Goharian, T. S., Andersen, L. B., Franks, P. W., Wareham, N. J., Brage, S., Veidebaum, T. ... Grøntved, A. (2014). Examining the causal association of fasting glucose with blood pressure in healthy children and adolescents: a Mendelian randomization study employing common genetic variants of fasting glucose. *Journal of Human Hypertension, 29*, 179-184.

Title

Examining the causal association of fasting glucose with blood pressure in healthy children and adolescents: a Mendelian randomization study employing common genetic variants of fasting glucose

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Sources of funding

This study obtained funding from the following sources; The Danish Heart Foundation, The Danish Medical Research Council Health Foundation, The Danish Council for Sports Research, The Foundation in Memory of Asta Florida Bolding Renée Andersen, The Faculty of Health Sciences, University of Southern Denmark, The Danish Independent Research Council, The Estonian Science, and The UK Medical Research Council. NJW, UE, SB and RJFL work in a UK Medical Research Council funded unit and DAL works in a Centre that receives funds from the UK Medical Research Council (G0600705) and University of Bristol. The views expressed in this paper are those of the authors and not necessarily any funding body. None of the funding bodies influenced data collection, analysis or interpretation of results.

Abstract

OBJECTIVES

To determine whether genetically raised fasting glucose (FG) level is associated with elevated blood pressure (BP) in healthy children and adolescents.

DESIGN AND METHODS

We used 14 common genetic variants of fasting glucose discovered in genome-wide association studies, including the rs560887 SNP located in the *G6PC2* locus found to be robustly associated with FG in children and adolescents, as an instrument to associate FG with resting BP in 1,642 children and adolescents from the European Youth Heart Study (EYHS).

RESULTS

Rs560887 was associated with increased FG levels corresponding to an increase of 0.09 mmol/l ($P = 4.0 \times 10^{-10}$). FG was associated with elevated BP, independent of other important determinants of BP in conventional multivariable analysis [systolic BP z-score: 0.32 SD per increase in mmol/l (95% CI 0.20-0.43, $P = 5.4 \times 10^{-8}$), diastolic BP z-score: 0.14 per increase in mmol/l (95% CI 0.07-0.22, $P = 3.5 \times 10^{-4}$)]. This association was not supported by the Mendelian randomization approach, either from instrumenting FG from all 14 variants nor from the rs560887, where non-significant associations of glucose with BP were observed.

CONCLUSIONS

The results of this study could not support a causal association between FG and BP in healthy children and adolescents; however it is possible that rs560887 has pleiotropic effects on unknown factors with a BP lowering effect or that these results were due to lack of statistical power.

KEYWORDS

Glucose, hyperglycemia, blood pressure, hypertension, Mendelian randomization, children.

Introduction

Observational studies in children and adolescents have revealed a positive association between fasting glucose (FG) and blood pressure (BP) [1, 2]. Ten percent of adolescents with type 2 diabetes participating in the TODAY trial had hypertension, and the incidence during the average 3.9 years of follow-up was 22 percent [3]. Nevertheless, treatment with hypoglycemic agents could not lower the incidence of hypertension, and glycemic control was unrelated to the incidence of hypertension in this cohort of adolescents with type 2 diabetes [4]. In adults higher FG has been found to be associated with subsequent elevated BP in several prospective cohort studies [5-7]. However, some prospective studies do not support this association [8, 9] and as impaired FG and raised BP share common environmental and biological risk factors, residual and unknown confounding may explain the observed associations raising the possibility that hyperglycemia is not causally related to high BP.

Some evidence from randomized controlled trials among type 1 diabetics and individuals with impaired glucose metabolism support a causal role for glucose homeostasis in risk of elevated BP. One trial has shown that intensive insulin therapy reduces the long-term risk of hypertension in patients with type 1 diabetes [10] and a second that glucose-lowering therapy in patients with impaired glucose tolerance is effective in reducing the risk of hypertension [11]. Because hypoglycemic agents also target pathways extrinsic to that of glucose homeostasis, findings from these trials could also be explained by other biological factors. Further evidence regarding whether this association is causal would be valuable since it would point to potential interventions for reducing raised FG levels in those at risk of, as well as with, diabetes in order to prevent hypertension.

