2.13 (2.08,2.18)
Female	2,739	2,650,387	1.03 (0.99,1.07)

Disclosure: **A. Déruaz-Luyet:** Employment/Consultancy; Employee of Boehringer Ingelheim.

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Less cortical folding in adult patients with longstanding type 1 diabetes is related to cognition and diabetes duration

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Background and aims: In adults with type 1 diabetes (T1DM) mild subcortical volume loss with intact thickness has been previously found. Until early adulthood the cortical structure shows fast developmental changes. Cortical gyrification, the folding of the cortex, on the other hand takes place in the 3rd trimester of pregnancy and remains stable during childhood development. Folding is important for intellectual and cognitive functioning in other populations and may also be related to cognitive decrements in T1DM.
Materials and methods: Thus, cortical gyrification was calculated using FreeSurfer5.3 in 51 adult T1DM patients with and 53 without proliferative retinopathy, and 49 controls. Additionally, the longitudinal 4-year trajectory of folding changes was determined in 25 randomly selected patients with complications and 25 matched controls. Differences in folding were correlated to cognition and disease variables in the patient group as a whole. Analyses were corrected for age, sex, systolic blood pressure, and depressive symptoms. Corrections for multiple vertex testing was performed using the strict Family Wise Error (FWE) method.
Results: At baseline, relative to controls, all patients as a group showed lower gyrification in the left supramarginal and inferior parietal cortices, as well as in the right supramarginal, frontal, and temporal lobe. Patients with complications drove these effects, although in the temporal lobe folding was lower in patients without complications versus controls as well (all $P_{FWE}<0.05$). There were no between patient group differences or changes in gyrification over 4 years in the 25 patients with complications relative to controls. Lower right hemisphere cortical folding at baseline was related to lower attention ($\beta=0.248$), higher age ($\beta=-0.197$), being female ($\beta=-0.292$), longer disease duration ($\beta=-0.235$), having proliferative retinopathy ($\beta=-0.214$), and greater left carotid intima media thickness ($\beta=-0.220$), with age and sex as the strongest predictors (all $P<0.05$), but did not predict changes in cognition after 4 years.
Conclusion: Cortical gyrification, which is characterized by the relative absence of developmental changes, is affected by T1DM and additionally proliferative retinopathy. No progression in loss of cortical folding was found in this middle-aged adult sample of patients with proliferative retinopathy.
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Disclosure: **R.G. Ijzerman:** None.

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hMT/V5 is more affected with age than other brain regions in patients with type 2 diabetes

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Background and aims: Type 2 Diabetes (T2D) may derive potential complications to several organs in humans. In this study, we investigated T2D brain complications using spatially separated speeds as stimuli to infer if the age is associated with complications in the visual cortex. The results are compared to Brodmann Area 7 (BA7; which has a role in locating objects in space) and the cingulate gyrus (CG; which has a role in emotion formation and processing, learning and memory). hMT/V5, BA7, and CG showed higher significance than more than 30 other brain regions that we have investigated in this study.
Materials and methods: Each stimulus contained two spatially separated dots moving back-and-forth in a maximally ambiguous difficult task, where both dots moved with the same speed and the subjects clicked a button to indicate the side of the faster dot. The other control-stimulus was a fixation point. The two dots are projected on different visual hemifields and hemispheres. The size of the image in the screen-pad was 22.62º × 17.06º visual angles and the subjects viewed the image at 50cm distance through a mirror, had their head movements restricted and viewed the stimuli monocularly with the dominant eye while the other eye was covered with an opaque eye-patch. Using a 3 Tesla scanner, functional and structural MRI have been acquired for 56 T2D patients aged (mean: 47.9

± 6.2; max: 73) and 73 healthy aged (mean: 58.2 ± 8.6; max: 71) healthy controls.

Results: For each functional MRI volume, we performed de-trending; skull stripping using FSL (FMRIB Software Library); motion correction, coregistration and normalization using SPM8 (Statistical Parametric Mapping). Anatomical ROIs have been generated in MNI space using the WFU PickAtlas. Moreover, we used pattern classification (Princeton MVPA toolbox) to predict the stimuli presented to each subject. We performed N-way Analysis of Variance (ANOVA) on the classification results that showed differences, via independent samples t-test, between T2D and controls in BA7, p(BA7L)=0.00003 and p(BA7R)=0.002; CG, p(CGL)=0.0006 and p(CGR)=00.04; and hMT/V5, p(hMT/V5-L)=0.00005 and p(hMT/V5-R)=0.00005. The results showed that the age significantly affects hMT/V5 more than BA7 and CG in T2D patients, as illustrated in Table 1.

Conclusion: This study has shown that the age may provoke more complications to hMT/V5 than other brain regions in T2D. We also performed similar analysis on the insula and BA6 and we found no relation with age in T2D (not reported due to lack of space). To confirm how much the age-provoked brain complications are related to diabetic retinopathy that exists in some patients of the used T2D sample, diabetic retinopathy scores have been used in the analysis. N-way ANOVA showed no relation between T2D and controls with regard to the age and the scores of diabetic retinopathy estimated using ETDRS schema scaled from 10 to 90 (age factor; F = 0.1, Prob>F is 0.7526). This clearly indicates that the detected hMT/V5 age-complications in T2D are not necessarily related to diabetic retinopathy.

Table 1. N-Way analysis of variance of T2D versus healthy subjects

		Left Hemispheric Region				Right Hemispheric Region			
		Sum Sq.	Mean Sq.	F	Prob>F	Sum Sq.	Mean Sq.	F	Prob>F
BA7	Group	0.08	0.08	10.38	0.0016	0.04	0.04	5.07	0.026
	Age	0.002	0.002	0.27	0.60	0.025	0.025	3.06	0.08
	Error	0.98	0.008	-	-	1.01	0.008	-	-
	Total	1.10	-	-	-	1.12	-	-	-
CG	Group	0.07	0.07	9.17	0.003	0.05	0.05	6.22	0.014
	Age	0.0017	0.0017	0.22	0.64	0.0015	0.0015	0.19	0.66
	Error	0.95	0.007	-	-	1.01	0.008	-	-
	Total	1.05	-	-	-	1.08	-	-	-
V5	Group	0.055	0.055	6.4	0.0012	0.036	0.036	4.05	0.046
	Age	1.07	1.07	12.4	0.0006	0.09	0.09	10.1	0.002
	Error	1.08	0.008	-	-	1.136	0.009	-	-
	Total	1.37	-	-	-	1.35	-	-	-

Degrees of freedom; Group (1), Age (1), Error (126), Total (128)

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The implications of insulin like growth factor 1 receptor in type 2 diabetes-related injuries of brain structure and function

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Background and aims: Patients with type 2 diabetes (T2D) showed significantly increased risk for various neurological and psychiatric disorders, such as major depression and dementia, which result from injuries of brain structure and function. Previous epidemiological and molecular investigations have highlighted the importance of insulin like growth factor 1 receptor (IGF1R) in maintaining normal brain function. However, the corresponding in vivo neurobiology of IGF1R was poorly understood. The present study aimed to reveal the associations between IGF1R and brain structure and neural function in T2D patients via genetic-imaging approach.

Materials and methods: A total of 168 patients with T2D were enrolled in the study, and every participant went through clinical evaluations, structural and functional magnetic resonance imaging (MRI) scans. High-throughput sequencing of *IGF1R* gene was utilized, and 23 single nucleotide polymorphisms (SNPs) entered the further genetic-imaging analysis after quality control. The associations with *IGF1R* polymorphisms and brain structure and function were investigated.

Results: In terms of brain structure, both rs17847195 (β = -0.44, FDR-corrected P = 0.023) and rs2684788 (β = -0.38, FDR-corrected P = 0.039) showed significantly associations with total brain volume. Furthermore, rs1815009 had significantly influence on volume of entorhinal cortex (β = -0.36, FDR-corrected P = 0.043). Regarding the neural activity, interactions were detected with rs2016347 and neural activity within right praecuneus (β = -0.58, FDR-corrected P = 0.013). Moreover, rs1815009 also modified the neural activity within left medial prefrontal cortex (β = -0.49, FDR-corrected P = 0.019).

Conclusion: The genetic-imaging approach expanded our understanding for the mechanisms underlying the implications of IGF1R in T2D-related injuries of brain structure and function, and alleviating the dysfunction of IGF1R may be a promising approach to relieve neurological and psychiatric disorders for patients with T2D.

SNPs	Minor allele	Minor allele frequency	Location	Functional activity	β	FDR-corrected P
rs17847195	A	0.09	Intron	Reduced volume of total brain	-0.44	0.023
rs2684788	A	0.05	3' UTR	Reduced volume of total brain	-0.38	0.039
rs1815009	A	0.11	3' UTR	Atrophy of entorhinal cortex	-0.36	0.043
				Reduced neural activity within medial prefrontal cortex	-0.49	0.019
rs2016347	A	0.06	3' UTR	Reduced neural activity within praecuneus	-0.58	0.013

Disclosure: **F. Su:** None.

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The relationship between blood glucose control and xanthine oxidoreductase activity in patients with diabetes

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Background and aims: The enzyme xanthine oxidoreductase (XOR) catalyzes the formation of uric acid (UA) from hypoxanthine and xanthine, which in turn are products of purine metabolism starting from ribose-5-phosphate. Besides the synthesis of UA, basic research has suggested that XOR is involved in the regulation of reactive oxygen species, adipogenesis, and peroxisome proliferator-activated receptor-γ (PPAR-γ). Indeed, it has been reported that XOR activity is associated with development of cardiovascular events and the progression of nephropathy. XOR activity has been shown to be much lower in humans than in rodents, which makes its accurate measurement difficult. A novel method that uses mass spectrometry to measure [$^{13}C_2$,$^{15}N_2$]UA using [$^{13}C_2$,$^{15}N_2$]xanthine as a substrate has been recently established to accurately determine plasma XOR activity in humans. We applied this novel method to accurately measure plasma XOR activity in patients with diabetes and evaluated its relationship with clinical parameters including glycemic control, diabetes type, body mass index (BMI), and uric acid metabolism.

Materials and methods: A total of 71 patients (females, 38; males, 33; mean age, 48.5 ± 16.3 years), including 36 with type 1 diabetes and 35 with type 2 diabetes, were enrolled. Blood samples were collected under fasting conditions in the early morning to measure plasma XOR activity and other parameters.

Results: The natural logarithmic value of XOR activity (ln-XOR) in the plasma was 3.8 ± 1.0 pmol/h/mL, while serum UA levels were 5.0 ± 1.5 mg/dL. Positive significant correlations ($p < 0.05$) of ln-XOR with HbA1c and BMI were observed (r = 0.249; HbA1c, r = 0.336; BMI). Multiple regression analysis revealed that both HbA1c ($\beta = 0.276$, $p = 0.031$) and BMI ($\beta = 0.326$, $p = 0.022$) were useful predictors of ln-XOR activity and that serum UA levels had a negative correlation with HbA1c ($\beta = -0.256$, $p < 0.01$).
Conclusion: Plasma XOR activity is associated both with worsening of glycemic control and adiposity, but not with serum UA in patients with diabetes. This raises the possibility that even with low serum UA levels, increased XOR activity may induce oxidative stress in patients with poor glycemic control.
Clinical Trial Registration Number: No. 2135
Disclosure: **K. Washio:** None.

1202

Development of the metabolic syndrome after orthotopic liver transplantation and relation with morbidity and mortality: a five year follow-up study

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Background and aims: The metabolic syndrome (MS) is a well-known complication of orthotopic liver transplantation (OLT) and may contribute to morbidity and mortality, especially by increasing the risk of cardiovascular disease (CVD). The present is a prospective study that was conducted to evaluate the development of the MS after OLT and its impact on morbidity and mortality over a 5-year follow-up.
Materials and methods: Eighty-four cirrhotic patients (75% male, mean age 53.9 ± 9.3 years) were evaluated at baseline and yearly for 5 years after OLT. The MS was defined according to the 2004 Adult Treatment Panel-III criteria. Complications, including major CVD events, infections, de novo malignancy and acute graft rejection, were recorded.
Results: Prevalence of the MS increased from 14/84 (16.6%) at baseline to 32/81 (39.5%) at year 1. During this period, 2 patients died (one with and one without the MS) and one was lost at follow-up. During the following 4 years, prevalence of the MS further increased to 34/73 (46.6%). Of patients with the MS at year 1, 9 reversed it, 18 did not, and 3 died. Of those without the MS, 14 developed the MS, 20 did not, and 5 died. Prevalence of obesity, arterial hypertension and hypertriglyceridemia progressively increased from year 1 to year 5 (obesity from 14.8% to 19.2%; arterial hypertension from 56.8% to 68.5%; hypertriglyceridemia from 25.9% to 30.1%).Conversely, prevalence of diabetes mellitus (DM) and low-HDL decreased from year 1 to year 5 (DM from 44.4%, 36/81, to 34.2%, 25/73; low-HDL from 34.5%, 28/81, to 27.4%, 20/73). Of patients with DM at year 1, 8 (22.2%) regressed and 4 died. DM regression was more frequent in patients without than in those with the MS (17.1% vs. 3%, p=0.05) and correlated inversely with the presence of the MS (r=-0.24, p=0.04). Of patients without DM at year 1, only one, who was affected by the MS, developed DM de novo. Age (p=0.04), BMI (p<0.001) and DM (p=0.03) at year 1 post-OLT were identified as independent variables associated with development of the MS during the subsequent 4 years. Patients who did not have the MS at any time from year 1 to year 5 showed a lower prevalence of major CVD events (10.0% vs.16.2%; p=0.31) and infections (40.0% vs. 55.8%; p=0.09) and a higher prevalence of graft rejection (30% vs. 27.2%; p=0.50) and de novo malignancy development (16.6% vs. 9.3%; p=0.30), as compared with those with the MS, though these differences did not achieve statistical significance. Major CVD events positively correlated with cyclosporine-based immunosuppressant therapy.
Conclusion: Even if development of the MS is an early phenomenon, its prevalence continues to increase for many years after OLT. Life-style intervention and individualization of immunosuppressant therapy may lead to improved outcomes in OLT patients.
Disclosure: **M.E. Lunati:** None.

1203

Type 2 diabetes is complicated by obesity and sleep apnoea syndrome: focussing in comorbidities

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Background and aims: Diabetes is characterized by high levels of blood glucose, which can cause both microangiopathies, including retinopathy and nephropathy, and macroangiopathies, including cerebral infarction, angina pectoris/myocardial infarction, and peripheral arterial disease. On the other hand, it has been reported that patients with sleep apnea syndrome (SAS), which is characterized by repeated episodes of apnea and hypopnea, are more likely to suffer from stroke, diabetes, and circulatory diseases, including hypertension, angina pectoris, myocardial infarction, chronic heart failure, and arrhythmia, compared to healthy subjects. Vascular complications are a common comorbidity in diabetes patients, which often results in absences from the workplace. It is therefore important to routinely evaluate and, where appropriate, treat diabetes patients for SAS. In this study, we focused on obesity, a known risk factor for SAS, and investigated the relationship between demographic and clinical variables on the one hand, and SAS and associated comorbidities on the other, in clinically obese type 2 diabetes mellitus patients.
Materials and methods: We evaluated 1367 ambulant patients with type 2 diabetes mellitus with a BMI ≧ 30 kg/m2. Their demographic and clinical data were recorded, as well as the presence of any systemic diseases. Their Apnea-Hypopnea Index (AHI) was determined using a portable sleep polygraph, and they were assessed for SAS and associated comorbidities.
Results: Fifty-six patients (33 males, 23 females) were retained for this study. The characteristics of the sample were as follows: average age, 56.7 ± 14.0 years; disease duration, 11.4 ± 8.4 years; BMI, 33.6 ± 2.9 kg/m2; waist circumference, 110.3 ± 9.7 cm; blood pressure, 136 ± 13 mmHg (systolic) and 78 ± 11 mmHg (diastolic); 25% (14/56) were alcohol consumers; 50% (28/56) were smokers; FBS, 165 ± 74 mg/dL; HbA1c, 7 .7±1.7%; fasting blood CPR, 4.3 ± 1.9 ng/mL; HOMA-IR, 12 .7 ± 20.4; Cr, 0.88 ± 0.29 mg/dL; and AHI 27.9 ± 22.1 (male, 30.3 ± 19.1; female, 24.6 ± 25.5). We found that 6 patients (10.7%) had a history of intracranial lesions, 7 cases (12.5%) had a respiratory comorbidity, 17 cases (30.4%) suffered from a circulatory disease, and 4 cases (7.1%) had comorbid cancer. The prevalence of SAS (AHI ≧ 15) was 75.8% (25/33) in males and 56.5% (13/23) in females. Among patients with an AHI ≧ 15, there was a higher proportion of intracranial lesions, including a history of cerebral infarction and encephalitis, subarachnoid hemorrhage or subdural hemorrhage, and pituitary tumor, than among patients with an AHI < 15 ($p < 0.05$). In addition, multivariable analysis adjusted to age, sex, and BMI identified intracranial lesions as an independent risk factor for the development of SAS.
Conclusion: There was a high prevalence of SAS in both male and female patients with type 2 diabetes mellitus and a BMI ≧ 30 kg/m2. In addition, our study demonstrates that SAS is found in patients with intracranial lesions, and that an important proportion of patients with type 2 diabetes mellitus have a history of intracranial lesions. This is likely related to the ageing of the Japanese society and strongly indicative of a need to routinely evaluate and treat diabetes patients for SAS.
Disclosure: **S. Kawasaki:** None.

1204

Pancreatic size and fat content in diabetes: a systematic review and meta-analysis of imaging studies

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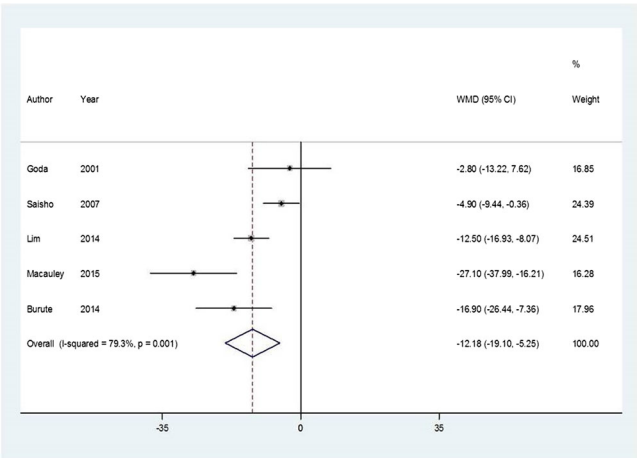
Background and aims: Imaging studies are expected to produce reliable information regarding the size and fat content of the pancreas. However, the available information have produced inconclusive results. The aim of this study was to perform a systematic review and meta-analysis of imaging studies assessing pancreas size and fat content in patients with type 1 and type 2 diabetes.
Materials and methods: Medline and Embase databases search was performed. Studies evaluating pancreatic size (diameter, area or volume) and/or fat content by ultrasound, computed tomography, or magnetic resonance imaging in patients with type 1 diabetes and/or type 2 diabetes as compared to healthy controls were selected. Sixteen studies including 2,593 subjects (284 with type 1 diabetes, 1,069 with type 2 diabetes, and 1,240 control subjects) were included in meta-analyses. Pancreas diameter, area, volume, density, and fat percentage were evaluated.
Results: Pancreatic volume was reduced in type 1 diabetes (-38.72 cm3, 95%CI: -52.25 to -25.19, I2=70.2%, p for heterogeneity=0.018) and type 2 diabetes (-12.18 cm3, 95%CI: -19.1 to -5.25, I2=79.3%, p for heterogeneity=0.001) in comparison with controls (FIGURE). Fat content was higher in type 2 diabetes vs. controls (+2.73%, 95%CI 0.55 to 4.91, I^2=82.0%, p for heterogeneity<0.001).
Conclusion: Individuals with both types of diabetes have reduced pancreas size in comparison with control subjects. Patients with type 2 diabetes have increased pancreatic fat content. Further longitudinal studies are required to elucidate the cause and effect relationship between pancreatic size and diabetes, as well as the possible causes of pancreas shrinkage and fat deposition. A better understanding of the mechanisms of altered pancreas morphology and fat deposition in diabetes may lead to new insights in preventing, predicting, and treating diabetes.



Supported by: FIPE
Disclosure: **C.B. Leitao:** None.

1205

Risk of DKA with Hamman's syndrome: a case control study and a systematic review with a single case report

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Background and aims: Hamman's syndrome is spontaneous mediastinal emphysema which often occurs during labor. It is also reported as a rare complication of diabetic ketoacidosis (DKA). The primary component of the pathophysiology is believed to be air leak from alveoli followed by high intrathoracic pressure from vomiting or hyperventilation. Impaired surfactant of alveolar surface induced by severe acidemia may also be another cause. We recently experienced a 40 year-old female diagnosed with DKA presenting Hamman's syndrome. She showed severe acidemia and compensatory hyperventilation (pH 6.956, pCO2 13.7mmHg, respiratory rate 32/min). With an aim to reveal the clinical characteristics and the risk factors of DKA with Hamman's syndrome, we conducted a systematic literature review and a case control study.
Materials and methods: The case was defined as DKA with Hamman's syndrome. In addition to our case, we searched case reports through two electronic databases (PubMed and Ichushi) with language restriction to English and Japanese. Keywords were "Hamman's syndrome", {"mediastinal emphysema" and "diabetes"} or {"pneumomediastinum" and "diabetes"}. We manually reviewed the literature by two physicians to confirm the diagnosis of DKA and Hamman's syndrome. As for DKA control without Hamman's syndrome, we included all the patients treated in our hospital from April 2009 to January 2017. We excluded patients under age 20 years old since pediatric DKA is seldom referred to our hospital. We tested inter-group differences of the clinical characteristics by two-sided t test. We also performed logistic regression analysis to elucidate an independent risk factor of DKA with Hamman's syndrome.
Results: Among 100 cases pooled from 85 papers, 64% were male, median pH and pCO2 were 7.13 and 15.9 mmHg respectively. Median respiratory rate was 32/min. Adult cases accounted for 63% (n=63) and were compared with 85 controls as shown in the table below. Hamman's syndrome was more prevalent in younger age group and also in type 1 diabetes. The cases exhibited frequent heart rate and respiratory rate. Blood gas analysis showed excessive acidemia and hypocapnia in Hamman's syndrome. Logistic regression analysis revealed the independent risk factors were younger age (Coefficient= -0.25, p<0.0001), faster heart rate (Coefficient=0.07, p=0.01), lower pH and pCO2 (Coefficient= -22.1, p=0.02, Coefficient= -0.41, p=0.02). Respiratory rate was not a significant factor possibly due to limited available data (n=22).
Conclusion: Younger aged DKA with severe acidemia and hypocapnia may be at the increased risk of Hamman's syndrome.

	Hamman's syndrome (n=100)	Hamman's syndrome over 20 (n=63)	Controls over 20 (n=85)	p-value
Age (year)	21 (17, 30)	29 (22, 36)	64 (44, 77)	< 0.0001
male (n, %)	63 (64%)	43 (69%)	43 (43%)	0.02
Type 1/ Type 2 diabetes (n, %)	35 (35%) / 7 (7%)	23 (26%) / 4 (5%)	23 (26%) / 38 (43%)	< 0.0001
HR (beats/min)	129 (108 ,140)	120 (110 to 130)	97 (92 to 102)	< 0.0001
Resperatory rate	32 (29, 38)	29 (25 to 32)	25 (20 to 30)	0.21
Glucose (mg/dl)	732 (673 to 790)	694 (632 to 757)	713 (638 to 788)	0.74
HbA1c (NGSP, %)	12.5 (11.5 to 13.6)	13.0 (11.5 to 14.6)	11.3 (10.6 to 12.0)	0.02
BGA				
pH	7.13 (6.99, 7.23)	7.13 (7.08 to 7.17)	7.21 (7.18 to 7.25)	0.004
pCO2 (mmHg)	15.9 (10.0, 24.6)	20.2 (15.9 to 24.6)	27.2 (25.0 to 29.5)	0.006
HCO3 (mmol/L)	6.0 (4.0, 10.0)	8.1 (6.1 to 10.0)	12.2 (10.8 to 13.7)	0.002

Disclosure: **Y. Namiki:** None.