Genome-wide association studies have identified several common genomic variants associated with fasting glucose (FG) [12]. These include a variant (rs560887) located within the

third intron of the *G6PC2* locus. *G6PC2* is particularly expressed in pancreatic beta cells. *G6PC2* is thought to hydrolyze glucose-6-phosphate (G6P) to glucose and inorganic phosphate opposing the action of glucokinase (a glucose sensor in beta cells) which facilitates phosphorylation of glucose to G6P in the first step of glycolysis [13]. The risk allele of rs560887 is believed to increase the expression or functional activity of *G6PC2* [14]. As a consequence the flux of glucose into the glycolytic pathway is reduced and this may lead to a higher glucostatic set-point of the beta cell, possibly leading to a higher FG level as revealed in a recent report from the European Youth Heart Study (EYHS) [15]. The effect of this variant has been replicated and found to be robust in children and youth, [15, 16] as well as adults [14, 17, 18]. Importantly, there is currently no evidence showing that rs560887 has an effect on insulin levels, insulin sensitivity, or other biological parameters [12, 14-16], and with no obvious direct role of *G6PC2* in a BP regulating pathway [12], this variant is an attractive instrument variable for FG. We therefore aimed to examine the association of FG with BP using conventional multivariable adjusted linear regression and also to examine the causal effect via a Mendelian randomization approach [19] by utilizing the common variant rs560887 in the *G6PC2* gene and other confirmed FG variants from GWAS as instrumental variables (IV) for FG. We studied this in a population sample of healthy children and adolescent from the EYHS.

Methods

Study population

Data from the EYHS, a multi-country longitudinal population-based study in children, were used in this study. Details of the design and data collection for EYHS have been previously published [20]. DNA, relevant risk factors and outcome data were available from Estonian (city and county of Tartu) and Danish (city of Odense) participants. A proportional two-stage cluster sample of boys and girls aged 9-11 and 14–16 years were selected. In total, 2,025 children and adolescents agreed to participate, with a similar proportion participating in each country (76% accepted in Estonia and 75% accepted in Denmark). A total of 1,660 had DNA samples available and full data on all variables. Additionally 15 individuals were excluded due to glucose levels ≥ 7mmol/l (n = 3) or not fasting overnight (n =15) leaving a total of 1,642 individuals.

Anthropometry and maturity

Weight, height and waist circumference (WC) were measured while the participants were wearing light clothing, without shoes, using standard techniques [20]. Body-mass index (BMI) was calculated as weight (kg)/height^2 (m^2). Waist circumference was measured with a metal anthropometric tape midway between the lower rib margin and the iliac crest at the end of gentle expiration. Pubertal status was assessed according to Tanner [21]. Assessment in girls was performed according to breast development and in boys according to pubic hair growth. Maturity stage among the 8-10-year olds was almost exclusively stage 1 or 2 in both Danish and Estonian children and almost all adolescents were categorized as Tanner stage 3, 4 or 5. Thus, we collapsed maturity to a 3-point ordinal variable (Tanner 1-2, Tanner 3-4, and Tanner 5).

Fasting glucose, insulin and lipids

Intravenous blood samples were drawn from an antecubital vein after participants had fasted for at least 8 hours. These were separated and stored at -80°C until analysis. Blood samples were analyzed by one of two Clinical Pathology Accreditation (CPA) accredited laboratory located in Bristol or Cambridge, England. Glucose, high-density lipoprotein (HDL), total cholesterol (TC) and triglyceride (TG) concentrations were measured by standard methods using Olympus AU600 random access analyzers. Insulin was analyzed using an enzyme immunoassay (microtiter plate format; Dako Diagnostics, Ely, U.K.). Between- laboratories correlations were 0.94–0.98 for 30 randomly selected samples analyzed in both Bristol and Cambridge.

Genotyping

The selected 16 variants (loci) associated with FG were identified from published meta-analyses [12, 16]: rs4607517 (*GCK)*, rs340874 (*PROX1*), rs11920090 (*SLC2A2*), rs11605924 (*CRY2*), rs560887 (G6PC2), rs780094 (*GCKR)*, rs10885122 (*ADRA2A*), rs10830963 (*MTNR1B*), rs2191349 (*DGKB-TMEM195*), rs7944584 (*MADD*), rs7034200 (*GLIS3*), rs13266634 (*SLC30A8*), rs11708067 (*ADCY5*), rs174550 (*FADS1*), rs11071657 (*C2CD4B*), rs7903146 (*TCF7L2*). All of the SNPs were genotyped at the Medical Research Centre Epidemiology Unit Research Laboratory in Cambridge, England, using the TaqMan SNP Genotyping Assays (Applied Biosystems, Warrington, U.K.). The genotyping assay was undertaken on 10 ng of genomic DNA in a 5 μl 384-well TaqMan assay using a PTC-225 Thermal Cycler (MJ Research, Watertown, MA). The ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Warrington, U.K.) was used for end point detection and allele calling. The call rate was >98.9% and concordance of 44 duplicate samples was 100%.

Blood pressure

BP was measured with participants in the up-right sitting position after resting for five minutes. Five measurements were conducted with two minutes intervals between each (Dinamap model XL, Kivex/Critikron, Inc., Tampa, FL). The mean of the last three measurements was used in all analyses. The Dinamap monitor has been validated in children against direct radial artery readings (mean error 0.24 mmHg SBP, 1.28 mmHg DBP) [22]. BP measurements were converted to age-, height- and sex-specific z-scores according to National High Blood Pressure Education Program (NHBPEP) Working Group on Children and Adolescents [23].

Other covariates

Parental educational level, birth weight, and infant breastfeeding status were obtained through a parental questionnaire. Breastfeeding status was, as previously described [24], expressed in a variable with two categories based on the mother's response: i) children who were ever exclusively breastfed and ii) those whose mothers indicated that they were never exclusively breastfed. Parental educational level was classified according to International Standard Classification of Education (ISCED) (UNESCO 1997). However, as the details obtained of the description of education was insufficient, the ISCED level 1 and 2 were collapsed to one group, level 3, 4 were grouped, and level 5, 6 and 7 were grouped as one in the analysis. We used the highest maternal- or paternal level of SES reported in the analysis.

Statistics

We used multivariable linear regression to first examine the association of FG with BP, adjusting for potential confounding by age, age group, gender, country, maturity, parental education, birth weight, breastfeeding, BMI, waist circumference, triglycerides, total cholesterol, HDL and insulin. We also assessed the size and precision of the coefficient of rs560887 (per allele) on BP changes

with additional adjustment for FG. We used linear regression to examine the association of individual instruments with FG, adjusting for age, age group, gender and country. We then undertook an instrumental variables regression to determine the causal association of FG with BP. The IV analysis was performed using a two-stage least squares regression and we compared results from the instrumental variable regression to that from the standard linear regression using the Durbin form of the Durbin-Wu-Hausman statistic [25]. To evaluate the strength and robustness of rs560887 and the GRS to instrument FG we calculated the first stage F-statistic from our instrumental variables analysis. An F-value>10 has been proposed as a cut point for evaluating sufficiently strong instruments in instrument variables regression [26]. We also examined whether rs560887 was associated with any of the observed confounders.

Because we studied two populations with possible different ancestry we explored the possibility for population stratification by conducting the rs560887-FG association separately in each of the two populations and comparing the point estimates before pooling the data in a single analysis. This was done by testing the rs560887-by-country interaction on glucose and evaluating the point estimates for each country. A p-value below 0.1 was considered indication of interaction. In all analyses the genetic model used was per allele. All statistical analyses were performed in STATA 11.0 (STATA Corporation, College Station, TX).

Genetic risk scores

The unweighted genetic risk score (GRS) was calculated by adding the number of risk alleles divided by the number of SNPs, assuming that each allele has an equal effect. The weighted genetic risk score (wGRS) was created by applying weights representing the effect size each allele carries on FG (extracted from the Barker et al. meta-analysis in healthy children and adolescents) [16]. Linear regression was performed, assuming an increase in risk with the accumulation of risk alleles,

adjusted for the possible confounders. The IV analysis was performed as described for rs560887. None of the SNPs were associated with insulin in this study, however other studies report an effect on insulin secretion by GCKR [12] and MTNR1B [27] respectively. MTNR1B may also have a protective effect on change in glucose concentrations [17]. Thus, excluding these two SNPs resulted in a significant increase of the F-value for both GRS and wGRS, Supplementary Table 1, leading us to proceed with genetic risk scores consisting of a total of 14 SNPs.

Results

General characteristics by age group and gender are shown in Table 1. The frequency of the minor allele (MAF) of rs560887 was 29.73% and 31.01% in Danish and Estonian samples, respectively. Table 2 shows the association of FG and other potential BP determinants with BP. FG was positively associated with BP in the basic model controlling for age, age group, gender and country. The association somewhat attenuated, but remained statistically significant after including all other determinants of BP. Fasting insulin, TC, triglyceride, BMI, waist circumference, and birth weight were significantly associated with either SBP or DBP in multivariable adjusted analyses including all potential determinants of BP (Table 2). Supplementary Table 2 details the association of potential confounding factors with rs560887 and supplementary Table 3 lists associations of potential confounding factors with FG. None of the confounders were associated with rs560887. By contrast, all of the confounding variables, with a few exceptions (HDL, birth weight and parental education) were associated with glucose.