PS 108 The bones in diabetes

1206

Usefulness of FRAX to predict the risk of fractures in a diabetic Portuguese population

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Background and aims: Several studies have shown that Diabetes Mellitus is associated with an increased risk of fracture. In Portugal, it is estimated that diabetes affects about 13% of the adult population. Osteoporosis is characterised by an increased risk of fracture due to a decrease in bone mass and/or deterioration of bone microarchitecture. The WHO fracture risk assessment tool (FRAX), used in the decision to institute anti-osteoporotic therapy, is an algorithm that estimates the probability of occurrence of fracture (major and hip) at 10 years taking into account variables such as age, gender, previous fracture, family history, low weight, body mass index, continued glucocorticoid therapy, rheumatoid arthritis, smoking, excessive alcohol consumption, adjusted, or not, with bone mineral density (BMD). The aim of this study is to analyse the power of the FRAX algorithm in the Portuguese diabetic population without/with Dual-energy X-ray absorptiometry in the subpopulation - diabetic women over 50.
Materials and methods: Individuals from the EpiReumaPt study (2011-2013, 10,661 adults) with FRAX data (n=3545) were selected and divided according presence/absence of diabetes. The population was further stratified by gender and age and the data analysed by non-parametric Mann-Whitney test (males) and t-student. A 95% confidence interval was considered.
Results: The EpireumaPt data revealed a sample population with a diabetes prevalence of 13,3%. The FRAX computed without BMD data, was higher in the diabetic group (n=474) for both major and hip fractures compared to non-diabetic (n=3071) (5.42±0.24 vs 3.90±0.08, p<0.05 and 2.10±0.16 vs. 1.26±0.05, p<0.05, respectively). Nonetheless, the subpopulations aged 50 or less years reveal a similar 10-year likelihood of both types of fractures between diabetic and non-diabetic males (1.95 ±0.45 vs 0.92±0.05, p=0.569 and 0.22 ±0.08 vs 0.11±0.01, p=0.325), females (2.07±0.32 vs 1.89±0.03, p=0.577 and 0.21±0.06 vs 0.16±0.01, p=0.470) as well as for males aged over 50 years (3.60±0.19 vs 3.29 ±0.11, p=0.180 and 1.49±0.14 vs 1.28±0.08, p=0.217). Simply diabetic women aged >50 years (n=248) have a significantly higher FRAX for major and hip fractures compared to non-diabetic (n=1161) (6.80±0.41 vs 5.65±0.17, p=0.005 and 2.67±0.29 vs. 2.06±0.11, p=0.028). However, when FRAX was adjusted with bone mineral density data, a similar 10-year likelihood of both fractures types between diabetic (n=57) and non-diabetic (n=189) is observed (7.92±0.89 vs 9.11±0.47, p=0.226 and 3. 09 ±0.53 vs 3.83±0.36, p=0.300).
Conclusion: In a population sample with the same diabetes prevalence as the Portuguese population, FRAX underestimates the risk of fractures in diabetic patients except in diabetic women over 50. However, in those diabetic women when bone mineral density is accounted the FRAX power of discerning fracture risk is lost. These results empower the recently suggested risk underestimation of the FRAX algorithm in diabetic population primarily when computed with bone mineral density.
Disclosure: **C. de Mello-Sampayo:** None.

1207

Estimating the risk of osteoporosis related fractures in a post-menopause type 2 diabetic Portuguese population

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Background and aims: Like diabetes (with an estimated prevalence of 13% of the adult population in Portugal), in particular type 2 diabetes, osteoporosis is an increasingly prevalent disease. Post-menopause osteoporosis is characterised by an increased risk of fracture due to an accelerated decrease in bone mass and/or deterioration of bone microarchitecture. Several studies have shown that type 2 diabetes is associated with a poor bone quality and increased risk of fragility fractures. The WHO fracture risk assessment tool (FRAX), adapted for Portuguese population in 2013, and used in the decision to institute anti-osteoporotic therapy, is an algorithm that estimates the probability of occurrence of fracture (major and hip) at 10 years taking into account, or not, the bone mineral density (BMD). FRAX provides valid prediction without BMD values although its accuracy increases when BMD is considered. The aim of this study is to verify the accuracy of the FRAX algorithm when computed, or not, with BMD in a Portuguese post-menopause female population with and without type 2 diabetes.
Materials and methods: A female sample population aged between 50 and 81 years old (2016-17, n=159) from Sintra, a neighbourhood city of the Portuguese capital, was assessed for the FRAX algorithm variables (age, gender, previous fracture, family history, low weight, body mass index, continued glucocorticoid therapy, rheumatoid arthritis, smoking, excessive alcohol consumption) and the Dual-energy X-ray absorptiometry evaluated using GE-Lunar. Patients with co-morbidities were excluded and the data divided according presence (n=67)/absence (n=84) of type 2 diabetes. The data was analysed by (paired or not) t-student. A 95% confidence interval was considered.
Results: The FRAX computed without BMD data was higher in the type 2 diabetes female group for both major and hip fractures compared to non-diabetic (6.5±0.5 vs 4.7±0.4, p=0.004 and 2.0±0.2 vs. 1.4±0.2, p=0.043). When FRAX was adjusted with BMD data, a similar 10-year likelihood of both fractures types between type 2 diabetes and non-diabetic was observed (5.9±0.4 vs 5.1±0.4, p=0.158 and 1.6±0.2 vs 1.4 ±0.2, p=0.446). In order to further understand these results, the undertaken analysis within each population group revealed a significantly lower FRAX for major and hip fractures in type 2 diabetes group when using DMO to compute it (5.9±0.4 vs 6.5±0.5, p=0.037 and 1.6±0.2 vs. 2.0 ±0.2, p=0.025) while that of controls was significantly higher for major fractures (5.3±0.3 vs 4.7±0.4 p=0.028) and similar for hip fractures (1.4 ±0.2 vs 1.4±0.2 p=0.841).
Conclusion: The obtained results suggest that in a female population sample over 50 years old, with no other identified morbidities except for type 2 diabetes, the use of DMO as a FRAX variable underestimates the risk of osteoporosis related fractures, which contrasts with the significant impact of its use to compute the major fractures FRAX of non-diabetic patients (increase). However the accuracy of FRAX to predict hip fractures is not affected by the use of BMD when considered morbidities are absent. These results suggest that for type 2 diabetes patients BMD might be a confounding factor to estimate the risk of fractures.
Disclosure: **M.C. Marques:** None.

1208

Low skeletal muscle mass is at increased risk of all-cause mortality in patients with type 2 diabetes

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Background and aims: Skeletal muscle is known to be an important tissue for insulin action and glucose metabolism. It is considered that the disturbance of glucose metabolism leads to loss of skeletal muscle in type 2 diabetes mellitus (T2DM) and vice versa. Thus, loss of muscle mass is a serious problem in patients with T2DM. It has been shown that patients with diabetes have low muscle mass of limbs, which may affect the increased mortality. However, there is no evidence to show whether the muscle mass reduction is associated with the mortality risk in patients

with T2DM. This study thus aimed to examine whether skeletal muscle mass index (SMI) were associated with the mortality rate in patients with T2DM.

Materials and methods: This is a historical cohort study with the endpoint of all-cause mortality in T2DM. A total of 141 postmenopausal women and 163 men (mean age of 66.1 and 64.4 years at baseline, respectively) were recruited whose appendicular skeletal muscle mass (ASM) was evaluated by dual-energy X-ray absorptiometry at Shimane University Hospital. SMI was calculated by the following formula: ASM / height^2. Low skeletal muscle mass was defined as SMI <5.4 kg/m^2 for women and <7.0 kg/m^2 for men according to the criteria of Asian Working Group for Sarcopenia. The participants were observed up to 7 years from the start of this study, and the association between SMI at baseline and the mortality rate was examined by the Kaplan-Meier method, the logrank test, and Cox regression analysis.

Results: The numbers of postmenopausal women and men with lower SMI were 17 (12.1%) and 69 (42.3%), respectively. During the follow-up period (average 6.2 years for postmenopausal women and 5.9 years for men), 14 postmenopausal women (9.9%) and 32 men (19.6%) died, respectively. Postmenopausal women with lower SMI had significant lower body mass index (BMI) compared to those with higher SMI (20.1 ± 2.4 kg/m^2 v.s 25.3 ± 4.4 kg/m^2, $p < 0.001$). Men with lower SMI had significant lower BMI and lower fasting C-peptide levels compared to those with higher SMI (BMI: 20.8 ± 2.5 kg/m^2 v.s 24.6 ± 2.6 kg/m^2, $p < 0.001$; C-peptide, 1.4 ± 0.7 ng/mL v.s 1.8 ± 1.1 ng/mL, $p = 0.021$). The Kaplan-Meier method and the logrank test indicated that lower SMI levels were significantly associated with higher mortality risk both in men and postmenopausal women ($p = 0.008$ and $p = 0.014$, respectively). In the Cox regression analysis adjusted for age, duration of T2DM, HbA1c, serum creatinine, and fasting C-peptide, SMI was significantly and inversely associated with the mortality risk [for postmenopausal women, hazard ratio (HR) = 6.20, 95% confidence interval (CI) = 1.46-26.28, $p = 0.013$; for men, HR = 2.46, 95%CI = 1.13-5.37, $p = 0.023$]. Moreover, after additional adjustment for BMI, the association remained significant in postmenopausal women (HR = 5.97, 95%CI = 1.04-34.37, $p = 0.045$) and marginal association was shown in men (HR = 2.38, 95%CI = 0.92-6.14, $p = 0.074$).

Conclusion: The present study showed that lower SMI was associated with the increased all-cause mortality in patients with T2DM. Furthermore, BMI-independent association was found in postmenopausal women. Therefore, the preservation of skeletal muscle mass is important to protect against the increased mortality risk in patients with T2DM, especially postmenopausal women.

Disclosure: **H. Miyake:** None.

1209

Age at first childbirth and sarcopenic obesity in postmenopausal women with diabetes: the Korean National Health and Nutrition Examination Survey 2009-2010

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Background and aims: Sarcopenic obesity is a double burden for older people because it carries the cumulative risk of functional abnormality, metabolic, cardiovascular risk, and mortality. The objective of the present study was to determine whether there was an association between age at first childbirth and sarcopenic obesity in postmenopausal women with diabetes.

Materials and methods: This study was based on data from the Korean National Health and Nutrition Examination Survey (KNHANES) from 2009 to 2010. Subjects were subdivided according to their age at first childbirth as follows: ≤ 19 years, 20-24 years, 25-29 years, and ≥ 30 years.

Results: Sarcopenic obesity prevalence differed significantly between the subgroups and increased with earlier age at first childbirth, with 11.7% in subjects ≥ 30 years at first childbirth and 30.7% in subjects ≤ 19 years at first childbirth. After fully adjusting for confounding factors, including chronic diseases, sociodemographic influences, lifestyle differences, serum 25(OH)D levels, and reproductive issues, women ≤ 19 years at first childbirth were significantly associated with sarcopenic obesity (odds ratio [OR] 1.719 [95% CI 1.091-2.711]).

Conclusion: Women's age at first childbirth influenced the sarcopenic obesity risk in postmenopausal women with diabetes, and adolescent pregnancy was independently associated with a higher risk of sarcopenic obesity in postmenopausal women with diabetes.

Disclosure: **J. Kim:** None.

1210

Osteoporosis and vertebral fracture are associated with deterioration of ADL and QOL in patients with type 2 diabetes independently of other diabetic complications

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Background and aims: Osteoporosis has been recognized as one of diabetic complications. However, because patients with diabetes usually have various complications such as micro- and macrovascular diseases, it is unclear whether the diabetes-related osteoporosis independently contributes to the deterioration of ADL and QOL. This cross-sectional study thus aimed to investigate the association between osteoporosis and assessment of ADL and QOL in type 2 diabetes mellitus (T2DM).

Materials and methods: The participants were 309 Japanese patients with T2DM (mean age; 65.3 years, mean duration of T2DM; 12.2 years, male; 63.4%). ADL and QOL were assessed by Barthel index (BI) and SF36 questionnaires, respectively. According to the Japanese diagnostic criteria, the presence of osteoporosis and vertebral fracture were diagnosed. Grading of vertebral fracture was performed using Genant Semiquantitative criteria. Statistical evaluations for differences among the groups were carried out using unpaired t test and Jonckheere-Terpstra trend test. Multiple logistic regression analyses adjusted for age, sex, T2DM duration, body mass index, HbA1c, estimated GFR, the presence of other diabetic complication (neuropathy, retinopathy, nephropathy, cardiovascular and cerebrovascular diseases, and peripheral artery diseases), and anti-diabetic treatments were performed to examine the association of the presence of osteoporosis or vertebral fracture with deteriorated ADL (below BI 99 scores) and QOL [below median for each component; physical functioning (PF), role physical (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role emotional (RE), and mental health (MH)].

Results: The numbers of patients with osteoporosis or vertebral fracture were 166 (53.7%) and 118 (38.2%), respectively. Compared to patients without osteoporosis, patients with osteoporosis were significantly older and had longer T2DM duration and lower scores of BI, PF, RF, SF, and RE ($p<0.05$ at least). Moreover, multiple logistic regression analysis showed that the presence of osteoporosis was significantly and independently associated with lower GH, SF, and RE [odds ratio (OR); 2.56, 1.79, and 1.92, respectively, $p<0.05$ at least] and trended to be associated with lower BI (OR 2.39, $p=0.068$). According to severity of vertebral fracture grade, patients with severe vertebral fracture were older and had longer T2DM duration and lower scores of BI, PF, RP, GH, and RE (p-trend<0.05 at least). Moreover, the presence of vertebral fracture grade 2 or 3 was significantly and independently associated with lower BI, BP, GH, VT, SF, and RE (OR; 2.58, 2.01, 3.64, 1.99, 2.18, and 1.97, $p<0.05$ at least).

Conclusion: The present study is the first to show that the presence of osteoporosis and severe vertebral fracture was associated with the deterioration of ADL and QOL independently of age, HbA1c levels, renal function, other diabetic complications and anti-diabetes treatments in patients with T2DM. Therefore, the management for diabetes-related

osteoporosis is an important task to protect against the deterioration of ADL and QOL in patients with T2DM.
Supported by: IK/Japan Osteoporosis Foundation
Disclosure: **I. Kanazawa:** None.

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Efficacy of anti-osteoporotic therapies in patients with type 1 and type 2 diabetes: a systematic review

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Background and aims: Both type 1 (T1DM) and type 2 diabetes mellitus (T2DM) have been associated with bone fragility and increased fracture risk. However, little is known regarding the effect of anti-osteoporotic treatment on bone mineral density (BMD) and / or fracture risk in patients with diabetes mellitus (DM). The aim of this systematic review was to investigate the efficacy of the available anti-osteoporotic therapies in patients with DM.
Materials and methods: MEDLINE and Scopus databases were searched (up to 31st March 2017) for the efficacy of anti-osteoporotic therapies in patients with DM versus controls.
Results: After excluding duplicates, 2,890 articles were identified, ten of which fulfilled inclusion criteria [patients with T2DM (n=9) or either DM type (n=1)]. *Bisphosphonates (n=2):* In one population-based study, DM group (n=3,482) exposed to bisphosphonates demonstrated similar vertebral and non-vertebral fracture risk compared with controls (n=94,028), without difference between the two DM types. In another study, bisphosphonates (mostly alendronate for 12 months) induced similar increases in spine BMD (no difference in hip) in DM (n=35) and control (n=35) post-menopausal women. *Alendronate (n=3):* In a post-hoc analysis of a randomized controlled trial (RCT), alendronate led to equal increases in spine and hip BMD (compared with placebo) in DM (n=148) and control groups (n=3,087) after 36 months of treatment. Data from two (n=52, n=151) retrospective studies showed comparable increases in spine BMD in DM and control groups, with no benefit in hip and non-vertebral fractures. *Risedronate (n=1):* In a post-hoc analysis of three RCTs, risedronate resulted in equal increases in spine BMD in both DM (n=53) and control groups (n=832) after 12 months of treatment. *Raloxifene (n=4):* In two post-hoc analyses of RCTs and the aforementioned retrospective population-based study, DM group exposed to raloxifene demonstrated the same vertebral fracture risk reduction compared with control groups without effect on non-vertebral fractures in either treatment group. In one small, observational study in patients on hemodialysis, the effect on spine BMD was comparable. *Teriparatide (n=1)*: DM (n=133) and control groups (n=196) demonstrated similar increases in spine and total hip BMD, after treatment for 24 months. No difference between the two groups was noticed in the incidence of non-vertebral fractures. No eligible study was found for zoledronic acid, ibandronate, strontium ranelate, denosumab or bazedoxifene. The effect of the aforementioned therapies on bone turnover in DM and control groups was comparable.
Conclusion: Despite heterogeneity of data, all anti-osteoporotic therapies are equally effective in patients with DM compared with those without DM, with respect to BMD and vertebral fracture risk. Thus, DM does not alter anti-osteoporotic treatment response.
Disclosure: **P. Anagnostis:** None.

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Impaired adhesion of human periodontal ligament fibroblast to collagen-I in model hyperglycaemia in vitro is corrected by Glo1 inducer

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Background and aims: Diabetes and inflammation enhances periodontal bone loss through enhanced resorption and diminished bone formation. It has been shown that human PDL fibroblast (hPDLF) attachment and function to type 1 collagen was impaired by methylglyoxal (MG) modification *in vitro.* MG is a reactive dicarbonyl metabolite produced mainly as a byproduct of glycolysis and precursor of advanced glycation endproducts (AGEs). We found that increased hPDLF detachment and dysfunction is partly mediated by increased exposure to high glucose concentration with increased formation of MG, impaired MG metabolism by glyoxalase 1 (Glo1) of the cytoplasmic glyoxalase system and increased formation of MG-derived AGEs. The aim of this study was to evaluate the effects of Glo1 inducer in high and low glucose concentrations on the hPDLFs adhesion efficiency.
Materials and methods: Primary hPDLFs were purchased from ScienCell, Carlsbad, USA. They were cultured in Modified Eagles Medium (MEM) supplemented with L-alanyl-L-glutamine (2 mM), 10% FBS and 100 Units/ml penicillin and 100 μg/ml streptomycin at 37ºC. Cells were passaged every 3 days. For experiments performed in low and high glucose conditions, culture media was supplemented with 8 mM and 25 mM glucose, respectively. Primary hPDLFs were incubated with high and low glucose media in triplicate for 3 days with or without Glo1 inducers (10 μM)): trans-resveratrol (tRES), hesperetin (HESP) and tREV and HESP combined (tRES-HESP). Cells were then incubated in a collagen-I coated cell adhesion assay plate for 3 h. After removing the unattached cells, adhesion efficiency was measured by quantifying attached cells.
Results: hPDLFs incubated in high glucose concentration gave a decreased adhesion to collagen-I: cell adhesion - 36 ± 9%, with respect to low glucose control ($P<0.05$, n = 3). Glo1 inducers, tRES, HESP and tRES-HESP combination improved cell adhesion in low glucose concentration cultures: cell adhesion + 52 ± 17%, +40 ± 7%, and + 57 ± 24%, respectively, with respect to low glucose control ($P<0.05$, n = 3). In high glucose concentration, Glo1 inducers corrected the decreased adhesion to collagen-I and improved cell adhesion to values higher than the low glucose control: tRES, + 87 ± 15%, HESP + 104 ± 20%, and tRES-HESP combination, + 60 ± 11%, with respect to low glucose control ($P<0.01$, n = 3; $P<0.001$ with respect to high glucose control). Similar incubations of control cells with collagen-I pre-incubated with conditioned medium with and without prior incubation with 500 μM aminoguanidine to scavenge MG suggested that MG in conditioned medium could also modified collagen-I to decreased cell adhesion. This was also prevented by Glo1 inducers.
Conclusion: We conclude that hPDLFs suffer dicarbonyl stress in high glucose concentration *in vitro* that impairs adhesion to collagen-I and this may be corrected by Glo1 inducer treatment. MG modification of collagen-I released from hPDLFs may also modify integrin binding sites and impair cell adhesion.
Supported by: Saudia Arabian PhD studentship
Disclosure: **A.A.D. Ashour:** Grants; Saudi Arabian studentship.

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Glyoxalase-1 inducer corrects disturbance of the cytosolic proteome in human periodontal ligament fibroblasts in model hyperglycaemia in vitro

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Background and aims: Hyperglycemia induces dysfunction in human periodontal ligament fibroblasts (hPDLFs) in diabetes which may be linked to increased risk of periodontitis. Cell dysfunction includes: abnormal accumulation of the glucose-derived reactive dicarbonyl metabolite,

methylglyoxal (MG) - dicarbonyl stress - and related increased formation of advanced glycation endproducts (AGEs), abnormal secretion and processing of extracellular matrix proteins, inflammation, oxidative stress, increased formation of advanced glycation endproducts (AGEs) and other processes. Insights into drivers of these processes may be gained by deep proteomics analysis and analysis of key proteomic pathways disturbed in hyperglycemia. The aim of this study was to analyse the cytosolic proteome of hPDLFs incubated in low and high glucose concentrations - the latter to model hyperglycemia - and study the possible reversible of effects in high glucose by treatment with glyoxalase inducer, trans-resveratrol-hesperetin combination (tRES-HESP).

Materials and methods: Primary cultures of hPDLFs were incubated in low and high glucose concentration media (8 mM and 25 mM, respectively) for three days with and without Glo1 inducer, tRES-HESP (10 μM); n = 3. Cytosolic protein was reduced, alkylated and digested with Lys-C and trypsin. Peptides were analysed by nanoflow liquid chromatography-Orbitrap Fusion™ (Thermo) mass spectrometry and a relative quantification of protein concentration was performed using non-conflicting peptides in Progenesis QI™ software (Nonlinear Dynamics, Newcastle upon Tyne, U.K.). Protein ontology was evaluated using literature and Web-based tools (http://www.reactome.org/) to identify functional annotation to characterise molecular functions and biologic processes. Protein identification was based on at least 2 unique peptides and protein sequence coverage range was 5 - 40%.

Results: In hPDLF cytosolic extracts, deep proteomics analysis detected 1077 proteins. Twenty-two cytosolic proteins were downregulated and 17 upregulated in high glucose incubations. Twenty of these proteins were significantly normalised by the Glo1 inducer. Many of the down-regulated proteins in high glucose concentration cultures that were normalised by Glo1 inducer were shown to be associated with the coatomer (COP)-mediated vesicle transportation pathway (P = 0.036). Additionally, in high glucose concentration cultures insulin-degrading enzyme (IDE) was decreased 43%, with respect to low glucose concentration cultures. This was normalised by Glo1 inducer treatment. IDE exhibits heat shock protein-like activity in association with the 26S proteasome to clear damaged proteins.

Conclusion: There were selected increases and decreases of proteins in the cytosolic proteins of hPDLFs exposed to high glucose concentration. Glo1 inducer corrected most of the changes. Disturbance of the cytosolic proteome in hyperglycemia may explain deficits in vesicle transport and proteostasis of hPDLFs in diabetes.

Supported by: Saudi Arabian PhD studentship

Disclosure: **N. Rabbani:** None.