Association between common genetic glucose variants and fasting glucose

We observed a strong association between the rs560887 and FG (increase in mmol/l per allele = 0.09, 95% CI 0.06 to 0.11, $P = 4.0 \times 10^{-10}$) adjusting for age, age group, gender and country. When adjusting for all confounders, there was a small increase in significance level ($P = 2.5 \times 10^{-11}$). The first stage f-statistics was 36.44 and rs560887 accounted for 2.2% of the variance in FG (unadjusted). The associations of individual variants with FG are listed in supplementary Table 4. There was no country specific association between rs560887 and FG (P = 0.26 for interaction). The increase in mmol/l FG per addition of risk allele was 0.07 (95% CI 0.03 to 0.11, $P = 3.3 \times 10^{-4}$) for Estonia and 0.10 (95% CI 0.06 to 0.14, $P = 2.2 \times 10^{-7}$) for Denmark, adjusting for age, age group

and gender. However, the instruments in Estonia were not strong (Supplementary Table 1), thus, sensitivity analyses were performed excluding Estonia in the IV regression analysis.

Mendelian randomization approach for the association between glucose and blood pressure

Rs560887 was negatively, and non-significantly associated with SBPz, decreasing SBPz by -0.05 SD (95% CI -0.11 to 0.02, P = 0.17) per allele, adjusted for age, age group, country and gender. A similar association was found with the genetic risk scores, decreasing SBPz with -0.01 SD per increase in risk alleles (95% CI -0.03 to 0.01, P = 0.36) and (95% CI -0.02 to 0.01, P = 0.26) for GRS and wGRS respectively. After adjusting for glucose, the effect on BP augmented and the P-value became significant for both rs560887 and wGRS, decreasing SBPz with -0.08 SD (per allele) (95% CI -0.15 to -0.02, P = 0.01) and -0.02 SD (95% CI -0.03 to -0.003, P = 0.02) respectively, but not for GRS, decreasing SBPz with -0.02 SD (95% CI -0.03 to 0.001, P = 0.07).

Table 3 shows the results from the IV analyses and compares these with the results from the conventional multivariable regression analyses. Despite the positive association from the conventional FG-BP multivariable regression the IV analysis using rs560887, GRS and wGRS showed a negative non-significant association with SBPz. The estimates from the IV approach with rs560887 and wGRS on SBPz were significantly different from the conventional multivariable analysis (P value for difference <0.05), unlike the GRS. When excluding Estonia, the association from the IV analysis using rs560887 was significantly inversely associated with SBPz (P = 0.04), Table 4. When analyzing Estonia alone no significant association was found (results not shown).

Discussion

We determined the association of FG and BP with the help of the rs560887 common allele in the *G6PC2* locus and a genetic risk score consisting of 14 SNPs, previously shown to be associated with FG in adults [12], children and adolescents [15, 16]. In a population-sample of European children and adolescents, FG in the non-diabetic range was positively associated with SBPz and DBPz in multivariable analyses adjusted for major lifestyle-, socio-demographic- and biological determinants of FG, suggesting the association was not a consequence of known confounders. In contrast, genetically elevated FG, either instrumented from rs560887 or all FG SNPs did not show an association to BP. In fact we observed evidence that associations differed between conventional analyses and the Mendelian randomization approach. Collectively, our results from the Mendelian randomization approach could not confirm that FG is causally related to BP among healthy children and adolescent of European descent.

Comparison with other studies

Our findings from the conventional analysis is in line with previous observational studies in children and adolescents supporting a positive association between FG and BP, and with studies comparing BP levels between diabetic and non-diabetic children and adolescents [1, 2, 28, 29]. Furthermore, the majority of previous observational studies among healthy adults suggest a positive association between FG and BP levels [5-7]. We are not aware of any studies that have reported associations of genetically elevated FG with BP levels or hypertension; however, other MR analyses describing associations of glucose levels and related outcomes have been reported. A recent study among Danish men and women found that genetically elevated non-fasting glucose levels was associated with an increased risk of coronary heart disease independent of hypertension and other biological CVD risk factors [30]. A study among US men and women found that a

genetic risk score based on five GWAS FG variants was associated with intima media thickness in the same direction as influencing FG, however, it was not reported whether BP explained this association. Although these studies suggest a causal effect of elevated glucose levels on development of atherosclerosis and coronary heart disease, further MR studies with BP or hypertension are warranted to further refute or support the notion that elevated glucose levels is causally related to raised BP levels.

Limitations with Mendelian randomization

Firstly, if the genetic determinants of FG have pleiotropic effects (exert effects on other factors that are related to BP regulation), our results could be biased. Thus, an alternative explanation for our observations could be that the rs560887 common allele increases FG levels as well as decreases BP. Because we did not find associations of rs560887 or the genetic risk score with any other major determinants of FG, we are fairly confident that this is not the case. Secondly, another limitation of Mendelian randomization approach is the use of weak instruments. The first stage F-statistics for rs560887 was high, confirming a strong instrument. Thirdly, population stratification could lead to spurious results. Sensitivity analyses confirmed that genetic effects of FG SNPs were fairly similar between countries and because both populations are of European descent and MAF was similar in both populations, the existence of population stratification is unlikely [19]. Lastly, our study could be insufficiently powered to determine valid results from the Mendelian randomization approach.