1214

Serum potassium control and patiromer taken without or with food in hyperkalaemic patients with diabetes

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Background and aims: Patiromer, a sodium-free, nonabsorbed potassium (K^+)-binding polymer that uses calcium as the counter exchange ion, is approved in the U.S. for the treatment of hyperkalemia (HK) and is under review in the E.U. Patiromer has been shown to significantly lower serum K^+ in several clinical trials, including in HK patients with diabetes mellitus (DM) and CKD in the AMETHYST-DN study. The recommended starting dose of patiromer for hyperkalemia treatment is 8.4 g once daily (QD) with food. The recently completed TOURMALINE study was designed to assess the efficacy and safety of patiromer given QD without food compared to with food for the treatment of HK. Here we report the results of a pre-specified subgroup analysis of patients with Type 1 or 2 DM randomized in TOURMALINE.

Materials and methods: TOURMALINE was an open-label, randomized study of HK patients (2 screening K^+ values >5.0 mEq/L assessed locally using a point of care device). If patients were on RAAS inhibitors, beta-blockers, or diuretics, doses were required to be stable for 14 days before screening. Patients could have well-controlled DM (Type 1 or Type 2), heart failure, hypertension, and/or CKD but none were a requirement for study entry. Patients meeting eligibility criteria were randomized in a 1:1 ratio to receive patiromer 8.4 g QD initially, either without food (≥1 hr before or ≥2 hr after eating) or with food (from start of meal to 30 min after eating). Randomization was stratified by screening K^+, race, and history of DM. Endpoints were between-group comparison for the proportion (95% CI) of patients with week 3 or 4 serum K^+ in the target range (3.8-5.0 mEq/L) and the mean (±SE) change in serum K^+ from baseline to week 4.

Results: Of 114 randomized patients, 94 (82%) had DM (without food, n=48; with food, n=46); 84/94 (89%) completed the study. Two patients in the with-food group were excluded from efficacy analyses (1 did not receive ≥1 patiromer dose; and 1 had an important protocol violation and no post-baseline serum K^+); the former patient was also excluded from safety analyses. Of patients with DM, 87/92 had Type 2 DM; mean (SD) time since DM diagnosis was 17.2 (10.5) yr; 59% were on RAAS inhibitors and mean (SD) eGFR was 40.8 (25.1) ml/min/1.73 m^2. Overall, 83.7% (95% CI, 74.5-90.6) of DM patients achieved target serum K^+ at week 3 or 4 (81.3% [95% CI, 67.4-91.1] in the without-food group and 86.4% [95% CI, 72.6-94.8] in the with-food group; p=0.5072), consistent with results observed in the overall study population. The mean (±SE) change from baseline to week 4 in serum K^+ in the DM subgroup (-0.55 ±0.06 mEq/L) was consistent with the change in the overall population (-0.57±0.06 mEq/L). Consistent with prior patiromer trials, 45/93 (48%) of patients with DM had 1 or more adverse event (AE), with similar rates in the without-food and with-food groups. Gastrointestinal AEs were the most common AE class (none severe) in DM patients, including 6 (6.5%) with diarrhea and 3 (3.2%) with constipation. Four DM patients had serious AEs; none were considered by the investigator to be related to patiromer.

Conclusion: More than 80% of patients with DM achieved serum K^+ between 3.8-5.0 mEq/L with once-daily patiromer, with similar results when patiromer was given with or without food. Patiromer was generally well tolerated in patients with DM.

Clinical Trial Registration Number: NCT02694744

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PS 109 Diabetes in childhood

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Role of gluten free diet in the preservation of insulin reserve in newly diagnosed type 1 diabetic children with coeliac disease

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Background and aims: Experimental results suggest that dietary gluten may play a role in the progression of type 1 diabetes mellitus (T1DM). Based on clinical data, it can be assumed that gluten-free diet (GFD) could delay the progression of T1DM. T1DM is frequently associated with celiac disease (CD). We hypothesized that diabetic patients with CD on GFD would have different insulin requirement as compared to diabetic peers consuming gluten. Our aim was to assess whether GFD started at the diagnosis of T1DM has any impact on increasing insulin need in the remission phase of T1DM.
Materials and methods: Pediatric patients with T1DM and CD from the Ist Department of Pediatrics, Semmelweis University were enrolled in the study (31 girls, 15 boys, age at the time of T1DM diagnosis 6.9 ± 3.9 years). Four patients were also enrolled who were on GFD without the diagnosis of CD. Patients were divided in two groups: I. GFD started within two months after the diagnosis of T1DM (group GFD +, n = 27) and II. GFD started some years later or not kept (group GFD-, n = 19). Gluten consumption was presumed based on anamnestic data and/or positive serology for transglutaminase autoantibodies. Insulin requirements were evaluated retrospectively during the first three years after the diagnosis of T1DM, and the difference between the two study groups was compared every six months. Data were analyzed by parametric statistical tests and repeated measures analysis of variance.
Results: Mean (± SEM) insulin requirement of the GFD- group increased from 0.40 ± 0.18 U/kg to 0.72 ± 0.14 U/kg in 3 years ($p < 0.001$). In the GFD+ group the insulin dose increased similarly from 0.41 ± 0 ,20 U/kg to 0.71 ± 0.13 U/kg in 3 years ($p < 0.001$). The rise in insulin requirement was significant after 1 year in the GFD- group, and after 2 years in the GFD+ group. Insulin requirements were different in GFD+ and GFD- groups 1 and 1.5 years following the diagnosis of T1DM (0.43 ± 0.04 U/kg vs. 0.64 ± 0.07 U/kg, $p < 0.01$; and 0.5 ± 0.04 U/kg versus 0.7 ± 0.06 U/kg, $p < 0.01$, respectively).
Conclusion: Insulin requirements of diabetic children on GFD increases slower during the first two years post-diagnosis of T1DM as compared to diabetic children consuming gluten. Insulin requirements under GFD are lower after 1 and 1.5 years. During the remission phase, the increase in insulin requirement reflects the evolution of endogenous insulin reserve. GFD, therefore, likely helps to conserve endogenous insulin. The longer remission phase is of clinical relevance having impact on the long-term metabolic control of diabetic children.
Disclosure: **P. Toth-Heyn:** None.

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Preserved C-peptide levels in overweight or obese children with newly diagnosed type 1 diabetes

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Background and aims: The number of children with overweight and obesity has been rapidly increased in Poland. Some authors suggest that increased weight may be an accelerator of type 1 diabetes (T1D) onset, others did not support this hypothesis. The aim of our study was to investigate if Polish overweight or obese children might develop T1D early despite preserved C-peptide value.
Materials and methods: The study included 1098 children aged 2-17 with newly recognized T1D. The following data were retrospectively collected from seven Polish hospitals: date of birth, fasting C-peptide, HbA1c, sex, weight, height at the time of diabetes onset. Based on the WHO standards, patients were categorized as underweight (≤-2 Z-scores), normal weight (+1 to -1 Z-scores), overweight (≥+1 Z-score), or obese (≥+2 Z-scores).
Results: In our study 11.3% children were overweight or obese at T1D onset. Overweight or obese children had more residual beta-cell function and lower HbA1c than did normal weight or underweight children (Table 1). Children with obesity (1.8%) had the youngest median age and the highest fasting C-peptide levels. There was a correlation between fasting C-peptide and BMI-SDS ($p=0.0001$), age ($p=0.0001$), and a negative correlation with HbA1c ($p=0.0001$).
Conclusion: Children with obesity and overweight are diagnosed with type 1 diabetes at an early stage with largely preserved C-peptide levels and might be good responders to immune therapy.
Disclosure: **A. Szypowska:** None.

1217

Tight glucose control in critically ill pediatric patients: a network meta-analysis of randomised controlled trials

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Background and aims: Our recent network meta-analysis of randomized trials in critically ill adults showed no reduction of mortality by tight glycemic control (blood glucose: 4.4-<6.1 mmol/l) with intensive insulin therapy, although hypoglycemia was 5 times more frequent versus mild control (7.8-<10.0 mmol/l) or very mild control (10.0-<12.2 mmol/l) (Intensive Care Medicine 2017). On the other hand, it is still unclear whether tight glycemic control is warranted in critically ill pediatric patients. We employed network meta-analysis to examine the risk of secondary infection and hypoglycemia associated with different glycemic control targets in critically ill pediatric patients.
Materials and methods: Electronic databases were searched up to 2017 for randomized controlled trials comparing various insulin regimens in critically ill pediatric patients. Two reviewers extracted information and evaluated its quality with the Cochrane risk-of-bias tool. Three glycemic control groups were compared: tight (blood glucose: 4.4 < 6.1 mmol/l), mild (7.8 < 10.0 mmol/l), and very mild (10.0 to < 12.2 mmol/l). Network meta-analysis was performed by a frequentist-based approach with multivariate random effects meta-analysis. Inconsistency of the network model was estimated by using inconsistency factors and their uncertainty. In addition, ranking plots (rankograms) were constructed using the probability that a given treatment had the highest event rate for each outcome. The surface under the cumulative ranking curve (SUCRA), which is a simple transformation of the mean rank, was used to set the hierarchy of the treatments.
Results: Four randomized trials were identified (3,235 patients, 359 secondary infection events, 391 hypoglycemia events, and 127 severe hypoglycemia events). Compared with very mild control, there was borderline reduction of the risk of infection (sepsis) with tight control [pooled odds ratio (OR) 0.79 (95% CI 0.62-1.00), $p=0.05$]. However, hypoglycemia and severe hypoglycemia were more frequent with tight control than very mild control [OR 5.9 (1.63-21.3), $p=0.007$ for hypoglycemia; OR 4.76 (2.84-7.97), $p<0.001$ for severe hypoglycemia]. There was also a higher risk of severe hypoglycemia with tight control than mild control [OR 3.76 (1.58-8.96), $p=0.003$].

Conclusion: In critically ill pediatric patients, network meta-analysis showed a borderline benefit of reduced secondary infection risk with tight glycemic control relative to very mild control, but the risk of hypoglycemia and severe hypoglycemia was 5 times higher versus very mild control.

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Disclosure: **T. Yamada:** None.

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IGF-I at diagnosis and subsequent years in adolescents with type 1 diabetes

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Background and aims: Type 1 diabetes (T1D) in adolescents is associated with alterations in the IGFsystem probably caused both by a deranged metabolism and insulinopenia in the portal vein.We aim to study how the circulating IGF-I is affected at diagnosis and subsequent years in adolescents with T1D.

Materials and methods: Ten girls and ten boys with type 1 diabetes (T1D), age 13.0 ±1.4 (mean ±SD) years at diagnosis took part in the study. Blood samples were drawn at diagnosis, and after 3, 9, 18 and 48 months. HbA1c, total IGF-I and C-peptide were measured.

Results: At diagnosis the patients had high HbA1c, low IGF-I and measurable C-peptide. After start of insulin treatment maximal improvement in glycemic control and IGF-I occurred within 3 months and then both tended to deteriorate, i.e. HbA1c to increase and IGF-I to decrease. C-peptide decreased with time and after 4 years half of the patients were C-peptide negative. At diagnosis C-peptide correlated positively to IGF-I ($r=0.50$; $p<0.03$). C-peptide correlated negatively with insulin dose (U/kg) after 18 and 48 months from diagnosis ($r=-0.50$; $p<0.03$ and $r=-0.72$; $p<0.001$, respectively).

Conclusion: In newly diagnosed adolescents with type 1 diabetes and deranged metabolism IGF-I levels are low but improves with insulin treatment and are positively influenced by residual beta cell function. It is concluded that it is important to preserve beta cell function to keep low HbA1c and IGF-I as normal as possible.

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Disclosure: **S. Chisalita:** None.

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Analysis of rehabilitation for 2001 children and adolescents with diabetes

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Background and aims: Medical rehabilitation is a special concept in the treatment of diabetes mellitus: It plays an important role in the therapeutic concepts of children and adolescents. Services can be applied, which are difficult to implement in an out-patient or an acute in-patient setting. The study analyzes rehabilitation in 2001 patients over a period 12 years.

Materials and methods: All (n = 2001) children and adolescents with diabetes mellitus (52% girls, age 12.6 ± 4.9 years) were examined, who were admitted to a specialist clinic for rehabilitation during the period 01/2004-12/2016.

Results: The duration of medical rehabilitation was 27.3 ± 6.1 days. 1980/2001 (98.9%) children and adolescents had type 1, 21/2001 (1.1%) had type 2 diabetes mellitus. Mean HbA1c was 7.87±1.47%. 1897/2001 (95%) patients had an intensified insulin therapy, of which 633 (32%) used insulin pumps (CSII). They injected 0.86±0.47 I.E. Insulin/kg body weight/d and performed 37.6±11.4 blood glucose self-tests/week by. Over the follow-up period of 12 years parameters of the metabolic control (HbA1c, incidence of acute complications) changed hardly. The proportion of patients with CSII increased ($p<0.05$). In particular young children used CSII more frequently (age <4 59% vs 24% age ≥16 <17 years, $p<0.05$). Changes also occurred in cultural status: the percentage of patients from German families decreased ($p<0.05$), the proportion of patients from mixed-cultural families increased ($p<0.05$). The number of patients living together with both parents also decreased ($p<0.05$ for the tendency), the number of patients living with single parents increased ($p<0.05$ for the tendency). In young children HbA1c values were the lowest. From the beginning of puberty (about 10 years) HbA1c increased (8.5±1.9% in 16 to 17-year-olds). There were no correlations/associations between metabolic control and the incidences of hypoglycaemia/ketoacidoses.

Conclusion: There is a change in medical rehabilitation: the proportion of patients using CSII increased, the number of patients living with single parents and the percentage of patients from a culturally mixed families increased too. Hence, more and more patients showed psychological alterations. These need special care and therapy

Disclosure: **R. Schiel:** None.

1220

Delivery of exenatide by subdermal placement of ITCA 650 in a nonclinical juvenile rabbit model to determine exposure and tolerability

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Background and aims: The incidence of type 2 diabetes (T2D) in children and adolescent is increasing globally. The deterioration of beta cell function is more progressive in pediatric T2D compared to adult T2D. This, combined with the obesity component, highlights the need for a safe and efficacious treatment with a high adherence rate in the pediatric population. ITCA 650 is an osmotic mini-pump that delivers a continuous rate of exenatide subcutaneously, following subdermal placement in the abdominal wall for up to 6 months. Juvenile toxicity data are required in support of a planned human pediatric study. For the class of GLP-1 RAs and use in a pediatric T2D population, potential concerns relate to accelerated sexual maturation, precocious puberty and growth. Studies with ITCA 650 in juvenile rats are not feasible due to their small size postnatal. The rabbit was chosen because the stages of development/puberty most closely match those of the rodent and based on historical data and direct experience with ITCA 650 in rabbits.

Materials and methods: In a dose-finding juvenile toxicity study, rabbits received ITCA 650 placed subdermally at exenatide doses of 0 (ITCA placebo), 0 (pair fed) 3, 10, 20, 40, and 60 mcg/d from postnatal days (PND) 28 through 90 to determine tolerability, exposure, and immunogenicity of exenatide.

Results: Plasma exenatide levels increased in proportion to dose and were similar in males and females. Anti-exenatide antibodies were detected at low levels (titer ≤45) in 33% of the ITCA 650 animals on PND 90 with no relationship between incidence, dose or gender. Juvenile rabbit-human exposure multiples for the 3, 10, and 20 mcg/d dose levels ranged between 0.41-6.45. There was no indication of acceleration in sexual maturity. Sexual maturation was comparable between groups for vaginal patency (32.0-35.0 d) in females and for preputial separation (79.3-91.7 d) in males. Growth, measured by mean femur length, was comparable between groups (83.7-87.4 mm in males and 83.0-87.4 mm in females). No gross lesions related to ITCA 650 occurred. Body weights (BW) on PND 90 ranged from 89.9-94.9% of the control group 0 (ITCA

placebo) for the 0 (pair fed), 3, 10, and 20 mcg/d groups in males, and from 97.2-102.7% in females. Doses of ITCA 650 as high as 60 mcg/d did not affect the organ weight or ratio of the organ weight to the terminal BW but did result in identifiable toxicity. Adverse clinical observations (thin appearance, dehydration, ungroomed fur, liquid feces and weight loss) were observed in the 40 and 60 mcg/d group (either individual rabbits or all rabbits in a group) and resulted in early death or animals to be euthanized.

Conclusion: ITCA 650 at doses up to 60 mcg/d did not accelerate sexual maturation or impact growth. Adverse clinical reactions, reduced BW gains and food consumption occurred in all groups exposed to exenatide due to its known pharmacological activity. These results will inform the definitive juvenile rabbit toxicity study in support of the clinical pediatric program.

Disclosure: **D.T. Zane:** None.

PS 110 Cancer risk and diabetes

1221

Can we identify people with higher pancreatic cancer risk who present with type 2 diabetes?

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Background and aims: Type 2 Diabetes (T2D) is associated with increased risks of developing several cancer types, including pancreatic cancer. A unique relationship may exist between new onset T2D, together with other symptoms like weight loss, and pancreatic cancer diagnosis. This would suggest an opportunity to develop risk models after diagnosis of T2D as an early cancer diagnosis strategy. Previous studies on this topic have been limited by using primary care datasets only, failing to link with cancer registries, and therefore (i) commonly underestimate cancer incidence by 9% to 25%; and (ii) may misclassify date of cancer diagnosis.

Materials and methods: We used primary care data from the Clinical Practice Research Datalink (1990-2011), on 330,311 individuals with T2D from 372 GPs in the UK, and linked these with the National Cancer Registration and Analysis Service, yielding 57,910 individual cancers.We developed logistic regression models to determine the pancreatic cancer occurrence within 6 months of T2D diagnosis. Covariates included were sex, age (continuous and in 5 year bands), smoking status, body mass index (BMI), weight loss, alcohol, government office region and Index of Multiple Deprivation (IMD).We benchmarked our results against the UK National Institute of Health and Care Excellence (NICE) 3% Positive Predictive Value (PPV) in their guidance on immediate referral with symptoms suspicious for cancer.Performance of the model was assessed using the area under the ROC curve (AUC_{ROC}).

Results: There were 130015 men and 141075 women with a new diagnosis of T2D, with 88 and 101 pancreatic cancers (respectively) within 6 months. In models that incorporated age, sex smoking status, current BMI, alcohol consumption, region, and deprivation, we attained an AUC_{ROC} of 0.7962, but no individuals had a risk above the 3% threshold. In models where indicators of weight loss were added this improved the AUC_{ROC} to 0.8034; here, 16 people were classified as having a cancer risk above the 3% threshold, but only one was a true positive. All models had poor sensitivity and high specificity.

Conclusion: There are opportunities to develop a risk model of pancreatic cancer occurrence within the first 6 months after diagnosis of T2D, but this was at a price of poor sensitivity and high specificity. Future developments will include external validation approaches and testing emerging hypotheses for example, a new diagnosis of T2D without the presence of obesity may indicate a strategy of enhanced cancer investigations. Pancreatic cancer has a poor prognosis, and any opportunities for earlier detection should be explored to improve survival.

Supported by: MRC CRUK

Disclosure: **E.L. Badrick:** None.

1222

The effect of type 2 diabetes on selected clinical parameters and disease outcome in patients with colorectal cancer

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Background and aims: Recent epidemiological evidence indicates increased incidence of certain cancer types in patients with type 2 diabetes

mellitus (T2DM), worse outcomes and also certain modifying effect of various glucose-lowering therapies. Given the population prevalence of both T2DM and colorectal cancer (CRC) their relationship is particularly relevant. Study objective was to analyse tumour-, patient- and treatment-related characteristics explaining possible difference in selected outcomes between CRC subjects with and without pre-existing diabetes by means of in-depth epidemiological analysis of all consecutive patients diagnosed and treated for CRC over the past 10 years (stratified by the presence/absence of T2DM) in the largest cancer centre in Czech Republic - Masaryk Memorial Cancer Institute (MMCI) in Brno.

Materials and methods: Retrospective data-mining of all available data from electronic health records of MMCI Brno and National Cancer Registry revealed a total of 8860 CRC cases during 2004-13 period, of which a subset of 3371 CRC subjects underwent the entire CRC treatment including diagnosis in MMCI. Inclusion criteria were: CRC and eventual T2DM diagnosis classified by ICD-10 code as C18 - C20 and E11 and/or presence of anti-diabetic medication (ATC group A10B (oral antidiabetics) or A10A (insulins)). Exclusion criteria: other co-existing tumour (as a disease outcome confounder). Statistical analyses based-on on probabilistic and time-to-event analysis to determine the effect of pre-existing diabetes, its treatment modality and their interactions on following outcomes: disease-free interval, recurrence rate, cancer-specific mortality, all-cause mortality were used.

Results: 41.2% of CRC cases were in stage I+II and 44.8% in stage III+IV. Prevalence of T2DM was approximately 10% in this sample and time period, median survival time 8.4 years. T2DM subjects were more frequently men, significantly older and had higher clinical CRC stage ($P<0.05$, chi-square or Mann-Whitney test). Significant differences in the overall and CRC-specific survival between diabetic and non-diabetic ($P<0.001$, log-rank test) independent of age and stage at baseline were revealed with T2DM having significantly shorter median survival.

Conclusion: Using a large cohort of clinically well characterised CRC subjects we confirmed that T2DM represents significant risk of adverse CRC outcomes. Detail analysis of tumour type- and site- and treatment-specific effects is warranted in this cohort to provide a complex and personalised estimation of the risk existing T2DM possess at the time of CRC diagnosis.

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Disclosure: **L. Pacal:** Grants; GA16-14829S and MEYS – NPS I – LO1413.

1223

Pre-existing diabetes and all-cause mortality of cancer patients: a register-based study in Latvia

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Background and aims: Most studies from high income countries consistently report that pre-existing diabetes reduces overall survival of cancer patients. Our preliminary analysis, presented at the EASD meeting in 2012, showed atypical results: in Latvia, among men, diabetes was associated with a better survival in the first years of follow-up. We re-examined this research question in a retrospective cohort study by using two different population-based data sets.

Materials and methods: The Cancer Register in linkage with the Diabetes Register and the Causes of Death Data Base was the first data source. The analysis included 25,043 men and 27,081 women registered with incident cancers from 2009 to 2013; of those 1,910 (7.6%) men and 2,802 (10.4%) women had prior diabetes. Follow-up ended on 28 February 2015. The National Health Service data served as the second source for selection of 10,725 men and 13,779 women, discharged from specialized oncology hospitals from 2009 to 2012, with cancer as the main diagnosis. Of those, 951 (8.9%) men and 1,521 (11.0%) women had records of dispensed reimbursed antidiabetic medications before the hospital admission, indicating prior diabetes. Their follow-up ended on 31 December 2013. The Cox proportional hazards model was used to assess association between pre-existing diabetes and all-cause mortality, adjusted for age, presented as HR (95% CI).

Results: In men, according to the disease-registers data, cancer patients with diabetes experienced lower mortality than their non-diabetic counterparts (age-adjusted HR 0.86; 0.79-0.93) during the first year after cancer diagnosis. The mortality difference levelled off in the next two years; but after the third year of follow-up, diabetes was associated with a higher risk of dying (HR 1.60; 1.28-1.99). In the health service data, diabetic men also had lower mortality than cancer patients without diabetes during the first two years after the hospital discharge (HR 0.89; 0.80-0.98). After the first two follow-up years, diabetes had no association with mortality (HR 1.03; 0.74-1.43). In women, pre-existing diabetes was associated with slightly higher all-cause mortality during the entire follow-up: age-adjusted HR was 1.17 (1.10-1.24) in the disease-register data and 1.11 (1.02-1.21) in the health service data.

Conclusion: Our study confirmed that pre-existing diabetes was associated with a worse prognosis among women with cancer; whereas among men, the better overall survival of diabetic cancer patients in the first years after cancer diagnosis is an unusual finding. We hypothesize that limited access to health services, including preventive care, might give an advantage to diabetic patients who are in close contact with the healthcare system.

Disclosure: **I. Strele:** None.

1224

Within-class sulfonylurea differences and cancer risk (ZODIAC-55)

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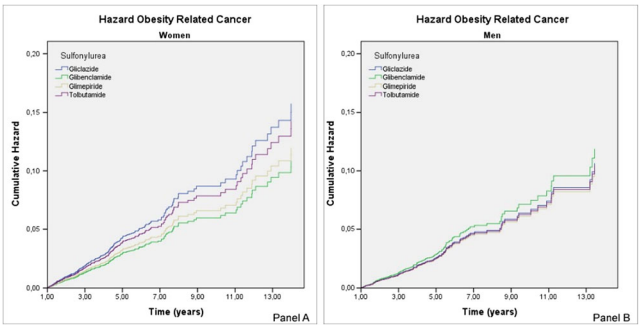
Background and aims: Patients with type 2 diabetes (T2D) are at increased risk for developing cancer. There are within-class sulfonylurea (SU) differences concerning hypoglycaemia risk and probably also for cardiovascular safety. Several studies also reported within-class differences in cancer risk. In what way and to what degree cancer risk is modified by the use of specific SUs or whether methodological issues have been accounted for in previous studies is unclear. The aim of this study was to investigate within-class SU differences in obesity and all cancers risk.

Materials and methods: A primary care diabetes cohort, the prospective observational ZODIAC (Zwolle Outpatient Diabetes project Integrating Available Care) cohort study with annually collected diabetes and medication data was linked to the Dutch National Cancer Registry. Patients who received their first prescription of SU treatment at any point within ZODIAC where selected, this moment was defined as baseline. Patients using insulin at baseline were excluded. Primary outcomes were obesity related and all cancer risk, evaluated for men and women separately. Cox proportional hazard analyses adjusted for age, HbA1c, diabetes duration, BMI, creatinine, smoking, and metformin use were used for analysing the relationship between baseline SU use and cancer risk.

Results: Of the 28.096 patients selected, 49% were female, with a mean (SD) age of 67 (12), median (IQR) diabetes duration 7.6 (4.4 - 11) year, median HbA1c 6.8% (6.3 - 7.5), median BMI 28.9 (26.0 - 32.5) and a median follow-up of 3.3 years (1.3 - 5.6). Cumulative hazard ratios (HR) for women (panel A) and men (panel B) are shown in figure 1. Gliclazide was chosen as reference. For obesity related cancers, HR (95%CI) for women and men for glibenclamide were 0.69 (0.40 - 1.18) and 1.12

(0.64- 1.96), for glimepiride 0.76 (0.54 - 1.07) and 0.96 (0.65 - 1.42), and for tolbutamide 0.91 (0.67 - 1.23) and 0.92 (0.68 - 1.41). For all cancers, HR (95%CI) for glibenclamide were 0.80 (0.53 - 1.21) and 1.21 (0.87 - 1.68), for glimepiride 0.86 (0.66 - 1.13) and 0.95 (0.74 - 1.21), and for tolbutamide 0.96 (0.76 - 1.22) and 1.03 (0.83 - 1.28), for women and men respectively.

Conclusion: No significant within-class differences between baseline SU use and obesity related and all cancer risk were present. We could not rule out relevant differences, i.e. type 2 error. Fortunately, an update of the linkage in the next month will double the amount of patient-years and events. Another step is to integrate exposure duration by accounting for medication and clinical variables during follow and to perform time-dependent Cox regression analyses.



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1225

The influence on survival of glucocorticoid induced diabetes in cancer patients with metastatic spinal cord compression

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Background and aims: Glucocorticoids are used to treat edema in patients with metastatic spinal cord compression (MSCC) before and during radiotherapy. Hyperglycemia and diabetes are well known side effects to treatment with glucocorticoids, but the influence of hyperglycemia on survival is unknown.

Materials and methods: In a prospective, observational cohort study 131 patients with MSCC referred to radiotherapy, 30 Gy in 10 fractions, and treated with ≥ 100 mg prednisolone a day were followed with daily blood glucose measurements during radiotherapy and categorized as having no diabetes, diabetes by definition or insulin treated diabetes. The patients were followed for at least 5 months.

Results: During follow-up a total of 56 patients 43% (95% CI = 35%-52%) presented plasma glucose values diagnostic of diabetes. Sixteen patients who developed diabetes were treated with insulin, 12% (95% CI = [6%; 18%]) of the total population. In six months, from the first day of prednisolone, 60% of the patients died. The patients developing diabetes with need for insulin therapy during glucocorticoid therapy had a significantly increased mortality compared to those with normal glucose metabolism and with diabetes without need for therapy, hazard ratio = 2.1 (95% CI =1.08- 4.09, p = 0.0285).

Conclusion: The literature on the effect of glucocorticoid induced hyperglycemia/diabetes on survival in cancer patients is sparse and inconclusive. To our knowledge this is the first prospective study to describe the influence of glucocorticoid induced diabetes on survival in patients with MSCC from different primary tumors. The results indicate that development of diabetes during high-dose glucocorticoid therapy needing insulin treatment in patients with MSCC from different primary tumors is associated with reduced survival.

Disclosure: **H. Schultz:** None.

1226

Metformin inhibits vistatin gene expression via HIF1 in PC3 prostate cancer cells: a potential role for visfatin as a non-invasive biomarker

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Background and aims: Hypoxia plays a pivotal role in the aggressiveness of solid cancers, including prostate cancer (PC). During cancer progression, hypoxia stimulates hypoxia-related gene expression and the production of glycolytic intermediates useful for cancer growth. In this setting, hypoxia-inducible factor 1 (HIF1) is the major transcription factor regulating hypoxia adaptive responses. Among the HIF1-inducible gene products, visfatin, both a NAD+ cycle enzyme, and an adipokine, plays an important role in promoting cancer cell proliferation. Metformin, commonly used for the management of type 2 diabetes and insulin resistance, has shown anti-proliferative effects in vitro and in vivo, leading to increasing interest in its potential use as an anti-tumoral agent. How metformin inhibits cell growth, however, remains still poorly understood. Aim of this study was to evaluate the effects of metformin on the HIF1/visfatin axis in prostate cancer PC3 cells. Furthermore, our purpose was to investigate the potential use of visfatin as a non-invasive prognostic biomarker in patients with PC.

Materials and methods: PC3 cells were cultured in RPMI plus 10% FBS, in the presence or absence of metformin, in normoxia or hypoxia (CoCl2 or 2%O2). Visfatin gene expression was evaluated by qRT-PCR. Reporter gene analysis was performed by dual luciferase assay after 48h transfection, using lipofectamine, with a plasmid containing the visfatin gene promoter. Western blots were carried out using a HIF1-α polyclonal antibody. Serum specimens were obtained from the "Biobanco" service of the IMIBIC Institute, and patients were engaged for the presence or absence of PC, metformin treatment, and sorted by Gleason score and BMI. Visfatin was measured by a competitive EIA visfatin kit.

Results: In PC3 cells, treatment with metformin caused a consistent, dose-dependent decrease in visfatin mRNA levels, both in normoxia and hypoxia ($p<0.05$; Mann-Whitney test). Also, in these cells, either under normoxia or hypoxia, metformin induced a significant, dose-dependent decrease in visfatin promoter activity ($p<0.001$; Mann-Whitney test), demonstrating a role on gene transcription. In parallel experiments, metformin-treated cells showed a decrease in HIF1α protein content ($p<0.001$; Mann-Whitney test). In patients with PC and poorer prognosis (Gleason score 9/10 vs Gleason score 6), serum visfatin was more elevated (2.64± 1.5ng/mL vs 1.05± 0.3ng/ml, respectively, p=0.0067; Kruskal-Wallis test; n=176). Furthermore, in metformin-treated patients, serum visfatin was significantly lower in each BMI range, compared to metformin-untreated patients. Visfatin levels were: in the normal weight group (BMI<25), 0.56±0.1 ng/mL vs 3.42±1.3 ng/mL p=0.005; in the overweight group (25<BMI<30), 0.98±0.18 ng/mL vs 1.14±0.23 ng/mL p=0.032; and in the obese group (BMI>30), 0.81 ±1.9 ng/mL vs 0.98±2.0 ng/mL p=0.033 (metformin-treated vs untreated patients, respectively; Mann-Whitney test).

Conclusion: Our data in PC3 cultured cells indicate that the anti-proliferative activity of metformin could be mediated, at least in part, through the HIF1/visfatin axis. Our findings also suggest that visfatin could serve as a non-invasive biomarker of aggressiveness in patients with PC.

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Disclosure: **S. Messineo:** None.

1227

Complex interplay between androgen, estrogen and insulin receptor gene expression in prostate cancer depending on diabetes status

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Background and aims: In contrast to many other malignancies, incidence of prostate cancer (PCa) is not elevated in patients with diabetes mellitus. However, PCa survival is markedly reduced in diabetic men. Prostate cell growth and prostate carcinogenesis are not only mediated by androgens, they are also depending on functional estrogen and insulin receptor signaling. To address potential underlying mechanisms for the reduced survival in diabetic men we looked for gene expression patterns of the androgen receptor (AR), insulin receptor A (IRa) and B (IRb), IGF1R, and the classical nuclear estrogen receptor β (ERβ) *(ESR2)* both in diabetic and nondiabetic patients. Moreover, as in diabetic men testosterone levels are reduced, the expression of the regulating enzymes for the non-canonical androgen receptor ligands 27-hydroxycholesterol and 24(S)-hydroxycholesterol, Cyp27A1 and Cyp46A1, respectively, were analyzed.
Materials and methods: 80 prostate tissue samples of diabetic patients (45 cancer, 35 tumor-adjacent benign tissue, and 86 samples of patients without diabetes (35 cancer, 51 tumor-adjacent benign tissue), who underwent a radical prostatectomy, carefully matched for age and BMI, were included in the study. mRNA expression of target genes was analyzed by RT-qPCR and normalized to *UBC* mRNA in duplicate.
Results: *AR* expression in tumor tissue was elevated only in men with diabetes (p=0.025), which went along with higher IRa/IRb ratio (p=0.001) and lower IRb/IGF1R ratio (p<0.0001). When correlated to the tumor content in the biopsies, we found an impressive reduction in *Cyp27A1* and *Cyp46A1* gene expression with increasing tumor content (p<0.0001 and p=0.0002, respectively). Only in diabetic men, the reduction of *Cyp27A1* expression went along with enhanced cell proliferation, measured by the expression of the proliferation marker Ki67 (p=0.0007), while the inverse correlation of *Cyp46A1* expression with Ki67 showed a trend (p=0.11). *ESR2* was inversely associated with IRa/IRb ratio (p<0.0001), and only in men with diabetes, inversely with *AR* (p=0.0007). Finally, *ESR2* expression went along with elevated *Cyp27A1* and *Cyp46A1* expression in diabetes (p=0.003 and 0.012, respectively).
Conclusion: In this study we could demonstrate an elevated *AR* expression in tumor only in diabetic men, associated with alterations of the insulin signaling cascade, and showing inverse association with the ERβ. Our results suggest a complex interplay between these signaling pathways in PCa, especially when diabetes is present. These changes may contribute to the reduced survival of diabetic men with PCa.
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Disclosure: **S.Z. Lutz:** None.

PS 111 Cancer: in vitro studies

1228

Glucose impairs tamoxifen responsiveness modulating CTGF (Connective Tissue Growth Factor) in breast cancer cells

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Background and aims: Type 2 diabetes (T2D) is associated with a 20% increased risk of breast cancer (BC) and up to 16% of BC patients suffer from T2D or impaired glucose tolerance. T2D promotes a more aggressive BC phenotype. Particularly, hyperglycemia is associated with a poorer therapeutic outcome and reduced drug response in BC. Thus, glucose may directly affect cancer cell responsiveness to chemotherapy. Moreover, glucose may affect surrounding stromal cells. BC cells are embedded in a adipocyte-rich microenvironment. Glucose is able to modify the secretory capability of adipocytes, enhancing BC cell proliferation and invasiveness. Nevertheless, adipocyte-derived factors potentially able to alter BC drug response have not been identified yet. Identifying the mechanisms that govern it will pave the way to new targeted strategies to overcome chemoresistance in T2D patients with BC. Thus, the aim of this study was to investigate the direct and indirect (i.e. adipose tissue-mediated) impact of glucose on BC cell responsiveness to tamoxifen (tam).
Materials and methods: MCF7 BC cells (ER^+) were cultured in high (MCF7 HG;25 mM) or in low (MCF7 LG;5.5 mM) glucose medium. Adipocyte conditioned media (hAdipo CM) system and co-coltures with human adipocytes were established. Cell viability upon tam treatment was assessed by sulforhodammine B assay or crystal violet. Cell transcriptome was characterized by RNA Sequencing on a Next Generation Sequencing platform (Illumina HiSeq 2000).
Results: In LG, tam reduced cell viability by about 50% while in HG drug sensitivity was 2 fold reduced (Pval<0.05). Shifting MCF7-HG to LG restored tam sensitivity, whereas the shift of MCF7-LG to HG reduced drug responsiveness. RNA-Sequencing revealed that glucose deregulates the expression of cell cycle-related genes (Pval<0.05). Particularly, *CTGF* expression was 2-fold reduced upon the shift in LG (Pval<0.001). Of note, in MCF7-HG responsiveness to tam was 2-fold increased (Pval<0.05) upon the knockdown of *CTGF* by siRNA. Consistently, the treatment with human recombinant CTGF (1μg/mL) reduced by about 4-fold MCF7-LG responsiveness to tam (Pval<0.05). Moreover, *CTGF* expression in MCF7-HG was 2.5-fold increased (Pval<0.05) in presence of CM collected from adipocytes pre-incubated in HG. Notably, MCF7-HG responsiveness to tam was worsened both in presence of hAdipo-CM and in co-colture with adipocytes (Pval<0.05). The inhibition of adipocyte-released IL8 reduced the effect of hAdipo-CM of about 2-fold (Pval<0.05) both on CTGF expression and on tam responsiveness. Accordingly, cell treatment with human recombinant IL8 (1μg/mL) induced CTGF expression (≈50%;Pval<0.05) and increased tam responsiveness (≈15% reduction of cell viability; Pval<0.05) mimicking the hAdipo-CM effects on BC cells
Conclusion: Glucose affects tam responsiveness modulating *CTGF* in BC cells, both directly and indirectly through adipocyte-released IL8. CTGF may be a diabetes-associated predictive marker for chemosensitivity and may represent a potential therapeutic target to overcome tam resistance in BC.
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Disclosure: **M.R. Ambrosio:** None.

1229

The link between diabetes and colorectal cancer: effect of diabetic microenvironment, metformin and 5-fluorouracil on AMPK activation, apoptosis and autophagy

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Background and aims: Epidemiologic studies showed that (i) type 2 diabetes mellitus is associated with increased risk of development of certain cancers including colorectal cancer (CRC)and (ii) result of CRC treatment are worse in diabetics including higher mortality rates. On the other hand, antidiabetic treatment, specifically metformin, was associated with better prognosis and also increased efficacy of standard chemotherapeutic treatment of CRC. Despite the historical use, molecular mechanisms of anticancer effect of metformin are not fully understood yet. Activation of AMP-activated protein kinase (AMPK), the key regulator of glucose metabolism, orchestrates the pleiotropic effects of AMPK including cell cycle arrest, autophagy induction or apoptosis but also activation of survival metabolic pathways. It is therefore obvious that the final outcome of metformin action in cancer critically depends on the functionality of p53 as a key regulator of all above mentioned processes. Little is known about how hyperglycaemia/diabetic milieu affects AMPK pathway. Aim of the study was to study the effect of (i) diabetic microenvironment, (ii) metformin and (iii) first line CRC cytostatic agent 5-fluorouracil on AMPK signalling, autophagy and apoptosis *in vitro* in CRC with defined p53 status.
Materials and methods: HCT116 cell line with and without p53 (p53+/+ and p53-/-) was cultured 30 hours in following settings: 7.5 mmol/l or 25 mmol/l glucose in the medium with or without the addition of metformin (500 μmol/l) or 5-fluorouracil (5-FU, 5 μmol/l). Cell viability was assessed using resazurin. AMPKα activation was monitored using Western blot with phospho-specific antibody against threonine 172 (Cell Signaling Technology). Autophagy induction was detected using antibody against LC3 and apoptosis was detected using caspase-3 antibody (both from Cell Signaling Technology).
Results: Regardless commonly used milimolar concentrations of metformin in experiments we initially determined IC50 for metformin (and 5-fluorouracil), because physiologically relevant metformin concentrations are tens of micromoles. IC50 concentrations 500 μmol/l for metformin and 5 μmol/l for 5-FU were subsequently used in all experiments with cell line HCT116. We found that metformin decreases viability of HCT116 cells more profoundly in 53-/- compared to +/+ even in normoglycaemia. We also confirmed AMPKα activation by metformin, again more significant in HCT116 p53-/- cells. Caspase-3 expression was increased by 5-FU and also by combination of 5-FU + metformin in both normo- and hyperglycaemia in p53-/- but not in p53+/+ cells.
Conclusion: We confirmed stronger effect of metformin on viability and AMPKα activation in cancer cells without functional p53. Combination of metabolic/energetic stress and exposition to cytotoxic agents (5-fluorouracil as a typical first line chemotherapeutic for CRC treatment) might represent potentially effective approach to treatment of p53 deficient cancers.
Supported by: GA16-14829S and MEYS – NPS I - LO1413
Disclosure: **K. Kuricová:** Grants; Study was supported by the grant GA16-14829S from the Czech Grant Agency and by project Grant MEYS - NPS I - LO1413.

1230

Expressions of microRNA-30, 196 and 423 distinguish type 2 diabetes from diabetes associated with pancreatic cancer and chronic pancreatitis

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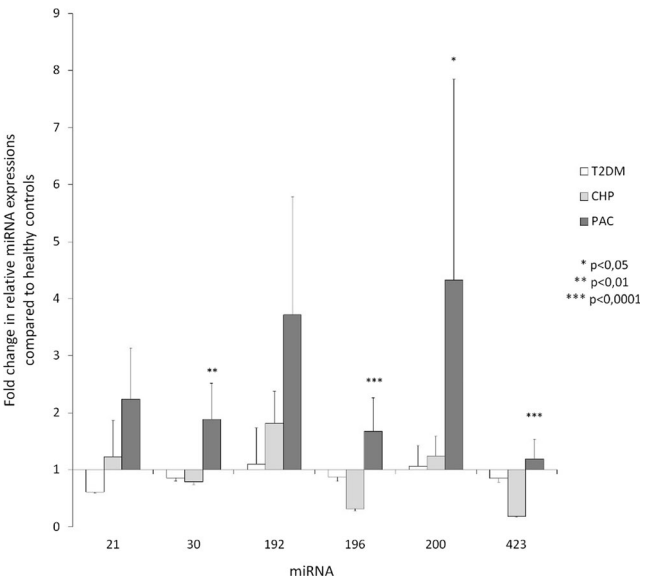
Background and aims: Diabetes mellitus (DM) is frequently the first symptom of pancreatic cancer (PAC). The prevalence of DM in chronic pancreatitis (CHP) is elevated too. CHP is simultaneously one of the main risk factors for developing PAC. The aim of this study was to estimate if microRNA (miRNA) profiling in DM patients could improve PAC diagnosis and its distinguishing from CHP.
Materials and methods: Fifty eight diabetic patients with PAC, 15 diabetic patients with CHP, 39 type 2 diabetic patients (T2DM) without PAC or CHP, and 30 healthy controls were enrolled in our study. Diagnosis of PAC and CHP was done by high resolution imaging methods and confirmed by histological examination. Expressions of 8 miRNAs (miR-21, 30, 191, 192, 196, 200, 423 and 454) were determined in the serum by real-time PCR. MicroRNAs were then related to miRNA-191 and 454 that were set as endogenous controls. Kruskal-Wallis ANOVA test was performed to evaluate the results.
Results: The mean expressions of miR-21, 30, 192, 196 and 200 were significantly elevated (1,8-4,3 times) in PAC patients compared to healthy controls and T2DM group ($p<0,05$ to $p<0,0001$). On the contrary, miR-423 was significantly lower (4,6-6,5 times) in CHP patients compared to each of other groups ($p<0,0001$). MicroRNA-30, 196, 200 and 423 were able to distinguish PAC patients from CHP group. MicroRNA expressions in PAC, CHP and T2DM groups were compared with healthy controls having expression equal to 1,0. Significant differences between PAC and CHP groups are shown in the Figure.
Conclusion: Our study is the first that compares expressions of selected miRNAs in diabetic patients with PAC or CHP. While patients with CHP should be examined for tumour development, it is not easy to distinguish the cancer in the early stage. MicroRNA-30, 196 and 423 seem to become powerful markers in patients with diabetes mellitus in distinguishing pancreatic cancer from chronic pancreatitis.



Disclosure: **P. Skrha:** None.

1231

Type 2 diabetes: induced MMPs promote pancreatic cancer progression

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Background and aims: Increasing epidemiologic evidence suggests that incidence of pancreatic cancer is associated with type 2 diabetes as well as certain risk factors of diabetes. In pancreatic cancer, diabetic conditions promotes the growth of tumor and increases the mortality rate. However,

the underlying mechanisms are poorly understood at present. Diabetes associated molecular changes in tumor potentially contribute to the cancer progression in type 2 diabetes patients. In most cancers, the expression of matrix metalloproteinases (MMPs) increased which involved in cancer progression, including angiogenesis, invasiveness, and metastasis. But MMPs in diabetic pancreatic cancer has not been previously explored.This study aims to investigate the key molecules MMPs in type 2 diabetes that may promote the pancreatic cancer using molecular imaging methods and assess whether these pancreatic-cancer-promoting molecules can be used as targets for targeted specific imaging and treatment in pancreatic cancer with type 2 diabetes. We expect to demonstrate and develop a specific approach that is individualized to treat the diabetes associated pancreatic cancers.

Materials and methods: Orthotopic pancreatic cancer models were established in db/db diabetic mice, Steptozotocin(STZ) induced diabetic mice and C57BL/6 mice. Based on the analysis of clinical human pancreatic tumor tissue samples collected in patients and the animal models for type 2 diabetes and pancreatic cancer, we performed the MMPs molecular imaging experiments and MMP inhibitors tumor therapy experiments in animal models followed by immune-histo-chemistry and pathological analyses.

Results: Pancreatic cancer grew faster in both diabetic mice models than in wild type mice. MMP-2 and MMP-9 expressed in pancreatic cancer with type 2 diabetes mellitus was significantly higher than in those without type 2 diabetes mellitus in clinical patients ($P < 0.05$). Besides, the expression of MMPs in diabetic pancreatic cancer mice models were also higher than that in wild type mice using MMPs NIRF imaging followed by immune-histo-chemistry and pathological analyses ($P < 0.05$). What is beyond our expectations is that tumor inhibition rate of selective MMP-2 and MMP-9 inhibition in wild type mice, STZ diabetic mice and db/db diabetic mice was 12.18%, 36.94%, 54.89%, respectively. It indicates that selective MMP-2 and MMP-9 inhibition reverses diabetes-induced pancreatic cancer growth monitored by MR imaging and MMPs molecular imaging in vivo.

Conclusion: In a conclusion, it suggests that type 2 diabetes aggravates pancreatic cancer by stimulating the expression of MMPs. MMPs can be used as targets for targeted specific imaging and treatment in pancreatic cancer with type 2 diabetes.

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Disclosure: **X. Tingting:** None.

1232

Insulin activates AKT pathway and promotes ECM production of PSC: implication for pancreatic fibrosis and cancer in type 2 diabetes

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Background and aims: Type 2 diabetes has been demonstrated to be an independent risk of pancreatic cancer (PC). Insulin resistance, hyperglycemia, hyperinsulinemia, and abnormalities in insulin/IGF receptor pathways have been suggested to be related to diabetes-associated pancreatic cancer, and activated pancreatic stellate cells (PSC) are reported to be crucial effector cells responsible for development of pancreatic fibrogenesis and pancreatic cancer progression in type 2 diabetes. However, few researches have illuminated how high insulin promotes PSC proliferation, which plays a central role in fibrogenesis associated with pancreatitis and pancreatic cancer. AKT/mTOR/p70S6K and AKT/FoxO1 signaling pathway are classical pathways regulating cell proliferation, differentiation and protein synthesis. The aim of this study was to investigate the effects of high insulin on AKT/mTOR/p70S6K and AKT/FoxO1 signaling pathway enhancing proliferation and fibrosis responses in PSC.