Strengths of the study

One of the strengths of this study is the use of a common variant robustly associated with FG. Further, no evidence of pleiotropy from our own investigations or other studies has been found. Another strength of this study is that the study population consists of individuals in the early stages

of life (healthy children and adolescents). Studies with an adult population may have a higher prevalence of preclinical diseases (due to accumulation of environmental and behavioral influences), potentially interfering with a possible causal association. However if the cumulative effect of common genetic glucose variants on FG adds up with time, there is a chance that we could detect effects of larger magnitude in an adult population, as suggested by Renstrom et al. [17].

Conclusion

Although conventional multivariable adjusted analyses showed that FG was positively associated with BP amongst healthy children and adolescents, this association could not be confirmed by the Mendelian randomization approach using either the common variant rs560887 in the *G6PC2* locus or several FG SNPs jointly as instruments. Because, our study was moderate in size we cannot exclude that our finding was due to statistical power reasons and additional studies among populations of children, youth, and adults are needed to further exclude this possibility.

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Table 1. Characteristics of the participants by age group and gender, n = 1,642

	Children (*n* = 1,001)		Adolescents (n = 641)	
	Boys (*n* = 477)	Girls (*n* = 524)	Boys (*n* = 291)	Girls (*n* = 350)
Age (years)	9.7 ± 0.4	9.6 ± 0.4	15.5 ± 0.5	15.4 ± 0.5
Height (cm)	139.0 ± 6.7	138.8 ± 6.8	174.4 ± 7.2	165.0 ± 6.0
Weight (kg)	33.1 ± 6.0	33.1 ± 6.9	62.9 ± 10.1	56.0 ± 8.4
BMI (kg/m^2)	17.1 ± 2.2	17.1 ± 2.6	20.6 ± 2.6	20.5 ± 2.7
Waist circumference (cm)	59.4 ± 5.4	58.4 ± 6.7	71.5 ± 6.0	66.7 ± 5.9
Maturity*- No (%)				
1	477 (100.00)	513 (97.9)	11 (3.8)	2 (0.6)
2	-	11 (2.1)	127 (43.6)	191 (54.6)
3	-	-	153 (52.6)	157 (44.9)
SBP (mm Hg)	102.6 ± 8.9	101.0 ± 8.7	115.9 ± 11.3	107.7 ± 9.4
SBPz	0.02 ± 0.8	-0.10 ± 0.8	-0.03 ± 1.0	-0.62 ± 0.9
DBP (mm Hg)	60.0 ± 7.1	60.0 ± 6.5	63.0 ± 6.8	64.0 ± 6.6
DBPz	-0.10 ± 0.6	-0.06 ± 0.6	-0.23 ± 0.6	-0.22 ± 0.6
Glucose (mmol/l)	5.1 ± 0.4	5.0 ± 0.4	5.2 ± 0.4	5.0 ± 0.4
Insulin (pmol/l)	39.7 ± 22.0	46.4 ± 24.6	70.6 ± 36.1	78.2 ± 37.0
TC (mmol/l)	4.4 ± 0.7	4.6 ± 0.8	4.0 ± 0.7	4.4 ± 0.8
HDL (mmol/l)	1.6 ± 0.4	1.5 ± 0.3	1.3 ± 0.3	1.4 ± 0.3
Triglycerides (mmol/l)	0.7 ± 0.3	0.8 ± 0.3	0.8 ± 0.3	0.9 ± 0.4
Birth weight (g)	3519 ± 626	3413 ± 602	3528 ± 591	3323 ± 549
Breastfeeding- No (%)	406 (85.1)	453 (86.5)	242 (83.2)	269 (77.9)
Parental education**- No (%)				
1	31 (6.50)	26 (5.0)	16 (5.5)	31 (8.9)
2	120 (25.2)	137 (26.2)	73 (25.1)	83 (23.7)
3	326 (68.3)	361 (68.9)	204 (69.4)	237 (67.4)
Unweighted risk score (GRS)	18.2 ± 2.3	18.2 ± 2.5	18.1 ± 2.2	18.3 ± 2.2
Weighted risk score (wGRS)	20.7 ± 3.0	20.7 ± 3.1	20.6 ± 3.1	21.0 ± 3.0

Data are means ±SD or %. BMI= body mass index, SBP= systolic blood pressure, DBP= diastolic blood pressure, TC= total cholesterol, HDL= high-density lipoprotein cholesterol.
*Maturity 1= Tanner stage 1-2, Maturity 2= Tanner stage 3-4 and Maturity 3= Tanner stage 5.
** According to International Standard Classification of Education (ISCED) (UNESCO 1997). Parental education 1= ISCED level 1-2, Parental education 2= ISCED level 3-4 and Parental education 3= ISCED level 5-7.

Table 2. Associations of fasting glucose, and potential confounders, with blood pressure, *n* = 1,642

	Difference in SBPz per unit of category of exposure			Difference in DBPz per unit of category of exposure		
	Effect size	95% CI	P-value	Effect size	95% CI	P-value
Glucose (mmol/l)[a]	0.40	0.28 ; 0.51	6.6×10^{-12}	0.21	0.13 ; 0.28	1.5×10^{-7}
Insulin (pmol/l) [a]	0.01	0.004 ; 0.01	5.2×10^{-15}	0.003	0.002 ; 0.004	1.4×10^{-8}
TC (mmol/l) [a]	0.13	0.07 ; 0.18	1.0×10^{-5}	0.10	0.06 ; 0.13	8.4×10^{-7}
TG (mmol/l) [a]	0.40	0.28 ; 0.53	4.4×10^{-10}	0.27	0.19 ; 0.36	3.2×10^{-10}
HDL (mmol/l) [a]	-0.08	-0.21 ; 0.06	0.26	0.002	-0.09 ; 0.09	0.96
BMI (kg/m^2) [a]	0.08	0.06 ; 0.10	1.5×10^{-20}	-0.002	0.00 ; 0.02	0.12
WC (cm) [a]	0.02	0.02 ; 0.03	1.2×10^{-11}	-0.001	-0.01 ; 0.004	0.60
Birth weight (g) [a]	-1.1×10^{-4}	-1.9×10^{-4} ; -4.3×10^{-5}	0.002	1.6×10^{-5}	-6.4×10^{-5} ; 3.3×10^{-5}	0.52
Glucose (mmol/l)[b]	0.32	0.20 ; 0.43	5.4×10^{-8}	0.14	0.07 ; 0.22	3.5×10^{-4}
Insulin (pmol/l) [b]	1.8×10^{-3}	2.7×10^{-4} ; 3.4×10^{-3}	0.02	1.5×10^{-3}	4.5×10^{-4}; 2.6×10^{-3}	5.0×10^{-3}
TC (mmol/l) [b]	0.08	0.02 ; 0.15	0.01	0.07	0.02 ; 0.11	3.0×10^{-3}
TG (mmol/l) [b]	0.12	-0.01 ; 0.26	0.10	0.16	0.06 ; 0.25	1.9×10^{-3}
HDL (mmol/l) [b]	-0.04	-0.20 ; 0.11	0.60	-0.02	-0.12 ; 0.09	0.77
BMI (kg/m^2) [b]	0.08	0.05 ; 0.11	7.2×10^{-8}	0.02	5.5×10^{-5} ; 0.04	0.049
WC (cm) [b]	-0.01	-0.02 ; 0.01	0.26	-0.01	-0.02 ; -4.7×10^{-3}	2.5×10^{-3}
Birth weight [b] (g)	1.4×10^{-4}	2.1×10^{-4} ; 6.7×10^{-5}	1.3×10^{-4}	4.7×10^{-6}	4.4×10^{-5} ; 5.3×10^{-5}	0.85
	Difference in SBPz by category			Difference in DBPz by category		
Breast-fed [a]	-0.17	-0.28;- 0.05	0.004	-0.05	-0.13 ; 0.02	0.17
Maturity* [a]						
1	reference	-	-	reference	-	-
2	0.09	-0.27 ; 0.44	0.63	-0.09	-0.33 ; 0.15	0.48
3	0.47	0.11 ; 0.84	0.01	0.13	-0.12 ; 0.38	0.29
Parental edu** [a]						
1	reference	-	-	reference	-	-
2	-0.08	-0.27 ; 0.11	0.41	-0.06	-0.19 ; 0.07	0.36
3	-0.14	-0.31 ; 0.04	0.14	-0.11	-0.23 ; 0.02	0.09
Breast-fed [b]	-0.14	-0.25 ; -0.03	0.01	-0.04	-0.11 ; 0.04	0.32
Maturity* [b]						
1	reference	-	-	reference	-	-
2	-0.11	-0.45 ; 0.23	0.54	-0.10	-0.34 ; 0.14	0.41
3	0.19	-0.16 ; 0.55	0.28	0.10	-0.15 ; 0.35	0.42
Parental edu** [b]						
1	reference	-	-	reference	-	-
2	-0.08	-0.26 ; 0.10	0.39	-0.06	-0.18 ; 0.07	0.36
3	-0.08	-0.25 ; 0.09	0.34	-0.09	-0.21 ; 0.03	0.16