Materials and methods: In primary mouse PSC and a novel immortalized mouse pancreatic stellate cell line (imPSC), the effects of insulin on AKT/mTOR/p70S6K and AKT/FoxO1 signaling pathway were examined. Cells were stimulated with high insulin (100 nmol/L), and cultured with metformin (2mmol/L). We measured extracellular matrix (ECM) synthesis of collagen I (Col-I) and fibronectin (FN), determined the expression signaling pathway components, analyzed their operation as profibrotic and/or proliferative pathways and assessed the potential contributions of PSC to a fibrogenic microenvironment.

Results: High insulin significantly increased Col-I and FN production ($P < 0.01$). Besides, the expression of FoxO1 in PSC was ascended by insulin ($P < 0.01$). Activated phosphorylation of the Akt, mTOR, P70S6K and inhibited phosphorylation of AMPK were completely caused by high insulin stimulation in PSC ($P < 0.01$). Meanwhile, high insulin promoted phosphorylation of FoxO1 in PSC ($P < 0.01$). However, metformin obviously activated AMPK and reduced AKT/mTOR/P70S6K phosphorylation ($P < 0.01$).

Conclusion: Our study showed that high insulin activated AKT/mTOR/p70S6K and AKT/FoxO1 signaling pathway and promoted ECM production of PSC, which suggested a potential key role of AKT pathway in development of pancreatic fibrosis responses in type 2 diabetes. Metformin is a hope for prevention of pancreatic fibrosis and cancer in type 2 diabetes.

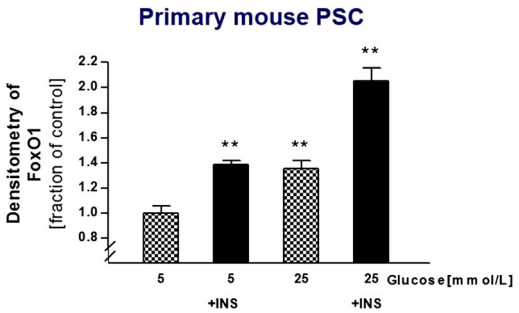

Fig. Expression of FoxO1 induced by insulin and glucose

Disclosure: **M. Zhi:** None.

1233

Dietary advanced glycation end-products activate NF-κB, AKT1 and ERK1/2 linking cancer risk and diabetes

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Background and aims: Recent evidence has suggested a positive correlation between type 2 diabetes and increased colorectal cancer risk. We explored the hypothesis that high dietary intake of advanced glycation end products (AGEs) may play a fundamental role in this association.

Materials and methods: Casein was glycated in vitro (30 min at 70 ºC, 4 sec at 140 ºC, in the presence of 116 mM lactose - 55 mM glucose-fructose. C2BBe1 colorectal carcinoma cells (enterocytes) were treated with 200 μg/ml glycated or control casein for 3 and 24 h. Additionally, we used anti TNF-α, anti RAGE and anti IL-1β antibodies to block the AGEs induced signaling that may involve these respective proteins. Phosphorilated extracellular signal-regulated kinases 1/2 (Erk1/2), RAC-alpha serine/threonine-protein kinase (AKT1), the p50 subunit of NF-κB, receptor for advanced glycation end products (RAGE) and interleukin 1β (IL-1β) protein expression were evaluated by western blot.

Results: AGEs content in glycated casein was 17.5 μg/mg protein. The 3 h AGEs exposure induced a significant ($p<0.05$) increase by 1.7 fold in Erk1/2 expression, and after 24 h Erk1/2 returned to control levels. The initial increase was fully prevented by the anti-TNF-α antibody co-

treatment, however, no significant contribution by the RAGE or IL-1β blocking antibodies. A similar expression profile was observed for p 50 subunit, albeit, the initial high expression was more pronounced (it increased by over 2.5 fold) and it was abolished in the presence of all antibody treatments. Both the AGEs and the anti-RAGE antibody - AGEs co-treatment induced at 24 h increased p 50 expression, which could be explained by the co-dependent nature of the NF-κB - RAGE relationship, also highlighted by the merely identical expression profile we noted for the RAGE receptor. AKT1 expression was unaffected after 3 h, although AGEs exposure increased its expression significantly after 24 h, and could not be controlled by any antibody treatments applied. IL-1β expression was increased dramatically at the 3 h interval by AGEs exposure and was diminished to control level by the antibody treatments. Notably, cell proliferation was increased after exposure to glycated casein by 1.4 fold after 24 h ($p<0.05$).

Conclusion: On the one hand, enterocytes are involved in creating a non-belligerent microenvironment in the gut, along with local immune-competent cells. They are routinely stimulated by antigens, and do not respond by triggering inflammation. However, our data seem to suggest, that AGEs can readily induce transient pro-inflammatory changes. These changes, such as the increased Erk1/2 expression, known to induce metalloproteinases, may contribute in the long term to compromise the barrier represented by enterocytes, and allow antigen access to the lamina propria, where full-scale inflammation could develop. On the other hand, the changes we observed in AKT1 expression could have profound implications, given the carcinogenic origin of the C2BBe1 cells we used. AKT1 is known to inhibit apoptosis and to activate NF-κB and antioxidant enzymes, and it is currently considered a primary target of cancer therapies. Its increase after 24 h could enhance cancer cells ability to cope with stressful stimuli and promote survival. In this way, a link between high dietary AGEs intake, human cancer biology and chronic inflammatory diseases involving the gut may be supported.

Supported by: CNCS-UEFISCDI, PN-II-RU-TE_2012-3-0034

Disclosure: **A.I. Serban:** None.

PS 112 Pathogenesis of non-alcoholic fatty liver disease: from mice to men

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Two mtDNA point mutations in complexes of the respiratory chain synergistically reduce mitochondrial fission and impair metabolism in liver

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Background and aims: Mutations in complexes of the respiratory chain, which are encoded in the mitochondrial genome (mtDNA), can facilitate the production of reactive oxygen species (ROS) and affect the mitochondrial live cycle during aging. Due to these effects on mitochondrial viability mtDNA mutations may result in metabolic liver dysfunction and thus, can contribute to the pathogenesis of type 2 diabetes mellitus (T2DM). Previous studies indicate that multiple mutations in the OXPHOS system predispose to hepatic steatosis. The aim of this study was to investigate fat and ROS accumulation as well as mitochondrial dynamics in liver tissue from conplastic mice carrying mutations in the cytochrome c oxidase (complex IV) and the NADH dehydrogenase (complex I).

Materials and methods: The conplastic mouse strains C57BL/6NTac-mtBPL/1J (NADH dehydrogenase mutation und cytochrome c oxidase mutation, mtBPL) and C57BL/6NTac-mtAKR/J (control; mtAKR) were analyzed at the age of 3, 6, 9 and 12 months. After in vivo MitoSox injection the hepatic mitochondrial ROS production was quantified in liver sections using fluorescence microscopy. In addition, we analyzed the gene expression of fission and fusion regulating proteins, namely MFN1, MFN2, OPA1, FIS1, MFF, DMN1L in liver tissue. Fat accumulation was determined by Oil Red O staining of liver sections. Total body weight and, liver and fat mass were ascertained. Furthermore blood glucose was measured.

Results: Blood glucose levels, body weight and liver fat accumulation of 12 months old mtBPL mice were significantly increased compared to the mtAKR control strain (6.8 vs. 5.7 mmol/l, $p < 0.05$; 39.3 vs. 36.2 g, $p < 0.05$; 18260 vs. 4100 pixel, $p < 0.001$). With aging, mtBPL mice showed a more pronounced increase in ROS accumulations as observed in mtAKR mice. The gene expression of FIS1 and MFF showed a significantly lower level in mtBPL mice compared to controls at all examined time points. In addition, at the age of 3 and 9 months the DNM1L gene expression in mtBPL mice was significantly reduced compared to controls. In contrast to these differences in fission proteins, the gene expression of fusion proteins was comparable between both mouse strains.

Conclusion: The mtDNA point mutations in two OXPHOS complexes, the NADH dehydrogenase and cytochrome c oxidase resulted in lipometabolic dysfunction in liver and higher blood glucose levels in 12 months old mtBPL mice without manifestation of diabetes. Our data suggest that these processes are triggered by reduced mitochondrial fission. Thus, OXPHOS mutations can induce tissue-specific impairments via changes in the mitochondrial network regulation. Whether hepatic steatosis implies insulin resistance and development of T2DM in old mtBPL mice is the purpose of our current research.

Disclosure: **S. Schroeder:** None.

1235

PTP1B deficiency in mice induces changes in gut microbiota and gut barrier permeability during NASH

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Background and aims: Mice deficient in protein tyrosine phosphatase 1B (PTP1B) have increased insulin sensitivity in the liver. However, the role of PTP1B in the dynamics of the gut barrier and associated changes in microbiota during non-alcoholic steatohepatitis (NASH) is unknown. We analyzed the effects of PTP1B deficiency in gut microbiota and intestinal permeability to understand the relation of the gut-liver axis in NASH.
Materials and methods: NASH was induced in 16-20 week-old male wild-type (WT) or PTP1B-deficient (KO) mice (C57Bl/6J) fed choline/methionine-deficient (MCD) diet up to 2 months. Control mice were fed chow diet. DNA was extracted from stool samples collected individually. Amplicons of the V3-V4 hypervariable regions of 16S were sequenced using Illumina-Myseq system. Microbiome analysis was performed with QUIIME program. In vivo permeability was measured through oral gavage of FITC-Dextran and its determination in blood 4 hours later.
Results: After one week on MCD diet, the PCoA analysis revealed a consistent change in microbiota composition due to differences in diet (MCD versus chow) and genotype (PTP1B KO versus WT).When we analyzed the beta diversity, the ratio Firmicutes/Bacteroidetes increased from 1.32 (±0.24) to 2.70 (±0.45) in WT and further in PTP1B KO 3.62 (±0.66). Also, in mice fed MCD diet changes in phyla Verrucomicrobia, Firmicutes and Bacteroidetes were time-dependent, whilst phyla Cianobacteria and Actinobacteria were dependent on the genotype. Histological analysis of duoden and colon did not show differences between groups. However, after one month on MCD diet, we observed a decrease in colon length in both genotypes ($p<0.001$) (WT chow 9.5 ±0.53cm, WT MCD 7.53±0.47cm; PTP1B KO chow 9.55±0.74cm, PTP1B KO MCD 7.4±0.46cm) and a reduction in colon diameter ($p<0.01$) which was more pronounced in PTP1B KO versus WT mice ($p<0.05$) (WT -27.85%±6.54; KO -38.51%±8.22). Both WT and PTP1B KO mice fed MCD diet showed increased circulating endotoxemia than their counterparts fed chow diet (WT chow 13.83ng/ml±2.77, WT MCD 29.51ng/ml±6.41; $p<0.001$), (PTP1B KO chow 13.67ng/ml±3.24; PTP1B KO MCD 19.70ng/ml±7.45; n.s), but endotoxemia was less elevated in PTP1B KO mice fed MCD compared to WT MCD group (WT MCD vs PTP1B KO MCD; $p<0.01$). Gut permeability was also measured by administration of FITC-Dextran by an oral gavage at different time-periods. Non significant differences were found in mice fed chow or MCD diet for 7 days. However, permeability was more elevated in WT mice fed MCD diet for one month compared to PTP1B KO mice ($p<0.05$) (WT basal 0.0024%±3,2E-06; WT 30 days 0.0056%±5,0E-06; PTP1B basal 0.0020%±2,1E-06; PTP1B 30 days 0.0050%±3,4E-06). Preliminary data showed increased proinflammatory markers (TNFα and IL1β) and a decline in tight junction proteins (E-cadherin and occluding) in WT fed MCD diet versus WT fed chow diet that were not observed in PTP1B KO mice.
Conclusion: Marked changes in gut microbiota composition and gut barrier permeability occur during the establishment of NASH in the MCD model. PTP1B deficiency induces substantial changes in gut microbiota and confers protection against alterations in gut barrier permeability during NASH.
Supported by: FPU (MECD); SAF201565267-R (MINECO/FEDER); CIBERdem (ISCIII), Spain; FIS
Disclosure: **C. Rubio:** Grants; FPU 2013 (MECD), SAF201565267-R (MINECO/FEDER), CIBERdem (ISCIII, Spain), FIS.

1236

JAZF zinc finger 1 ameliorates age- and diet-associated hepatic steatosis through SREBP-1c-dependent mechanism

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Background and aims: Hepatic steatosis is characterised by an aberrant accumulation of triglycerides in the liver. However,the drivers of progressive steatohepatitis remain incompletely defined. Juxtaposed with another zinc finger gene 1 (JAZF1) has been reported to be involved in glucose and lipid metabolism. Therefore, the aim of this study was to investigate the influence of JAZF1 on age- and adipose-associated hepatosteatosis and the mechanism.
Materials and methods: Expression levels of JAZF1 gene/protein were examined in the liver tissues from obesity-associated mice. In vitro, lipid metabolism was analysed in hepatocytes with JAZF1 overexpression and suppression. In vivo, Wild-type C57BL/6 and age-matched JAZF1-Tg mice were randomized to chow or HFD. Liver triglyceride metabolism was analysed in mice by PCR , Histological examination, Oil Red O staining, and western blot. Culture of primary mouse hepatocytes and dual-luciferase reporter gene assay were used to further investigate the mechanism of JAZF1 activation in the regulation of triglyceride metabolism.
Results: In JAZF1-Tg mice, body fat content and hepatosteatosis were protected from HFD-induced steatosis, and accompanied by decreased lipogenesis gene expression. In hepatocytes, over-expression of JAZF1 attenuated, while knockdown of JAZF1 enhanced the expression of lipogenesis genes. The over-expressing of JAZF1 in hepatocytes displayed the increased AMPK phosphorylation and decreased sterol regulatory element-binding protein 1 (SREBP-1) expression. The roles of JAZF1 were partially attenuated by Compound C. Mechanistically, JAZF1 suppressed SREBP-1c expression through the inhibition of transcriptional activity of LXRE2 in the SREBP-1c promoter.we found that JAZF1 over-expression protected against the development of atherosclerosis in ApoE KO mice and attenuated the expression of pro-inflammatory cytokines in vivo and vitro. More recently, we further demonstrated that JAZF1 over-expression decreased hepatic glucose production (HGP) and increased phosphorylation of insulin signaling molecules in liver with a PI3-kinase/Akt-dependent manner.
Conclusion: Our data illustrate that JAZF1 plays a crucial role in the regulation of age- and nutrient- associated hepatosteatosis through an AMPK/SREBP-1-dependent mechanism.
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Disclosure: **X. Luo:** None.

1237

Circulating PCSK9 is associated with pentraxin 3 concentration in patients with type 2 diabetes

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Background and aims: Nonalcoholic fatty liver disease (NAFLD) is present in up to 80%f of type 2 diabetic (DM2) patients. NAFLD exacerbates hepatic insulin resistance and is associated with obesity, hypertension and atherogenic dyslipidemia. Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) is a serine protease secreted by hepatocytes and involved in the regulation of LDL receptor expression. There is evidence that circulating PCSK9 increases with hepatic fat accumulation and correlates with the severity of steatosis, independently of metabolic confounders and liver damage. Pentraxin 3 (PTX3) is an essential component of innate immunity and a member of the long pentraxin superfamily, which are soluble proteins induced by various inflammatory stimuli. PTX3 is independently associated with the risk of vascular events. There is gathering evidence that plasma pentraxin 3 level is a novel marker for nonalcoholic steatohepatitis and severity of liver fibrosis. There are data that PTX3 also possess anti-microbial, anti-inflammatory

and cardioprotective properties. In this study we aimed to determine factors associated with PCSK9 in a group of patients with DMt2.
Materials and methods: Material included 116 consecutive patients with DMt2, 79 with NAFLD and 39 with coronary artery disease (cad). In each patient standardized questionnaire, anthropometric measurements, fasting serum lipids, apolipoprotein C3 (apo C3), glucose, glycated hemoglobin HbA1c, cytokeratin-18 fragments (CK-18), a marker of NAFLD, PTX3 serum concentration and circulating PCSK9 were determined. NAFLD was diagnosed by ultrasonography. CK-18, PTX3, PCSK9 and apo C3 levels were determined by ELISA, serum lipids enzymatically using Roche reagents, glycated hemoglobin A1c by HPLC.
Results: Mean (SD) age of patients was 59.1 (11.1) years, mean duration of diabetes 9.8 (5.9) years, mean values of HbA1c 8.63 (2.4%), and of BMI 32.8 (5.6) kg/m^2. Mean values of serum LDL-cholesterol were 2.6 (1.16), of triglyceride concentrations 1.8 (1.0) mmol/l, and of HDL-cholesterol 1.14 (0.29) mmol/l respectively. The mean (SD) values of serum PCSK9 were 255,44 (106,967) ng/ml, of PTX 3 4.53 (1,77) ng/ml and did not differ between patients with and without NAFLD or cad. In the whole group of patients circulating PCSK9 correlated negatively with age (r=-0.21, p<.05), HbA1c (r=-0.21, p<.05), and positively with BMI (r=0.21, p<.05) and, as expected, with total cholesterol (r=0.59), LDL-cholesterol (r=0.50), triglyceride (r=0.35) and po C3 (r=0.31) concentration (p<.001 for all). Interestingly, strong positive correlation between PTX3 and PCK9 was observed (r=0.47, p<.001). We did not found any associations between PCSK9 and CK-18 fragments nor liver enzymes.
Conclusion: The results of the study indicate that circulating PCSK9 is significantly associated with PTX3, marker of cardiovascular risk and NAFLD fibrosis. In our group of DMt2 patients PCSK9 is also significantly positively associated with BMI and atherogenic lipid levels and apo C3 concentrations. The association between circulating PCSK9 and PTX3 might be of value in understanding the processes of increased cvd risk in NAFLD patients.
Supported by: K/ZDS/005595
Disclosure: **M. Waluś:** None.

1238

Correlations between patatin-like phospholipase domain containing 3gene (PNPLA3) polymorphisms and nonalcoholic fatty liver disease in type 2 diabetes patients

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Background and aims: Patatin-like phospholipase domain-containing protein 3 (PNPLA3) has been linked to the development and evolution of fatty liver, but not to insulin resistance. The aim of the study was to evaluate the relationship between PNPLA3 and fatty liver, metabolic syndrome and subclinical atherosclerosis.
Materials and methods: We evaluated a group of 130 subjects with type 2 diabetes treated with oral medication or diet, who were admitted to the Clinical Center of Diabetes, Nutrition and Metabolic Diseases. The subjects with hepatitis B, C and toxic hepatopathy were excluded. The study group was represented by 92 patients with a mean age of 60.38 ± 10.37 years, with 47.83 % men. We analysed the link between fatty liver and anthropometric indices, biochemical parameters, genetic marker and subclinical atherosclerosis assessed by measurement of carotid intima-media thickness (CIMT). Fatty liver and CIMT were assessed using ultrasonography. PNPLA3 rs738409 genotype determination was performed by high resolution melting analysis (HRM) with a Rotor-Gene 6000 instrument (Cornett Research), its software (Series Software 1.7.87) and uAnalyze web application that allowed three standards genotyping CC, CG and GG.
Results: More than 90% of the subjects showed various degrees of hepatic steatosis. Using the body mass index, 60% of the subjects were obese, but 90% of them had abdominal obesity (using abdominal circumference). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT), serum triglycerides, insulin resistance (evaluated using the Homeostasis Model Assessment index - HOMA-IR), high sensitive C Reactive Protein, systolic blood pressure were correlated with fatty liver. A negative correlation was found between fatty liver, magnesemia and high density lipoprotein cholesterol (HDLc). The degree of liver fat accumulation increased significantly with the increasing number of the metabolic syndrome components. CIMT values were correlated with the degree of fatty liver. The frequency of cases with normal liver or mild steatosis was significantly higher in subjects with normal CIMT (54.29% versus 25% above normal values, p = 0.003), and the frequency of cases with moderate or severe steatosis was significantly higher in subjects with abnormal CIMT (75% versus 45.71% of normal values, p = 0.003). Genotyping PNPLA3 showed that the difference between subjects without steatosis and subjects with hepatic steatosis is given by the higher frequency of genotype GG. Triglycerides, cholesterol, HDLc, AST and ALT were similar among genotypes CC, CG, GG. PNPLA3 genotypes were not associated with the components of the metabolic syndrome, subclinical atherosclerosis or insulin resistance.
Conclusion: In our study, the GG genotype was associated with an increase regarding hepatic fat content. The results confirm that PNPLA3 polymorphism has a major role on the content of triglycerides in the liver. The lack of association with the components of metabolic syndrome (components) suggest that the presence of the G allele is not linked to metabolic disorders in subjects with type 2 diabetes. The data we have obtained confirm that the polymorphism PNPLA3 is closely associated with hepatic fat, but not with insulin resistance and metabolic syndrome. Cardiovascular risk in diabetic fatty liver is not shown to be directly dependent on the genotype.
Clinical Trial Registration Number: A-17-2794-EASD
Supported by: SOPHRD/159/1.5/S/135760
Disclosure: **R.S. Gavril:** None.

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Role of the gut in glucose-induced suppression of bone resorption in patients with non-alcoholic fatty liver disease and/or type 2 diabetes

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is associated with decreased bone mineral density (BMD), while type 2 diabetes (T2D) is associated with normal to high BMD. Both conditions are associated with increased fracture risk. The pathophysiology underlying bone abnormalities in NAFLD and T2D remains uncertain, but may involve the gut-bone axis. We investigated the influence of the gut on postprandial bone resorption in healthy individuals and patients with T2D and/or biopsy-verified NAFLD.
Materials and methods: NAFLD patients with normal glucose tolerance [n=8, HbA_{1c}: 33 (32, 37) mmol/mol (median with interquartile range in brackets); BMI: 30 (26, 35) kg/m^2], NAFLD patients with T2D [n=8, HbA_{1c}: 50 (41, 53) mmol/mol; BMI: 30 (28, 32) kg/m^2], T2D patients without liver disease [n=8, HbA_{1c}: 43 (39, 48) mmol/mol; BMI: 28 (26, 28) kg/m^2] and healthy controls [n=9, HbA_{1c}: 36 (34, 37) mmol/mol; BMI: 28 (28, 29) kg/m^2] underwent a 4-hour 50g oral glucose tolerance test (OGTT) and an isoglycaemic intravenous glucose infusion (IIGI) with repeated measurements of the bone resorption marker C-terminal type I collagen telopeptide (CTX).

Results: Plasma glucose levels achieved during OGTTs were successfully matched on corresponding IIGI days. Patients with NAFLD+T2D exhibited similar CTX suppression (as assessed by $AUC_{0\text{-}4h}$ for percent change from baseline) on OGTT vs. IIGI [-94 (-117, -70) vs. -114 (-129, -68) %×h, p=0.46], while remaining groups showed greater (p<0.05) CTX suppression during OGTT vs. IIGI (healthy subjects [-111 (-136, -93) vs. -66 (-102, -30) %×h], NAFLD [-145 (-162, -124) vs. -97 (-108, -44) %×h], and T2D [-79 (-96, -49) vs. -20 (-37, 32) %×h]).
Conclusion: OGTT-induced potentiation of CTX suppression seems to be abolished in patients with NAFLD+T2D, but preserved in patients with either NALFD *or* T2D. This suggests that coexistence of T2D *and* NAFLD may disturb the gut-bone axis.
Clinical Trial Registration Number: NCT01492283
Disclosure: **H. Maagensen:** None.