SBP=systolic blood pressure, DBP=diastolic blood pressure, TC=total cholesterol, TG=triglyceride, HDL=high-

density lipoprotein cholesterol, BMI=body mass index, WC= waist circumference, edu= education.
[a]Adjusted for country, age, age group, gender.
[b]Adjusted for country, age, age group, gender, maturity, country, parental education, birth weight, breastfeeding, BMI, waist circumference, triglycerides, total cholesterol, HDL and insulin.
*Maturity 1= Tanner stage 1-2, Maturity 2= Tanner stage 3-4 and Maturity 3= Tanner stage 5.
** According to International Standard Classification of Education (ISCED) (UNESCO 1997). Parental education 1= ISCED level 1-2, Parental education 2= ISCED level 3-4 and Parental education 3= ISCED level 5-7.

Table 3. Instrumental variable analyses of the association of fasting glucose with blood pressure, using rs560887 and genetic risk scores, *n* = 1,642.

	Difference in SBPz per increase in mmol/l FG				Difference in DBPz per increase in mmol/l FG			
	Effect size	95% CI	P-value	P-value for difference with conventional approach*	Effect size	95% CI	P-value	P-value for difference with conventional approach *
IV analysis, rs560887[a]	-0.52	-1.27 ; 0.22	0.17	0.01	0.01	-0.49 ; 0.51	0.97	0.43
IV analysis, rs560887[b]	-0.42	-1.13 ; 0.30	0.25	0.03	4.9×10^{-4}	-0.49 ; 0.49	1.00	0.47
IV analysis, GRS[a]	-0.43	-1.34 ; 0.49	0.36	0.07	-0.01	-0.63 ; 0.60	0.97	0.47
IV analysis, GRS[b]	-0.39	-1.26 ; 0.49	0.39	0.11	-0.12	-0.72 ; 0.49	0.70	0.38
IV analysis, wGRS[a]	-0.41	-1.13 ; 0.31	0.26	0.02	-0.02	-0.50 ; 0.47	0.95	0.33
IV analysis, wGRS[b]	-0.34	-1.03 ; 0.35	0.34	0.04	0.10	-0.58 ; 0.37	0.67	0.25
MV analysis[a]	0.40	0.28 ; 0.51	<0.0001		0.21	0.13 ; 0.28	<0.0001	
MV analysis[b]	0.32	0.20 ; 0.43	<0.0001		0.14	0.07 ; 0.22	<0.0001	

SBP=systolic blood pressure, DBP=diastolic blood pressure, FG= fasting glucose, CI= confidential interval, IV= instrumental variable, MV= multivariable, GRS= unweighted genetic risk score, wGRS= weighted genetic risk score.

[a]Adjusted for country, age, age group, gender.

[b]Adjusted for country, age, age group, gender, maturity, country, parental education, birth weight, breastfeeding, BMI, waist circumference, triglycerides, total cholesterol, HDL and insulin. * P-value for difference in estimates between results from the IV approach and the conventional approach (MV analysis), using Durbin-Wu Hausman

Table 4. Instrumental variable- and multivariable regression analyses of the association of fasting glucose with blood pressure, using rs560887 and genetic risk scores, excluding Estonia, *n* = 873.

	Difference in SBPz per increase in mmol/l FG				Difference in DBPz per increase in mmol/l FG			
	Effect size	95% CI	P-value	P-value for difference with conventional approach*	Effect size	95% CI	P-value	P-value for difference with conventional approach*
IV analysis, rs560887[a]	-0.77	-1.58 ; 0.05	0.07	0.01	0.06	-0.51 ; 0.62	0.85	0.44
IV analysis, rs560887[b]	-0.82	-1.58 ; -0.06	0.04	0.03	-0.07	-0.62 ; 0.47	0.79	0.47
IV analysis, GRS[a]	0.01	-0.75 ; 0.77	0.98	0.047	-0.18	-0.70 ; 0.34	0.50	0.37
IV analysis, GRS[b]	-0.17	-0.89 ; 0.54	0.64	0.11	-0.37	-0.88 ; 0.14	0.16	0.38
IV analysis, wGRS[a]	-0.25	-0.90 ; 0.41	0.46	0.01	0.00	-0.45 ; 0.45	1.0	0.20
IV analysis, wGRS[b]	-0.35	-0.97 ; 0.27	0.27	0.04	-0.18	-0.62 ; 0.26	0.43	0.25
MV analysis[a]	0.25	0.11 ; 0.39	0.001		0.16	0.06 ; 0.3	0.002	
MV analysis[b]	0.13	-0.01 ; 0.28	0.08		0.08	-0.02 ; 0.18	0.13	

SBP=systolic blood pressure, DBP=diastolic blood pressure, FG= fasting glucose, CI= confidential interval, IV= instrumental variable, MV= multivariable, GRS= unweighted genetic risk score, wGRS= weighted genetic risk score, [a]Adjusted for country, age, age group, gender. [b]Adjusted for country, age, age group, gender, maturity, country, parental education, birth weight, breast-feeding, BMI, waist circumference, triglycerides, total cholesterol, HDL and insulin.