1240

Hypovitaminosis D is associated with insulin resistance in prediabetic individuals with nonalcoholic fatty liver disease

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Background and aims: Vitamin D deficiency due to impaired insulin action enhances excessive accumulation of fat within the hepatocytes and is a leading cause of nonalcoholic fatty liver disease (NAFLD). A number of studies claim the association of hypovitaminosis D with features of insulin resistance such as obesity, type 2 diabetes, and other metabolic syndromes. However, data examining the association of vitamin D deficiency with insulin resistance among prediabetic subjects with NAFLD are limited. In this context, the present study was assessed to explore the association of vitamin D deficiency with insulin resistance in prediabetic individuals with NAFLD.
Materials and methods: We studied 151 prediabetic subjects (M/F, 85/66; age in years, 45±9; BMI in kg/m^2, 25.9±4.4; M±SD) after confirming their oral glucose tolerance test. NAFLD was confirmed by upper abdomen ultrasonography comprising into 84 non-NAFLD (47/37; 44±9; 25.3 ±4.6) and 67 NAFLD (38/29; 46±9; 26.6±4.0) groups. Serum glucose was measured by glucose-oxidase method. HbA_{1c} was measured by high performance liquid chromatography. Serum insulin and vitamin D levels reflecting 25-hydroxyvitamin D [25(OH)D] were measured by ELISA techniques. Insulin resistance (HOMA-IR) was calculated by homeostasis model assessment (HOMA). Data were analyzed by univariate, bivariate and multivariate as appropriate.
Results: Compared to the non-NAFLD counterparts, NAFLD subjects had significantly lower levels of [25(OH)D] (30.01±6.46 vs. 49.12±14.27 nmol/L, P<0.001) as well as significantly higher levels of HOMA-IR (3.72±1.12 vs. 1.69±0.39, P<0.001). Pearson's correlation analysis showed a significant negative correlation of [25(OH)D] with HOMA-IR (r=-0.275, P=0.024) in NAFLD subjects. Multiple linear regression analysis showed a significant negative association of HOMA-IR with [25(OH)D] (β=-0.309, P=0.025) in NAFLD subjects after adjusting the effects of potential cofounders of age, body mass index (BMI) and glycosylated hemoglobin (HbA_{1C}) respectively. In binary logistic regression analysis, HOMA-IR (odds ratio, OR = 1.393, 95% CI: 1.025-1.892, P=0.034) and [25(OH)D] (OR = 0.898, 95% CI: 0.858-0.940, P<0.001) were found to be significant determinants of NAFLD when adjusted the effects of major confounders of age, BMI, and HbA_{1c} respectively.
Conclusion: NAFLD subjects seem to have an association with low [25(OH)D] levels and this relationship is mediated by insulin resistance which also the pathophysiological determinant of prediabetes.
Supported by: Bangladesh Medical Research Council (BMRC)
Disclosure: **M.R. Shah:** None.

1241

Non-alcoholic fatty liver disease and pancreatic beta cell function in non-diabetic patients

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) represents a histological spectrum ranging from benign hepatic steatosis to non-alcoholic steatohepatitis. NAFLD is closely associated with metabolic syndrome and insulin resistance (IR), and although the role of IR in NAFLD has been previously investigated, there are limited data on pancreatic β-cell function (BCF) in non-diabetic subjects. We aim to evaluate the association between NAFLD and IR/BCF assessed by oral glucose tolerance test (OGTT) derived indexes and analyse the risk of NAFLD when IR or beta-cell dysfunction is present.
Materials and methods: We recruited 218 non-diabetic obese subjects, who carried out an OGTT and at least one hepatic imaging test (ultrasonography or computed tomography scanning). Subjects were divided in two groups according to the presence or not of NAFLD. Insulin resistance was assessed by homeostasis model assessment (HOMA-IR), Quantitative Insulin Sensitivity Check Index (QUICKI) and insulin sensitivity index (ISI). Pancreatic BCF was assessed by insulinogenic index (IGI) and disposition index (DI). Anthropometric measurements and body composition study were performed in all patients. Bioelectrical impedance analysis was used to determine visceral fat by the ViScan system (Tanita Corp).
Results: The mean age (SD) of participants was 39.5 (14.3) years and 36% of participants were men. The prevalence of NAFLD was 42.2% in our population. The subjects with NAFLD compared with control group had higher insulin resistance (HOMA-IR 3.25vs2.37; QUICKI 0.33vs0.36; ISI 3.37vs5.53 (p<0.001)) and strained pancreatic BCF (DI 4.28vs4.59 (p=0.008) despite no differences in visceral adipose tissue between groups. An association between ISI, IGI and DI and the presence of NAFLD was statistically significant, showing a 32%, 71% and 18% increase in the risk of NAFLD for a one unit increase in ISI, IGI and DI, respectively.
Conclusion: NAFLD is associated with insulin resistance and strained pancreatic β-cell function in non-diabetic subjects. Presenting impaired OGTT derived indexes was associated with an increased risk of NAFLD. Future studies are necessary to evaluate the temporal relationship between insulin resistance, β-cell dysfunction and NAFLD and the potential application of the OGTT in clinical practice as a risk predictive tool for this condition.
Disclosure: **B. Pérez-Pevida:** None.

PS 113 Non-alcoholic fatty liver disease: clinical studies

1242

Reduced plasma NT-proBNP levels are predictive of non-alcoholic fatty liver disease among patients with type 2 diabetes

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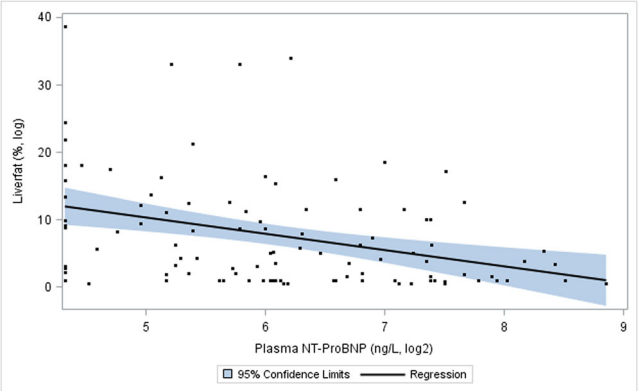
Background and aims: Plasma NT-proBNP is a well-established biomarker in heart failure. Increased plasma levels are associated with an increased cardiovascular mortality in the general population and in patients with heart disease. Notably, reduced concentrations of natriuretic peptides (NPs) are often reported in patients with type 2 diabetes (T2D), which in experimental settings, has been demonstrated to exaggerate insulin resistance and adipocyte dysfunction. Thus, decreased plasma levels of NPs could play a role in the accumulation of ectopic fat and development of non-alcoholic fatty liver disease (NAFLD) in T2D. We hypothesized that reduced plasma NT-proBNP level is associated with NAFLD among patients with T2D.
Materials and methods: We included 110 T2D patients from a randomized double-blind, placebo-controlled trial (The MIRAD study), investigating the effect of the mineralocorticoid receptor antagonist eplerenone on biomarkers related to adipocyte function. The patients had T2D and high cardiovascular (CV) risk defined as ischemic heart disease, history of myocardial infarction, stroke, peripheral artery disease or albuminuria. At baseline the patients underwent MR spectroscopy measuring liver fat content quantitatively with high precision by determining the fat peak content relative to the water content and abdominal fat in 1cm slice in the middle of L3 using 3.0T MR imaging (Philips Medical Systems, Best, the Netherlands).
Results: The participants were predominantly male n=76 (69%), with a mean (±SD) age of 64 (±9) years. Mean duration of T2D was 12 (±7) years and mean HbA1c was 59 (±14) mmol/mol; BMI was 30.8 (±4.2) kg/m^2 with visceral fat volume of 269 (±98) cm^3. The median (IQR) level of NT-proBNP was 67 (37-128) ng/L. A total of n=52 (47%) had liver fat content ≥5.6 % which is the cut off value for (NAFLD). There was a clear association between low NT-proBNP level and increased risk of NAFLD OR 0.51 (95% CI 0.35; 0.76, p<0.001), in univariate logistics regression analysis. As expected high triglycerides and visceral fat volume increased the risk for NAFLD (p<0.0001, p=0.003 respectively). The predictive ability of NT-proBNP was assessed in a multivariate logistic regression analysis demonstrating that reduced concentrations were independently associated higher risk of NAFLD OR 0.48 (95 % CI 0.31; 0.76 p=0.001), adjusting for sex, HbA1c, BMI and visceral fat.
Conclusion: Decreased plasma NT-ProBNP concentration is independently associated with a high risk of NAFLD among T2D patients. Therefore, reduced levels of plasma NT-ProBNP, could be a marker of accumulation of ectopic fat in the liver and bear novel clinical implications regarding screening for NAFLD in T2D patients with high CV risk.



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Disclosure: **M.L. Johansen:** None.

1243

Increased liver fat content in totally pancreatectomised patients

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Background and aims: Lack of glucagon signaling has been shown to increase hepatic fat content in animal studies. Furthermore, administration of glucagon receptor antagonists are associated with increases in hepatic transaminases and fat content in type 2 diabetes patients. Here we evaluated hepatic fat content and aspartate transaminase (AST) and alanine transaminase (ALT) in totally pancreatectomised patients without glucagon-secreting pancreatic alpha cells.
Materials and methods: Fasting blood samples and transient liver elastography (Fibroscan 501®, EchoSensTM, Paris, France), as a measure for hepatic fibrosis and steatosis, were performed in 10 totally pancreatectomised patients (age [mean ± SD]: 59.8±9.9 years; BMI: 21.5±4.3 kg/m2; HbA_{1c}: 67.3±11.0 mmol/mol; time since operation: 4.6±4.5 years), and 10 age, sex and BMI-matched healthy control subjects (age: 58.4±5.0 years; BMI: 22.9±2.4 kg/m2; HbA_{1c}: 34.6±6.2 mmol/mol).
Results: In both groups hepatic elasticity were within normal range (Fibrosis stage 0) with no difference in transmission speed of the ultrasonic waves between the pancreatectomised group and the control group (5.1±0.34 vs. 4.8±0.52 kPa, p=0.52). In the pancreatectomised group, two patients had steatosis grade 3 (67-100% of hepatocytes with fat accumulation), two patients had steatosis grade 2 (33-66% of hepatocytes with fat accumulation), one patient had steatosis grade 1 (11-33% of hepatocytes with fat accumulation) and five patients had steatosis grade 0 (<11% of hepatocytes with fat accumulation). In the control group, two subjects had steatosis grade 2, and eight subjects had steatosis grade 0. Fasting concentrations of AST were higher in the pancreatectomy group compared to the control group (54.2±4.5 vs 35.0±3.0 U/L, p=0.003) while no difference was observed for ALT (33.6±4.5 vs 25.6±2.2 U/L, p=0.13).
Conclusion: Totally pancreatectomised patients are characterised by increased hepatic fat content and higher fasting plasma concentrations of AST, which may relate to surgical removal of glucagon-secreting alpha cells.

Clinical Trial Registration Number: NCT02006459
Supported by: EFSD/MSD
Disclosure: **J.I. Bagger:** None.

1244

Validity of hepatic steatosis indices for prediction of non-alcoholic hepatic steatosis in type 1 diabetic patients

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Background and aims: Non-alcoholic fatty liver disease in type 1 diabetes mellitus patients is associated with an increased risk of cardiovascular events and diabetic complications. Markers of hepatic steatosis, hepatic steatosis index (HSI) and fatty liver index (FLI) have demonstrated reasonable efficacy in several patient groups. The purpose of our work was screen the effectiveness of FLI and HSI in a observational pilot study of 40 type 1 diabetic patients.
Materials and methods: Data of 252 LatDiane study patients were analyzed. Patients with diabetes duration less than 5 years and with high-risk alcohol consumption were excluded. For remaining 201 patients, FLI and HSI indices were calculated. 40 patients with marginal FLI/HSI values were invited to take part in the liver magnetic resonance study. In-phase/opposed-phase technique of magnetic resonance imaging was used. Accuracy of FLI/HSI was assessed from the area under the receiver operating characteristic curve (AUROC).
Results: 12 (30.0%) patients had liver steatosis. These patients had higher body mass index, waist circumference, FLI, HSI, aspartate transaminase, gamma-glutamyl-transpeptidase, C-reactive protein, diastolic blood pressure, and metabolic syndrome was diagnosed more often in this group. For FLI, sensitivity was 90%, specificity was 74%, positive likelihood ratio was 3.46, negative likelihood ratio - 0.14, positive predictive value - 0.64; negative predictive value - 0.93. For HSI, sensitivity was 86%, specificity was 66%, positive likelihood ratio was 1.95, negative likelihood ratio - 0.21, positive predictive value - 0.50; negative predictive value - 0.92. AUROC for FLI was 0.86 (95% confidence interval [0.72; 0.99]); for HSI - 0.75 [0.58; 0.91]. Liver fat correlated with liver enzymes, waist circumference, triglycerides, C-reactive protein, but not with estimated glucose disposal rate or insulin dose/kg/24 h. In multiple regression model, only C-reactive protein (p=0.038), gamma-glutamyl-transpeptidase (p=0.047) and waist circumference (p=0.001) were independent determinants of microscopic fat content and taken together explained 45% of its variation (p=0.001). FLI correlated with C-reactive protein, alanine transaminase, aspartate transaminase, systolic and diastolic blood pressure. HSI correlated with waist circumference and C-reactive protein. Neither FLI, nor HSI correlated with estimated glucose disposal rate or total daily insulin dose. FLI exceeding 60 and HSI exceeding 36 were significantly associated with metabolic syndrome and nephropathy.
Conclusion: The tested indices can serve as surrogate markers for liver fat content and metabolic syndrome in type 1 diabetic patients. Parameters of insulin sensitivity were associated neither with indices, nor liver fat. C-reactive protein might be considered for development of novel biomarkers for liver steatosis assessment in type 1 diabetes.
Supported by: Scientific Council of Roche Academy, University of Latvia
Disclosure: **J. Sokolovska:** None.

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Association and predictability of different non-invasive scores with nonalcoholic fatty liver disease diagnosis in patients with type 2 diabetes

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Background and aims: Non - alcoholic fatty liver disease (NAFLD) is the commonest cause of abnormal liver function tests and liver disease in the western countries. NAFLD is usually asymptomatic. As a result some non - invasive diagnostic models have been proposed for the diagnosis and staging of NAFLD. The objective of this study was to evaluate whether some of the most common and easily assessed models can also be used to screen for the presence of nonalcoholic fatty liver disease (NAFLD) in patients with type 2 diabetes (T2D) in clinical practice.
Materials and methods: The study population included 110 patients with T2D (58 men) [mean age (±SD) 60.1±9.5 years, HbA1c 6.4 ±1.0%, body - mass index 28.6±4.8 Kg/m2, duration of diabetes 8.5 ±4.0 years] attending the outpatient diabetic clinic of our hospital. Anthropometric, clinical, and laboratory data were analyzed during regular health checkups. NAFLD was diagnosed using ultrasound. NAFLD liver fat score, HAIR (Hypertension, ALT, Insulin Resistance), BARD, APRI (AST to Platelet Ratio Index), FIB - 4 and LAP (Lipid Accumulation Product) scores are were estimated. Discrimination capability was assessed based on the area under the receiver operating characteristic curve (AUC), sensitivity and specificity, positive (PPV) and negative (NPV) predictive values were calculated.
Results: NAFLD, using ultrasound, was diagnosed in 77 patients (70%). Receiver operating characteristic analysis showed that for the NAFLD liver fat score a cutoff of ≥ - 1.44 had a sensitivity of 93%, a specificity of 72%, with an AUC of 0.95 and a PPV of 89% and a NPV of 82%. For the HAIR score a cutoff of ≥0.50 had a sensitivity of 62%, a specificity of 44%, with an AUC of 0.58 and a PPV of 72% and a NPV of 33%. For the BARD score a cutoff of ≥2.50 had a sensitivity of 51%, a specificity of 64%, with an AUC of 0.59 and a PPV of 77% and a NPV of 36%. For the APRI a cutoff of ≥0.23 had a sensitivity of 49%, a specificity of 64%, with an AUC of 0.55 and a PPV of 76% and a NPV of 35%. For the FIB - 4 score a cutoff of ≥1.00 had a sensitivity of 53%, a specificity of 54%, with an AUC of 0.52 and a PPV of 73% and a NPV of 33%. For the LAP score a cutoff of ≥30.93 had a sensitivity of 94%, a specificity of 82%, with an AUC of 0.89 and a PPV of 92% and a NPV of 85%.
Conclusion: The results of the present study showed that NAFLD liver fat and LAP scores showed good sensitivity and specificity for the presence of NAFLD in patients with T2D. Both scores are two simple, accurate and non - invasive tools to predict NAFLD in patients with type 2 diabetes. On the contrary, HAIR, BARD, APRI and FIB - 4 scores showed poor sensitivity and specificity.
Supported by: Hellenic Society of Lipidology, Atherosclerosis and Vascular Disease
Disclosure: **A. Papazafiropoulou:** None.

1246

Application of the new EASL-EASD-EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease (NAFLD) in people with type 2 diabetes

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Background and aims: Recently, the European Association for the Study of the Liver (EASL)-European Association for the Study of Diabetes (EASD)-European Association for the Study of Obesity (EASO) propose recommendations for the diagnosis, treatment and follow-up of non-alcoholic fatty liver disease (NAFLD) patients. In this study we evaluate the application of these clinical practice guidelines for the management of NAFLD in people with type 2 diabetes. We also compare the performance of steatosis biomarkers (fatty liver index and steatotest) to 1H-magnetic resonance spectroscopy.
Materials and methods: A total of 179 type 2 diabetic patients were included in this study. Liver fat (1H-magnetic resonance spectroscopy),

Fatty liver index (FLI), NAFLD fibrosis score (NFS), Steatotest and fibrotest were measured.
Results: One hundred and twenty three (68.7%) patients had steatosis (hepatic triglyceride content greater than 5.6%) with 1H-magnetic resonance spectroscopy evaluation. With FLI biomarkers, 162 (90.5 %) patients had a diagnosis of steatosis (FLI>60). In comparison with 1H-magnetic resonance spectroscopy, FLI diagnosed 43 patients in excess, and did not diagnose correctly 5 patients (sensibility 73.4%, specificity 73.6%). With steatotest biomarkers, 141 (78.7%) patients had a diagnosis of steatosis (steatotest >0.57). In comparison with 1H-magnetic resonance spectroscopy, steatotest diagnosed 36 patients in excess, and did not diagnose correctly 16 patients (sensibility 74.8%, specificity 55.5%). Fifty-six patients were found to have abnormal liver transaminases or elevated serum gamma-glutamyltransferase. The application of the EASL-EASD-EASO guidelines using NFS in combination with FLI results to a referral to a liver clinic for 152 (84.9%) people with type 2 diabetes (NFS >-1.455). The application of the guidelines using Steatotest in combination with Fibrotest results to a referral for 62 (34.6%) people with type 2 diabetes to a liver clinic.
Conclusion: The SteatoTest and FLI biomarkers are not valid predictors of steatosis in type 2 diabetic patients. The application of these new recommendations for the diagnosis, treatment and follow-up of NAFLD would lead to an excessive number of people with type 2 patients that should be referred to a liver clinic. It seems very difficult to apply these new guidelines in clinical routine practice. We suggest that future clinical and/or biological biomarkers of steatosis and fibrosis be specifically validated in type-2 diabetic patients.
Disclosure: **B. Bouillet:** None.

1247

Improvement in liver histology with obeticholic acid in patients with nonalcoholic steatohepatitis and type 2 diabetes

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Background and aims: Nonalcoholic steatohepatitis (NASH) is commonly associated with type 2 diabetes mellitus (T2DM) and the presence of both is associated with higher risk of poor clinical outcomes. Obeticholic acid is a potent farnesoid X receptor agonist that has been shown to improve liver histology in patients with NASH in the FLINT trial (N=283). 149 patients (61%) enrolled in FLINT had T2DM and within this population, 45% (67/149) were found to have the presence of advanced fibrosis (≥F3) which is further indicative of higher risk of poor outcomes. The purpose of this post-hoc analysis was to assess the efficacy of OCA in patients in the FLINT trial who had both NASH and T2DM.
Materials and methods: FLINT was a randomized, double-blind, placebo-controlled, 72-week study. This analysis included all patients who had a diagnosis of NASH and T2DM at Baseline, and were treated with OCA 25 mg (n=75) or PBO (n=74). The primary endpoint was ≥2-point improvement in NAFLD activity score (NAS) without worsening of fibrosis. Fibrosis improvement was defined as ≥1 stage improvement. Histologic changes were only evaluated in patients who had both Baseline and Week 72 liver biopsy (OCA 25 mg, n=54; PBO, n=53).
Results: A greater percentage of OCA-treated patients achieved the primary endpoint [OCA 25 mg: 31/54 (57%); PBO: 11/53 (21%); p<0.01] at Week 72. A larger percentage of OCA-treated patients achieved fibrosis improvement compared to PBO at Week 72 [OCA 25 mg: 22/54 (41%); PBO: 10/53 (19%); p<0.05]. OCA treatment also resulted in reduction in body weight from Baseline compared to PBO at Week 72 [LS Mean (95% CI); OCA 25 mg: -3.3 (-4.8, -1.8) kg; PBO: 0.3 (-1.1, 1.7) kg; p<0.01]. There were no statistical differences between OCA and PBO in glycemic parameters examined at Week 72.
Conclusion: In the FLINT trial, within the subgroup of patients with both NASH and T2DM, OCA treatment resulted in improvement in liver histology and reductions in body weight. Further prospective evaluation with OCA is necessary to confirm these findings.
Clinical Trial Registration Number: NCT01265498
Supported by: National Institute of Diabetes and Digestive and Kidney Diseases
Disclosure: **A. Liberman:** Employment/Consultancy; Intercept Pharmaceuticals, Inc. Stock/Shareholding; Intercept Pharmaceuticals, Inc.

1248

Dulaglutide decreases liver enzymes in people with type 2 diabetes: a post-hoc analysis of the AWARD programme

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Background and aims: GLP-1 receptor agonists may reduce hepatic steatosis and steatohepatitic liver histology in people with NAFLD/NASH. Higher plasma alanine transaminase (ALT) levels correlate with hepatic steatosis and regress in response to treatment. This post-hoc analysis evaluated the effects of once weekly dulaglutide 1.5 mg (DU) on plasma ALT, aspartate transaminase (AST) and gamma-glutamyl transpeptidase (GGT) in patients with type 2 diabetes (T2D) and compared the effect of DU vs placebo (PL) on several metabolic measures in all patients, as well as in a subset predicted to have higher prevalence of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis (enriched by baseline ALT thresholds: male, ≥30 IU/L; female, ≥19 IU/L) from 4 PL-controlled studies in the AWARD program (AWARD-1, AWARD-5, AWARD-8 and AWARD-9).
Materials and methods: Patients receiving DU or PL were included in this post-hoc analysis. The objectives were to assess: 1) changes from baseline in ALT, AST and GGT between patients treated with DU vs PL at 6 months and, 2) changes from baseline in ALT, AST, GGT, HbA_{1c}, fasting serum glucose (FSG), lipid profile and weight between DU vs PL after 6 months of treatment in a NAFLD/NASH enriched population.
Results: At baseline, the clinical characteristics of DU and PL groups were similar. At 6 months, changes from baseline in ALT, AST and GGT were: (LSM [SE]) -4.7 (0.6), -1.9 (0.5), -5.0 (2.8) on DU vs -3.0 (0.6), -0.8 (0.6), 1.6 (3.3) on PL, respectively. Treatment differences were significant for ALT, AST and GGT (-1.7 [0.6] p=0.003, -1.1 [0.5] p=0.037 and -6.6 [3.0] p=0.025, respectively). The NAFLD/NASH enriched population had more males, shorter duration of diabetes, higher AST and triglycerides at baseline. At 6 months, a more pronounced change in ALT was observed in patients with baseline ALT ≥ULN treated with DU vs PL (p=0.022) (table). Overall, DU-treated patients with baseline ALT ≥ULN demonstrated significantly greater change from baseline in HbA_{1c}, FSG, weight, and triglycerides vs PL (p≤0.014 all parameters; table). Similar differences between DU vs PL were observed in patients with ALT <ULN.
Conclusion: Results from this post-hoc analysis suggest that once weekly DU 1.5 mg improves hepatic enzymes compared to placebo, with greater reduction seen in ALT and GGT than AST, consistent with reduced liver fat in both the overall and NAFLD/NASH enriched population. The glycaemic and metabolic response of DU in the enriched population was consistent with the results observed in the overall population.

Table. Change from baseline in efficacy and safety parameters and select hepatic enzymes by ALT ≥ULN in AWARD-1, AWARD-5, AWARD-8 and AWARD-9

Parameter	Dulaglutide 1.5 mg N=939	Placebo N=488	Treatment Difference[a]
ALT (IU/L)	-8.8 (0.7)*	-6.7 (0.9)*	-2.1 (0.9)#
AST (IU/L)	-4.3 (0.6)*	-3.8 (0.8)*	-0.5 (0.8)
GGT (IU/L)	-7.4 (3.3)*	-3.6 (4.1)	-3.8 (4.1)
HbA_{1c}, mmol/mol	-16.1 (0.7)*	-3.6 (0.9)*	-12.4 (0.9)#
FSG, mmol/L	-2.6 (0.2)*	-0.7 (0.2)*	-1.9 (0.2)#
Weight, kg	-1.6 (0.2)*	0.0 (0.3)	-1.6 (0.3)#
Triglycerides, mg/dL	-29.0 (8.4)*	-2.5 (10.3)	-26.5 (10.8)#

Data presented as LSM (SE). *p<0.05 change from baseline, #p<0.05 vs placebo. [a]All parameters were analysed using ANCOVA (LOCF) with study, country, treatment, ALT subgroup, treatment by ALT subgroup interaction. Triglycerides not done in AWARD-8. Abbreviations: ANCOVA=analysis of covariance; HbA1c=glycated haemoglobin A1c; GLP-1=glucagon-like peptide-1; LOCF=Last observation carried forward; LSM=least squares mean; SE=standard error

Supported by: Eli Lilly and Company

Disclosure: **A. Haupt:** Employment/Consultancy; Eli Lilly and Company. Stock/Shareholding; Eli Lilly and Company.

PS 114 Animal models on non-alcoholic fatty liver disease

1249

Body weight lowering agents and their comparative metabolic and hepatic effects in obese mouse models of nonalcoholic fatty liver disease

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Background and aims: The GLP-1 analogue liraglutide is an established treatment for obesity and type 2 diabetes. Here we aimed to compare the body weight lowering effects of liraglutide and the peroxisome proliferator activated receptor (PPAR) α/δ agonist, elafibranor, in diet-induced obese (DIO) and genetically obese mouse models of nonalcoholic fatty liver disease (NAFLD) and steatohepatitis (NASH).
Materials and methods: Male wildtype C57BL/6J and leptin-deficient $Lep^{ob/ob}$ mice (5 weeks of age) were fed a diet high in trans-fat, fructose and cholesterol for a total of 26 weeks and 12 weeks, respectively, for induction of NASH. Only biopsy-confirmed steatotic and fibrotic animals were included and stratified into DIO-NASH and ob/ob-NASH treatment groups and treated for 8 weeks with vehicle (PO, QD), liraglutide (0.2 mg/kg, SC, BID) or elafibranor (30 mg/kg, PO, QD). At termination, blood samples were collected for plasma liver enzymes (alanine/aspartate aminotransferases; ALT/AST) and lipids (total cholesterol; TC, triglycerides; TG). Furthermore, liver post-biopsies and tissue samples were obtained for histological and biochemical analysis. Finally, a blinded histological evaluation of NAFLD Activity Score (NAS) (steatosis, inflammation, ballooning degeneration) including Fibrosis Stage was performed.
Results: Liraglutide and elafibranor treatment induced a weight loss of approximately 10% in both DIO-NASH and ob/ob-NASH mice, albeit with differential effect on hepatomegaly, plasma ALT/AST/TG/TC and liver lipids. Based on histopathological analysis, liraglutide reduced composite NAS in DIO-NASH (7/10 animals), but not in ob/ob-NASH (2/10 animals), mainly by reducing steatosis component. In contrast, Elafibranor induced resolution of NAS in both DIO-NASH and ob/ob-NASH (10/10 animals) by improving all three dimensions (steatosis, inflammation and hepatocyte ballooning). Furthermore, only elafibranor reduced liver fibrosis stage in DIO-NASH (6/10 animals) and ob/ob-NASH (10/10).
Conclusion: Pharmacological intervention with liraglutide and elafibranor induced a diverse metabolic and hepatic profile, irrespectively of similar weight-loss inducing effect in wildtype diet-induced and genetically obese mouse models of NASH. Notably, both treatments exerted an anti-steatotic action and improved liver histopathology by reducing NAFLD Activity Score in DIO-NASH mice. In addition, elafibranor improved steatohepatitis in ob/ob-NASH mice and exerted anti-fibrotic effects in DIO-NASH and ob/ob-NASH mice.These finding suggest that weight-loss inducing agents with a direct liver target effect exerts superiority in alleviating NASH.
Disclosure: **J. Jelsing:** Stock/Shareholding; Gubra ApS.

1250

Pirfenidone prevents and reverses hepatic insulin resistance and steatohepatitis by polarising M2 macrophages in mice

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Background and aims: Excessive hepatic lipid accumulation promotes the activation of macrophage/Kupffer cells, resulting in exacerbation of insulin resistance and nonalcoholic steatohepatitis (NASH). In our previous study, we developed a cholesterol- and saturated fatty acid-induced model of lipotoxic NASH and revealed that hepatic oxidative stress and

insulin resistance promotes hepatic inflammation and fibrosis. Pirfenidone is an antifibrotic agent used in the treatment of pulmonary fibrosis. In addition, pirfenidone suppresses bleomycin-induced increases in the pulmonary influx of T cells and macrophages. However, less attention has been focused on its anti-inflammatory effects. In the present study, we investigated the effect of pirfenidone in a lipotoxicity-induced NASH model.

Materials and methods: Eight-week-old C57BL/6 mice were fed a high-cholesterol, high-fat (CL) diet or CL diet with 0.2% pirfenidone (CL + PFD), for a total of 12 weeks. The liver histology, insulin sensitivity, and inflammatory/stress signal were examined. Next, we quantified intrahepatic immune cells by flow cytometry.

Results: After 12 weeks of feeding, histological examination revealed hepatic steatosis, inflammation and fibrosis in mice fed CL diet. They showed hyperinsulinemia even though weight and adiposity were similar. Pirfenidone administration reduced hepatic TG, TC, and NEFA levels by 24%, 23%, and 50%, respectively (all $p < 0.05$), as well as lipid peroxidation, which was assessed by TBARS. Pirfenidone improved glucose intolerance and hyperinsulinemia in the CL group and enhanced the insulin signal, assessed by IRβ and Akt phosphorylation, in the liver, which is associated with the attenuation of MAPK (ERK/p38MAPK) and NF-kB activation. To assess the effect of pirfenidone on hepatic inflammation further, intrahepatic leukocytes were quantified using flow cytometry. Hepatic macrophages identified as $CD45^+CD11b^+F4/80^+$ cells were markedly decreased in the CL + PFD group by 67% compared with mice fed the CL diet. In addition to a reduction of the total macrophage content in the liver, mice fed CL + PFD had 81% fewer $CD11c^+CD206^-$ (M1)-type macrophages, but 33% more $CD11c^-CD206^+$ (M2)-type macrophages than mice fed the CL diet, resulting in a predominance of M2 over the M1 macrophage population. Pirfenidone also reduced $CD4^+$ and $CD8^+$ T-cell contents by 55% and 41% respectively, which contributed to the improvement of insulin resistance and steatohepatitis. Moreover, pirfenidone downregulated LPS-induced M1 marker (*Tnfa, Il1b* and *Mcp-1*) mRNA expression in RAW264.7 macrophages but augmented IL-4-induced M2 marker (*Mrc2, Cd206* and *Mgl1*) mRNA expression in a dose-dependent manner. Additionally, pirfenidone reduced the activation of hepatic stellate cells and inhibited fibrosis, lowering hydroxyproline content by 40% ($p < 0.05$). Importantly, pirfenidone reversed insulin resistance, as well as hepatic inflammation and fibrosis, in pre-existing advanced NASH.

Conclusion: Pirfenidone prevents and reverses hepatic insulin resistance and steatohepatitis by polarizing M2 macrophages in mice. Pirfenidone might be a novel and promising treatment for NASH.

Supported by: MEXT, Japan

Disclosure: **T. Ota:** None.

1251

Allopurinol reduces hepatic fat accumulation through regulating lipid oxidation and ER stress signalling in high fructose-fed OLETF rats

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Background and aims: Excess fructose consumption is associated with development of metabolic syndrome, type 2 diabetes, and nonalcoholic fatty liver disease (NAFLD). Uric acid generation is a unique feature of fructose metabolism and may have a direct role in the development of NAFLD. However, its mechanism is unclear. Therefore, we examined the effects of uric acid lowering treatment on fatty liver using a high fructose-fed OLETF rats for evaluating the contributions of fructose and uric acid on NAFLD development.

Materials and methods: 30-week-old OLETF rats were randomly divided into three groups as follows: (1) normal chow-diet OLETF rats (NC group); (2) high fructose-diet OLETF rats (HFrD group); and (3) high fructose-diet OLETF rats with allopurinol treatment (HFrDAL group). After 16 weeks of treatment, body weight was measured and intraperitoneal glucose tolerance test was conducted. Liver tissue was collected at sacrifice and processed to assess histological characteristics. Real-time polymerase chain reaction (PCR) was performed to evaluate hepatic expression of genes involved in lipid metabolism and inflammation. Hepatic endoplasmic reticulum (ER) stress pathway was evaluated by Western blot. All animal experiments were conducted according to the institutional animal research regulations of our center.

Results: The HFrD group had significantly elevated body weight, as well as higher serum uric acid and triglyceride concentrations compared to the NC group ($P<0.05$). Blood glucose concentrations at 2 hours after glucose load were significantly increased in the HFrD and HFrDAL groups compared with the NC group ($P<0.05$). Hepatic lipid accumulation was significantly increased in the HFrD group versus the NC group. Allopurinol treatment significantly reduced body weight, decreased serum uric acid concentrations, and ameliorated hepatic steatosis in the HFrDAL group compared with the HFrD group ($P<0.05$). High fructose diet significantly suppressed hepatic expression of fat oxidation genes and elevated hepatic expression of proinflammatory cytokine genes, whereas allopurinol treatment restored it ($P<0.05$). Furthermore, high fructose diet significantly increased protein expression of immunoglobulin heavy chain-binding protein and phosphorylated inositol-requiring enzyme 1. And x-box binding protein 1, which is the downstream effector, was spliced by high fructose diet in PCR. However, allopurinol treatment attenuated these changes induced by high fructose diet.

Conclusion: Allopurinol ameliorates high fructose diet-induced hepatic steatosis via modulating not only hepatic lipid metabolism and proinflammatory cytokines expression but also ER stress signaling.

Disclosure: **I. Cho:** None.

1252

Protective effects of the TAZ modulator TM-25659 against palmitate-induced lipogenesis in HepG2 cells and fatty livers from DIO and MCD-diet fed mice

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Background and aims: The transcriptional co-activator with PDZ-binding motif (TAZ) binds various transcription factors to control many physiological processes including cell differentiation, organ development, and inflammation. The TAZ modulator 2-butyl-5-methyl-6-(pyridine-3-yl)-3-[2'-(1H-tetrazole-5-yl)-biphenyl-4-ylmethyl]-3H-imidazo[4,5-b]pyridine] (TM-25659) inhibits adipocyte differentiation by interacting with peroxisome proliferator-activated receptor gamma (PPARgamma). TM-25659 has anti-obesity and anti-diabetic effects in high fat (HF) diet-induced obese (DIO) mice. However, the effects of TM-25659 in non-alcoholic fatty liver disease (NAFLD) have not been well studied. In this study, we investigated the beneficial effects of TM-25659 and the molecular mechanism of palmitate-induced lipogenesis in HepG2 cells and in the fatty liver of DIO and mice fed a HF completely devoid of methionine and choline diet (MCD).

Materials and methods: To clarify the molecular mechanism of TM-25659 in palmitate-induced HepG2 cells and in the fatty liver of mice, we treated the cells and mice with TM-25659 and used mRNA sequencing to evaluate the expression of transcription factor,s such as PPARgamma, retinoid X receptor, and nuclear receptor corepressor 1 (NCOR1), as well

as gene expression-related lipogenesis and inflammation. To investigate the protective effects of TM-25659 on fatty liver, C57BL/6J mice were randomly divided into five groups: low-fat diet control group (LFD), 60% HF group, HF plus TM-25659 diet group, MCD diet group, and MCD plus TM-25659 group. Glucose homeostasis and insulin sensitivity were evaluated after 16 weeks of treatment in the HF diet mice and after 4 weeks in the MCD mice. Fasting insulin and serum lipid levels were measured at the end of the study. Genes involved in inflammation and lipid metabolism were analyzed by real-time polymerase chain reaction and immunoblotting. Body weight, fasting insulin, and plasma glucose were maintained in the TM-25659-treated animal group.
Results: We demonstrated that TM-25659 was significantly effective for ameliorating HF diet-induced glucose intolerance, insulin resistance, and fatty liver. In addition, TM-25659 significantly reduced hepatic steatosis and immune cell infiltration. TM-25659 also reduced fatty liver in MCD mice as well as the expression of several inflammation-related genes. TM-25659 led to TAZ nuclear localization and binding with transcription factors, such as PPARgamma and NCOR1, in HepG2 cells. Finally, TM-25659 inhibited palmitate-induced inflammatory cytokine and lipogenic gene expression in HepG2 cells.
Conclusion: This is the first report demonstrating that TM-25659 has protective effects on NAFLD through the enhanced interaction between TAZ and PPARgamma. Therefore, TM-25659 may be useful for preventing NAFLD.
Disclosure: **T. Kim:** None.

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Curcumin8, a novel curcumin analog, prevent liver steatosis in high fat diet-induced obese mice

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) occurs when excess fat is stored in the liver. NAFLD is highly prevalent in type 2 diabetes mellitus (T2DM), likely reflecting the frequent occurrence of obesity and insulin resistance in T2DM. Curcumin8 (CUR8) is a synthetic derivative of natural active curcumin (CUR), which has antioxidant and anti-inflammatory properties. Here, we investigated the effects of CUR8, a novel CUR analog, on liver steatosis in high fat diet (HFD)-induced obese mice.
Materials and methods: We categorized six groups such as regular diet mice (RD, n=10), RD fed with curcumin (RD+CUR, 100mg/kg/day, n=10), RD fed with curcumin8 (RD+CUR8, 100mg/kg/day, n=10), high fat diet-induced obese mice (HFD, n=10) and HFD fed with curcumin (HFD+CUR, 100mg/kg/day, n=10), HFD fed with curcumin8 (HFD+CUR8, 100mg/kg/day, n=10) for 12 weeks. Liver steatosis was detected in hematoxylin and eosin (H&E) stained sections.
Results: CUR8 prevented the increase of body and liver weights in HFD-induced obese mice. In addition, insulin resistance was significantly improved in HFD+CUR8 group than HFD group. Serum ALT level, an indicator of liver damage, also reduced after CUR8 treatment. Moreover, H&E stain revealed that CUR8 decreased liver steatosis in HFD-induced obese mice. Interestingly, the increase of liver TG resulting from HFD also decreased after the administration of CUR8 but not CUR.
Conclusion: Our data demonstrates that CUR8 ameliorated insulin resistance and reduced liver steatosis in HFD-induced obese mice
Disclosure: **C.H. Chung:** None.

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E2F1 participates in liver cholesterol metabolism and protects against liver fibrosis development

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Background and aims: Over the past 30 years, non-alcoholic fatty liver disease (NAFLD) has become the most common chronic liver disorder in the world. The liver is a central organ for the metabolism of cholesterol whose hepatic accumulation is an early even of NAFLD. In our laboratory, we work on the metabolic roles of the transcription factor E2F1 mainly known for its roles in cell cycle control. We found that E2F1 plays a crucial role in maintaining cellular cholesterol homeostasis and the development of hepatic fibrosis, via the regulation of PCSK9.
Materials and methods: The importance of E2F1 on cholesterol metabolism was assessed by studying the mice invalidated for E2F1. Analysis of the E2F1 ChIP-seq in hepatocytes allowed us to determine its direct targets, which were validated by promoter's studies. The cellular models of mouse hepatocytes and HepG2 cell lines, invalidated for E2F1, allowed us to determine the mechanisms involved. The influence of cholesterol overload was determined by subjecting these mice to an atherogenic diet, rich in cholesterol. The importance of PCSK9 in the liver phenotype observed was determined by re-expressing PCSK9 in the liver of E2F1 -/- mice (by adenoviral strategy). We also studied PCSK9 -/- mice under high cholesterol diet.
Results: E2F1-/- mice display reduced total plasma cholesterol levels and increased cholesterol content in the liver. We show that E2F1 deletion in cellular and mouse models leads to a marked decrease in PCSK9 expression and increase in LDLR expression. In addition to the up regulation of LDLR, we report that E2F1-/- hepatocytes exhibit increased LDL uptake. ChIP-Seq and PCSK9 promoter reporter experiments confirmed that E2F1 binds to and transactivates the PCSK9 promoter. Interestingly, E2F1-/- mice fed a high cholesterol diet (HCD) display a fatty liver phenotype and early liver fibrosis, which is reversed by re-expression of PCSK9 in the liver.
Conclusion: Collectively, these data indicate that E2F1 regulates cholesterol uptake and that the loss of E2F1 leads to abnormal cholesterol accumulation in the liver and the development of fibrosis in response to a high cholesterol diet. In our study, we not only describe a new regulator of PCSK9, whose inhibitors have recently been developed for the treatment of hypercholesterolemia in humans, but we also highlight for the first time potential liver problems that could result from this inhibition.
Supported by: FNS, FSC
Disclosure: **P. Denechaud:** None.

1255

A novel mouse model of maternal overnutrition-induced non-alcoholic steatohepatitis (NASH)/hepatocellular carcinoma

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Background and aims: Non-alcoholic steatohepatitis (NASH) is associated with hepatocellular carcinoma, which is the leading cause of cancer-related mortalities in Japanese patients with type 2 diabetes mellitus. NASH is believed to develop after two-hit process, and the first 'hit' may lie in early intrauterine exposures. To examine the impact of intrauterine nutrition on the pathophysiology of NASH, we have generated a novel mouse model.
Materials and methods: Female C57BL6J mice were fed either high-fat diet (H) or a control diet (C) during gestation. During lactation, all mice were fed the control chow. After weaning, the male offspring were fed the

control chow (C), generating two groups: H/C (n=7), C/C (n=7). At 15 weeks of age, offspring body weight, fasting blood glucose (FBS), HbA1c, nonesterified fatty acid (NEFA), alanine transaminase (ALT), systolic blood pressure (SBP) were measured. Following sacrifice, liver was dissected and assessed using histology and CT imaging using the beam line at the SPring-8 synchrotron radiation research facility in Japan. To address the underlying the epigenetic mechanisms, we performed a microarray experiment using formalin-fixed paraffin-embedded (FFPE) liver tissue. The target genes of microRNAs were predicted by TargetScan.

Results: Compared with C/C offspring, H/C offspring showed lower FBS (74.00±16.95 *vs*. 52.86±8.71 mg/dL, $p<0.05$), HbA1c (3.57±0.44 *vs*. 2.94±0.34 %, $p<0.05$) despite of more food intake (2.33±0.97 *vs*. 3.45 ±0.54 g/day, $p<0.05$). Body weight (21.98±0.90 *vs*. 22.55±1.82 g) and SBP (100±11.54 *vs*. 98.19±7.7 mmHg) were not different between both groups. Remarkably, plasma NEFA (608.0±66.61 *vs*. 911.0±242.4 microEq/L, $p<0.01$) and ALT (17.00±4.2 *vs*. 27.71±5.95 IU/L, $p<0.01$) were elevated in H/C. Consistent with metabolic changes, H/C had severe form of NASH, presenting the fibrosis evaluated by masson trichrome stain. Synchrotron micro-CT imaging at SPring-8 showed the architectural remodeling of the parenchyma in 6 out of 7 the H/C, not in the C/C. Intriguingly, melanocortin 4 receptor (MC4R), which plays a pivotal role in regulating the energy homeostasis, was over-expressed in the cytoplasm of hepatocellular carcinoma cells of H/C. Microarray analysis revealed that 140 of 1,900 miRNAs analyzed were differentially expressed in FFPE liver samples of H/C in comparison to C/C, i.e. 27 up-regulated and 120 down-regulated miRNAs. Of those miRNAs, miR-29c-3p, which targets MC4R, was down-regulated (ratio 0.52) in H/C.

Conclusion: This study suggests that the early exposure to an overnutrition in utero ("first-hit"), and subsequent neonatal nutritional alternation ("second hit"), which results in dynamic metabolic changes, could develop NASH in adult offspring. In addition, the epigenetic mechanism by miRNA which targets MC4R might be involved in NASH-based hepatocaricinogenesis. These results suggest that the intervention in the maternal nutrition should be important for preventing the future NASH/hepatocellular carcinoma in the next generation.

Supported by: The Akiyama Life Science Foundation

Disclosure: **Y. Takiyama:** Grants; The Akiyama Life Science Foundation.

1256

Hepatocyte-specific sirtuin 6 deletion predisposes to nonalcoholic steatohepatitis by upregulation of Bach1, an Nrf2 repressor

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Background and aims: Nonalcoholic steatohepatitis (NASH), ranging from liver steatosis, inflammation, and fibrosis is associated with insulin resistance and is a worldwide epidemic. Sirtuin 6 (Sirt6), a chromatin associated deacetylase has been implicated in negative regulation of inflammation and lipid metabolism, although its function in the progression from NASH to fibrosis remains to be defined.

Materials and methods: To explore the role of hepatocyte Sirt6 in diet-induced insulin resistance and NASH development, we generated hepatocyte-specific Sirt6 knockout (KO) mice that were fed a high-fat and high-fructose (HFHF) diet for 16 weeks.

Results: HFHF-fed KO mice had increased hepatic steatosis and inflammation and aggravated glucose intolerance and insulin resistance compared to wild type. Notably, HFHF-induced liver fibrosis and oxidative stress, as determined by Sirius-red staining, dihydroethidium fluorescence, and related gene expression analyses, were significantly elevated in KO mice. In the livers of KO mice, nuclear factor erythroid 2-related factor 2 (Nrf2) was downregulated and conversely BTB and CNC homolog 1 (Bach1), a nuclear repressor of Nrf2, were upregulated. We discovered that Sirt6, which interacts with Bach1 under basal condition, induces its detachment from the antioxidant response element (ARE) region of heme oxygenase 1 promoter. Furthermore, we found that Sirt6 promotes Nrf2 binding to ARE in response to oxidative stimuli, which leads to the expression of phase II/antioxidant enzymes. We also showed that HFHF-induced steatosis, inflammation, and fibrosis were ameliorated by adenoviral Sirt6 overexpression.

Conclusion: Sirt6 may be a useful therapeutic target for amelioration of NASH and liver fibrosis.

Supported by: 2017R1A2B2005730

Disclosure: **B. Park:** None.

PS 115 Diabetes and cognitive dysfunction

1257

Neuroprotective effects of HM15211, a novel long-acting GLP-1/Glucagon/GIP tri-agonist in the MPTP Parkinson's disease mouse model

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Background and aims: HM15211 is a novel long-acting GLP-1/glucagon/GIP tri-agonist that is being developed for the treatment of obesity and related complications. Recent studies have shown that obesity, type 2 diabetes, and non-alcoholic fatty liver disease increase the risk of developing progressive neurodegenerative disease such as Parkinson's disease (PD) and Alzheimer's disease (AD). The dysregulated metabolic pathways are shared in the metabolic syndrome (MetS). To date, no disease modifying drug has been developed for PD or AD. Therefore, it is hypothesized that a treatment improving MetS may be useful for PD and AD patients. It was reported that both GLP-1 and GIP analogs show neuroprotective properties in PD and AD mouse models. In this study, we demonstrated the neuroprotective effect of HM15211 treatment in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced PD mouse model. MPTP is a neurotoxin, which can selectively destroy nigrostriatal dopaminergic neurons and cause parkinsonism in humans, nonhuman primates and mice, therefore it is used to induce PD in mice.
Materials and methods: MPTP 30 mg/kg was intraperitoneally injected once-daily for 7 days. HM15211 (2.5 nmol/kg, 5 nmol/kg) was subcutaneously administrated once at the first day and liraglutide (30 nmol/kg), as comparative control, was subcutaneously treated once-daily for 7 days. HM15211 and liraglutide were dosed 30 min after the MPTP administration. For motor function evaluation, the traction test, pole test and rotarod test were conducted before sacrifice. To assess the histological changes, hemi brain of all mice were sectioned with cryotome and stained. And for the molecular changes, striata were dissected from the other hemi brain and lysed with RIPA buffer and assayed with ELISAs.
Results: HM15211 significantly improved the MPTP induced motor impairments in three behavior tests in a dose-dependent manner. Histologically, the tyrosine hydroxylase positive neurons in substantia nigra and the staining density in striatum were reduced by MPTP. However, they were protected by HM15211 and liraglutide. In addition, HM15211 showed an anti-inflammatory effect over MPTP and decreased lipid peroxidation in the MPTP PD model. These neuroprotective effects of HM15211 were similar to the liraglutide-treated groups while only a single injection of HM15211 was used.
Conclusion: Based on these results, the novel long-acting GLP-1/glucagon/GIP tri-agonist HM15211 could have therapeutic potential for PD.
Disclosure: **Y. Lee:** None.

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Alterations of brain energy metabolism in insulin-resistant Goto-Kakizaki rats measured in vivo by ^{13}C magnetic resonance spectroscopy

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Background and aims: Type 2 diabetes is associated with deterioration of brain structure and function, leading to cognitive impairments. We hypothesized that diabetes induces a reorganization of the brain metabolic networks that support brain function. In this work, we investigated alterations of neuronal and glial energy metabolism in the living brain of a model of type 2 diabetes, the insulin-resistant Goto-Kakizaki (GK) rat.
Materials and methods: ^{13}C magnetic resonance spectroscopy *in vivo* at 14.1 T was used to detect labeling incorporation in to aliphatic carbons of glutamate, glutamine and aspartate in the brain of GK (n=7) and control Wistar (n=13) rats during [1,6-^{13}C]glucose administration. Labeling of brain glucose and amino acids over time was analyzed with a two-compartment mathematical model of brain energy metabolism to determine the rates of metabolic pathways in neurons and glia.
Results: Insulin resistance in GK rats caused mitochondrial oxidation rate to be reduced in neurons (-9±6%, P=0.002) but increased in astrocytes (+90±45%, P<0.001), when compared to the fluxes in the brain of control Wistar rats. Additionally, GK rats displayed lower rates of brain glutamine synthesis (-24±8%, P<0.001) and glutamate-glutamine cycle (-32 ±11%, P<0.001) than controls. Rates of global brain glucose transport and consumption were similar in GK and control rats.
Conclusion: Insulin resistance alters brain energy metabolism and impairs the glutamate-glutamine cycle between neurons and astrocytes, in line with diabetes-induced neurodegeneration and astrogliosis underlying brain dysfunction.
Supported by: Swiss National Science Foundation
Disclosure: **J.M.N. Duarte:** None.

1259

GLUT1 and VEGF are highly heritable, regulated by fat intake and affect memory performance

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Background and aims: VEGF is required to restore and maintain brain glucose uptake across the blood brain barrier via GLUT1, which was shown to be acutely diminished in response to a high fat diet (HFD) in mice. We aimed to investigate the genetic and HFD-related regulation and association of GLUT1 and VEGF in humans in our NUtriGenomic Analysis in Twins (NUGAT) study.
Materials and methods: 92 healthy and non-obese twins were included. After they were standardized to a high-carbohydrate low-fat diet for 6 weeks they switched to a 6-week HFD under isocaloric conditions. Three clinical investigation days were conducted, after 6 weeks of low-fat diet (LF6) and after 1 and 6 weeks of HFD (HF1 and HF6). Serum VEGF and other cytokine levels were measured using ELISA. Gene expression in subcutaneous adipose tissue (SAT) was assessed by quantitative Real-Time PCR. Genotyping was performed on HumanOmniExpressExome BeadChips. Auditory verbal learning task was conducted to measure cognitive performance.
Results: In response to the HFD serum VEGF levels increased significantly (P=0.002) while GLUT1 (*SLC2A1*) mRNA expression in adipose tissue significantly decreased (P=0.001). Both, serum VEGF and GLUT1 gene expression showed very high heritability (>90%) and correlated negatively (HF1 ρ=-0.428, P=5.4x10^{-5}). The rs9472159 polymorphism explained up to 39% of the variation in circulating VEGF concentrations (P=1.4x10^{-11}). Homozygous carriers (AA-genotypes) showed significantly reduced serum VEGF levels (HF6 P=6.4x10^{-11}) but higher GLUT1 expression in SAT (HF6 P=0.009) compared to CC or CA-genotypes. AA-genotypes showed impaired consolidated memory scores after 6 weeks of HFD compared to CC and CA-genotypes (P=0.001).

Conclusion: The environmental regulation of GLUT1 expression and serum VEGF by high fat diet is inversely correlated and both factors show very strong heritability. The rs9472159 polymorphism affected both VEGF and GLUT1 levels and homozygous carriers showed declined memory performance in response to the HFD. High fat diet induces a genetically determined and correlated decrease of GLUT1 and increase of VEGF which may affect memory performance.
Clinical Trial Registration Number: NCT01631123
Supported by: BMBF
Disclosure: **R. Schüler:** None.

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Apolipoprotein E ε4 affects cognition, but not brain structure and functioning in patients with longstanding type 1 diabetes

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Background and aims: Apolipoprotein E (APOE) ε4 is a genetic risk factor for cognitive decline, brain alterations, and dementia, especially in older age. In type 1 diabetes (T1DM), the DCCT/EDIC study showed no effect of APOE ε4 on cognitive decline, whereas a cross-sectional study found a negative association between APOE ε4 and decrements in cognition, but only in T1DM women. Brain structure or functioning have not been examined in relation to APOE ε4 in T1DM.
Materials and methods: We therefore determined the APOE genotype in 104 T1DM patients and 51 controls. Interaction terms were used to determine the differential effect of APOE ε4 genotype in patients and controls for cognition, cortical thickness, default mode network (DMN) functional connectivity, and white matter integrity. Analyses were corrected for age, sex, and systolic blood pressure.
Results: Genotyping was not possible in 3 patients due to insufficient material. The prevalence of APOE ε4 was similar between patients (n=33; 32.7%) and controls (n=15; 31.4%; $P>0.05$). Corrected for confounding, there were significant interactions between APOE ε4 and group for general cognitive ability (GCA), information processing speed (IPS), attention (ATT), cortical thickness and DMN functional connectivity (all $P<0.05$), but not for white matter integrity ($P>0.05$). Performance on these cognitive domains was lower in T1DM patients with APOE ε4 compared to without (GCA: β=-0.267; IPS: β=-0.264; ATT: β=-0.218; all $P<0.03$), without differences in controls. Contrary, in controls but not T1DM, right superior frontal and temporal cortical thickness and left lateral occipital DMN functional connectivity were lower in APOE ε4 carriers relative to non-carriers (all $P<0.05$).
Conclusion: To conclude, in T1DM APOE ε4 affected cognition, but not brain structure and functioning, which is similar to results found in type 2 diabetes. In controls, brain structure and functioning, but not cognition, were affected by APOE ε4, suggesting a distinct difference in the effect of APOE ε4 on the brain in T1DM and controls, with behavioural performance being most vulnerable to the effects APOE ε4 in patients.
Supported by: DDRF / EFSD/Lilly
Disclosure: **E. van Duinkerken:** None.

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The interaction with insulin receptor substrate 1 and cognition for patients with type 2 diabetes: in vivo evidence from gene association analysis

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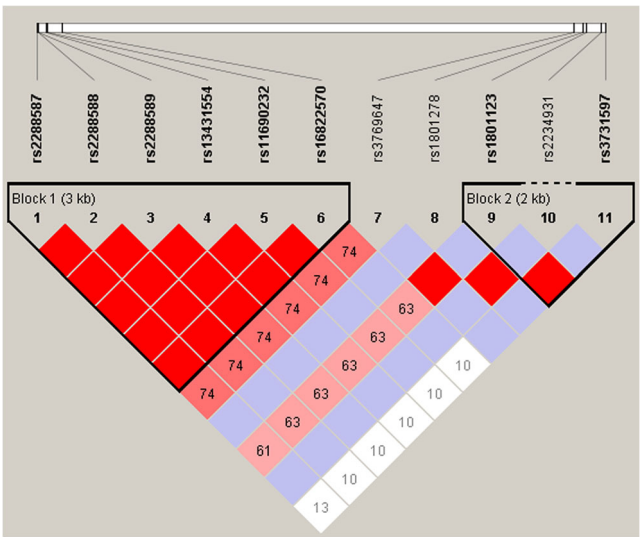
Background and aims: The dysregulations of insulin receptor substrate 1 (IRS-1) have been demonstrated to play vital roles in the interactions with brain insulin resistance and Alzheimer's disease by series of cellular and molecular investigations. However, little is known concerning its in vivo neurobiology underlying cognitive impairment. The present study aimed to clarify the associations between IRS-1 and brain functional network via genetic-imaging approach.
Materials and methods: 80 subjects with mild cognitive impairment (MCI) and 80 healthy controls (HCs) were employed. Every participants are patients with type 2 diabetes, and they went through the evaluation of cognition, scan of functional magnetic resonance imaging (MRI) and sequencing of the whole exome of IRS-1 gene. Haplotypes of IRS-1 were constructed to evaluate the integrations of multi-single nucleotide polymorphisms (SNPs). We explored the cognitive significance of IRS-1 haplotypes and corresponding influences on brain functional network.
Results: Two blocks of IRS-1 were detected. None of the haplotypes showed significantly different distributions between HCs and MCIs ($P > 0.05$), but the cognitive significance of block 1 was highlighted by the haplotype-based analyses (β = 0.39, raw P = 0.001, corrected P = 0.015). The further genetic-imaging analysis was performed to explore the effects of block 1 on brain default mode network. Regions related to the interactive effects of block 1 and disease statues were in frontal and temporal cortex. The behavioral relevance of the network was further indicated, as the functional connectivity within superior temporal cortex partially determine the cognitive performance for MCIs (β = 0.54, P = 0.005) .
Conclusion: The haplotype-based genetic-imaging analysis expanded our understanding for the mechanisms underlying the diabetes-related cognitive impairments, and relieving the dysfunctions of IRS-1 may be an effective method to alleviate the injuries of cognition related to both Alzheimer's disease and type 2 diabetes.



Disclosure: **J. Huang:** None.

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Serum ghrelin levels in elderly, diabetic patients with mild cognitive impairment

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Background and aims: Recent evidence has indicated that type 2 diabetes mellitus (T2DM) in the elderly is a risk factor for mild cognitive impairment (MCI) or dementia. The etiology of cognitive impairment in diabetes is unknown, but probably associated with many factors. One of the recent hypotheses suggests that ghrelin could be involved in cognitive impairment in diabetic patients. It has been reported that ghrelin signaling occurs in the hippocampus and improves memory and spatial learning. The aim of the study was to evaluate serum levels of ghrelin in elderly, diabetic patients with and without MCI and to determine the predictors (including ghrelin levels) of having MCI in elderly patients with T2DM.
Materials and methods: A survey was conduct among 276 elderly subjects with T2DM. 87 patients with MCI and 189 controls were selected according to the criteria proposed by the MCI Working Group of the European Consortium on Alzheimer's Disease (using the Montreal Cognitive Assessment: MoCA score). Data of biochemical parameters and biomarkers were collected. The serum levels of ghrelin were assessed using ELISA kit. The approval was obtained from the independent Local Ethics Committee.
Results: Serum levels of ghrelin were significantly lower in patients with MCI (239.03 ± 47.09 pg/ml) compared to controls (297.83 ± 39.18 pg/ml, p<0.001). In group of subjects with MCI serum ghrelin levels were positively correlated with MoCA score (r=0.555; p<0.001) and negatively correlated with HbA1c (r=-0.318; p<0.001) and BMI (r=-0.395, p<0.001). The univariate logistic regression models revealed that variables which increased the likelihood of diagnosis of MCI in elderly patients with T2DM were: lower levels of ghrelin and HDL cholesterol, higher levels of HbA1c, triglicerydes, previous cardiovascular disease (CVD), hypertension (HA) or use of antihypertensive drugs, hiperlipidaemia, retinopathy, nephropathy, increased number of co-morbidities, older age and less years of formal education. Lower levels of ghrelin (OR 0.97, 95% CI: 0.95-0.98, p<0.001), higher levels of HbA1c (OR 2.42, 95% CI: 1.17-5.01, P-0.017), HA or use of anti-HA drugs (OR 6.26, 95% CI: 1.28-30.7, p=0.024), previous CVD (OR 8.76, 95% CI: 3.42-22.4, p<0.001), increased number of co-morbidities (OR 1.23, 95% CI: 1.05-1.44, P-0.01), and less years of formal education (OR 0.62, 95% CI: 0.48-0.8, P<0.001) are the factors increasing the likelihood of having MCI in elderly patients with T2DM in multivariable analysis.
Conclusion: In summary, serum levels of ghrelin were decreased in MCI elderly diabetic patients compared to controls and associated with poor MoCA score. The results indicated that lower ghrelin levels may be a risk factor for a cognitive impairment in diabetic, elderly patients. Further prospective larger studies are needed to confirm the role of this marker in the progression to dementia.
Disclosure: **M. Gorska-Ciebiada:** None.

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Neuropsychological scores are correlated with the retinal sensitivity in type 2 diabetic patients with mild cognitive impairment and Alzheimer´s disease

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Background and aims: Epidemiological studies have demonstrated that type 2 diabetic (T2D) patients have a significantly higher risk of developing mild cognitive impairment (MCI) and Alzheimer's disease (AD) in comparison with age-matched non-diabetic subjects. The annual conversion rate from MCI to dementia ranges between 10-30%. The diagnosis of MCI is based on complex neuropsychological tests which makes unfeasible their incorporation in the daily practice. The retina is ontogenically a brain-derived tissue and it has been suggested that it may provide an easily accessible and non-invasive way of examining the pathology of the brain. Therefore, it seems reasonable to propose that the evaluation of retinal parameters related to neurodegeneration such as retinal function would be useful for identifying those T2D patients at a higher risk of developing AD. Fundus-driven microperimetry has emerged as a simple, non-invasive and rapid test that can be used in the clinical practice to evaluate the retinal function.On these bases, the aim of the present study was to determine whether retinal sensitivity assessed by microperimetry correlates with the neuropsychological tests in T2D patients MCI and AD.
Materials and methods: We have designed a prospective nested case-control study including 35 T2D patients with MCI matched by age (±4 years), diabetes duration (±4 years) and classic cardiovascular risk factors with 35 T2D patients with AD. All patients were functionally literate, without clinical evidence of diabetic retinopathy and cerebrovascular disease. Mini-mental state evaluation test (MMSE) and The Alzheimer's disease assessment scale (ADAS-cog) were performed for the neuropsychological evaluation. Retinal function was assessed by standard microperimetry (MAIA microperimeter 3rd generation).
Results: No differences were found between the two groups regarding the HbA1c levels, BMI, gender, family history of AD, and the APOEe4 allele genotype. Retinal sensitivity was lower in the T2D patients with AD in comparison with the MCI group (17.11±6.3dB versus 21.68±4,06, p<0.001). We found a significant correlation between retinal sensitivity and the MMSE and ADAS-cog tests (r=0,427, p<0,001 and r= -0.523, p<0,001 respectively).
Conclusion: Our findings suggest that retinal sensitivity may be a useful biomarker for cognitive changes in T2D patients, strongly correlated with actual neuropsychological scales used in the clinical practice. Retinal microperimetry is an effective, rapid and reliable tool for discriminating patients with AD from those with MCI.
Supported by: EFSD/Lilly
Disclosure: **A. Ciudin:** None.

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Metformin therapy improves cognitive function in women with Polycystic Ovary Syndrome (PCOS)

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Background and aims: Data is limited on the status of cognitive function in women with PCOS and whether or not this is affected by treatment with metformin. Therefore, we decided to investigate prospectively cognitive function in women with PCOS at diagnosis and after the administration of metformin.
Materials and methods: Sixty eight (68) women with PCOS and 27 healthy women serving as control subjects (mean ±SD: age 26.8±5.7 vs 29.3 ± 4.6, p=0.038; BMI 31.2 ±7.0 vs 27.9 ± 5.2, p=0.022; educational status p=0.568 respectively) underwent cognitive function testing using the Montreal Cognitive Assessment test (MoCA). MoCA test was done in PCOS patients at diagnosis (baseline) and repeated 3 months after treatment with metformin. In the control group, MoCA tests were also done on two occasions, 3 months apart to ensure lack of familiarity of the test. All MoCA tests were conducted by a trained nurse supervised by a neuropsychologist. Fifteen (15) patients were excluded (3 incomplete data, 2

intolerance to metformin, 1 undiagnosed diabetes, 9 successful pregnancy) leaving 53 patients with valid data for analysis.
Results: MoCA test score was found to be significantly lower in the patients with PCOS compared to the control subjects (23.7±3.2; 25.6 ±2.4, p=0.001). MoCA score however, improved significantly from baseline with metformin treatment (23.7±3.2, 25.9±2.4; p=0.0001) to levels comparable to those observed in the control group (25.6±2.3; 25.2±2.4, p=0.569). MoCA score did not change over time in the control group (25.8±2.6, 25.1±2.4, p=0.236).
Conclusion: Women with PCOS demonstrate impaired cognitive function at diagnosis but this is restored to normal level after 3-months treatment with metformin. Metformin may therefore have beneficial effect on cognitive function in women with PCOS, in addition to its well established metabolic effects.
Disclosure: **A. Alzaid:** None.

1265

Increased plasma homocysteine level is associated with executive dysfunction in type 2 diabetic patients with mild cognitive impairment

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Background and aims: Homocysteine (Hcy) is involved in the pathogenesis of type 2 diabetes mellitus (T2DM) and Alzheimer's disease. We aimed to investigate the role of Hcy in T2DM patients with mild cognitive impairment (MCI), and to determine whether methylene tetrahydrofolate reductase (MTHFR) C677T or cystathionine beta-synthase (CBS) 844ins68 polymorphism is related to T2DM-associated MCI.
Materials and methods: We recruited 285 T2DM patients and divided them into two groups, 140 patients with MCI and 145 healthy-cognition controls, on the basis of Montreal Cognitive Assessment (MoCA) scores. Demographic characteristics, clinical parameters and neuropsychological tests were assessed. MTHFR C677T and CBS 844ins68 polymorphisms were also analyzed.
Results: The MCI group exhibited a significantly higher plasma total Hcy (tHcy) level than the control group ($p < 0.001$). After adjusting some confounding factors, plasma tHcy level was negatively correlated with MoCA scores ($p = 0.002$), but positively associated with Trail Making Test A and B scores ($p = 0.044$; $p = 0.005$, respectively), which evaluated executive function. Multivariable logistic regression model showed that high tHcy level was an independent factor for MCI in T2DM patients. No significant difference was observed in the genotype or allele distributions of MTHFR and CBS between MCI and control groups. Increased MCI risk was found in MTHFR T allele compared with C allele, and in CBS I allele compared with D allele (OR = 1.361, $p = 0.067$; OR = 1.048, $p = 0.909$, respectively).
Conclusion: Increased plasma tHcy level was significantly related to T2DM-associated MCI, especially executive dysfunction. Further investigation with a large population size should be conducted to confirm these observed findings.

Table 1 Assessment results of the risk of having MCI in a multivariable logistic regression model in T2DM patients.

Variables analyzed	β	SE of β	P	OR	95%CI
Education Levels (years)	-0.062	0.078	0.426	0.939	0.806-1.095
Diabetes duration (years)	0.002	0.040	0.952	1.002	0.927-1.084
HbA1c (%)*	-0.571	0.112	<0.001*	0.565	0.453-0.704
FCP(nmol/L)	-0.353	0.204	0.083	0.703	0.471-1.048
HOMA-IR	1.394	0.988	0.158	4.033	0.582-27.945
tHcy (nmol/L)*	1.207	0.174	<0.001*	3.343	2.379-4.698
Vitamin B12 (pmol/L)*	0.042	0.013	0.001*	1.043	1.016-1.071
Folate (nmol/L)*	-0.439	0.142	0.002*	0.644	0.488-0.851

*Significance, $p < 0.05$. Abbreviations: β, regression coefficient; SE, standard error; OR, odds ratio; CI, confidence interval for odds ratio; MCI, mild cognitive impairment; HbAlc, glycosylated hemoglobin; FCP, Fasting c-peptide; HOMA-IR, the homeostasis model assessment of insulin resistance; tHcy, total homocysteine.

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Disclosure: **S. Tian:** None.

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High fat diet-induced mild insulin resistance accelerates memory dysfunction via Tau phosphorylation in an Alzheimer mouse model

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Background and aims: Type 2 diabetes mellitus (T2DM) and Alzheimer's disease (AD) are both more prevalent with aging, but it has generally been assumed that this is coincidental, not a reflection of comorbidity. However, evidence suggests that patients with T2DM that were hyperinsulinemic or insulin resistant as hallmarks of T2DM are at an increased risk of getting AD. In the present study, we investigate the effect of high-fat diet (HFD)-induced mild insulin resistance on memory function in a mouse model of genetically induced AD-like neuropathology (3xTg-AD).
Materials and methods: To induce mild insulin resistance in 3xTg-AD mice, mice received a HFD (60% kcal fat) for only a 8-week period starting at the age of 4 months. Food intake and body weight gain were followed up every 4 days. After 8-week HFD, we investigated cognitive dysfunction with water maze test. Cerebral amyloid-beta and Tau phosphorylation levels were determined by Western blotting. Plasma 50 cytokines were analyzed using a cytokine antibody array.
Results: HFD enhanced memory impairment and Tau phosphorylation levels compared to the normal diet (ND) in 3xTg-AD mice. However, the expression of cerebral amyloid-beta did not differ between ND and HFD in 3xTg-AD mice. 3xTg-AD mice with HFD showed significantly elevated plasma levels of L-selection, sTNF RII, IGFBP-2, MMP-3, resistin and osteopontin compared with 3xTg-AD mice with ND.
Conclusion: aken together, these findings suggest that HFD-induced mild obesity and insulin resistance may play an important role in the development of Alzheimer's disease via Tau phosphorylation. In addition, elevated cytokines in 3xTg-AD mice with HFD might be correlated with the development of Alzheimer's disease or may prove to be useful biomarkers in Alzheimer's disease.
Disclosure: **J. Park:** None.

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