* P-value for difference in estimates between results from the IV approach and the conventional approach (MV analysis), using Durbin-Wu Hausman

Supplementary Table 1. Association of rs560887, GRS and wGRS with FG, analyzed separately and jointly for countries, n = 1,642

	Difference in FG per increase of risk allele			
	mmol/l	95% CI	P-value	F-value
Denmark and Estonia, n = 1642				
Rs560887	0.09	0.06 ; 0.11	1.9 × 10-9	36.44
GRS-16	0.01	0.01 ; 0.02	4.4 × 10-4	12.38
wGRS-16	0.01	0.003 ; 0.02	0.003	8.65
GRS-14	0.02	0.01 ; 0.03	9.7 × 10-7	24.17
wGRS-14	0.02	0.01 ; 0.03	4.1 × 10-10	39.56
Denmark, n = 873				
Rs560887	0.10	0.06 ; 0.14	3.9 × 10-7	26.13
GRS-16	0.02	0.01 ; 0.03	3.1 × 10-5	17.55
wGRS-16	0.01	0.006 ; 0.02	0.001	12.03
GRS-14	0.03	0.02 ; 0.04	5.5 × 10-8	30.06
wGRS-14	0.03	0.02 ; 0.04	2.1 × 10-10	41.33
Estonia, n = 769				
Rs560887	0.07	0.03 ; 0.11	8.3 × 10-4	11.27
GRS-16	0.004	-0.01 ; 0.01	0.41	0.67
wGRS-16	0.003	-0.005 ; 0.01	0.46	0.56
GRS-14	0.01	-0.001 ; 0.02	0.08	3.05
wGRS-14	0.01	0.004 ; 0.02	0.003	8.62

Results are unadjusted estimates. GRS-16 and wGRS-16 include all 16 SNPs. GRS-14 and wGRS-14 include 14 SNPs, after excluding the ones that influence insulin levels. GRS= unweighted genetic risk score, wGRS= weighted genetic risk score, FG= fasting glucose, CI= confidential interval.

Supplementary Table 2. Confounding variables association with rs560887, n = 1,642.

Confounder	ß	95% CI	P-value
Age (years)	-0.1	-0.4 ; 0.06	0.17
Height (cm)	-0.6	-1.82 ; 0.61	0.33
Weight (kg)	-0.8	-1.9 ; 0.3	0.13
BMI (kg/m2)	-0.2	-0.43 ; 0.02	0.07
WC (cm)	-0.5	-1.1 ; 0.05	0.07
Insulin (pmol/l)	-1.0	-3.4 ; 1.5	0.44
Tc (mmol/l)	-0.01	-0.06 ; 0.05	0.82
TG (mmol/l)	0.01	-0.02 ; 0.04	0.46
Hdl (mmol/l)	-0.01	-0.04 ; 0.01	0.26
Birth weight (g)	-19.7	-63.9 ; 24.5	0.38
Maturity	-	-	0.37*
Breast-feeding	-	-	0.20*
Parental education	-	-	0.05*

Results are unadjusted estimates. *from chi2 test. CI= confidential interval, BMI=body mass index, WC= waist circumference, Tc=total cholesterol, TG=triglyceride, Hdl=high density lipoprotein cholesterol.

Supplementary Table 3. Confounding variables association with fasting glucose, n = 1,642.

Confounder	ß	95% CI	P-value
Age (years)	0.8	0.5 ; 1.2	<0.0001
Height (cm)	6.8	4.7 ; 8.8	<0.0001
Weight (kg)	5.8	3.9 ; 7.6	<0.0001
BMI (kg/m2)	0.7	0.3 ; 1.1	<0.0001
Waist (cm)	3.0	2.0 ; 4.0	<0.0001
Insulin (pmol/l)	26.7	20.7 ; 28.7	<0.0001
Tc (mmol/l)	0.1	0.01 ; 0.2	0.03
TG (mmol/l)	0.1	0.1 ; 0.2	<0.0001
Hdl (mmol/l)	-0.002	-0.04 ; 0.04	0.93
Birthweight (g)	-6.6	-82.0 ; 68.8	0.86
Maturity	-	-	0.003*
Breast-feeding	-	-	0.003*
Education	-	-	0.73*

Results are unadjusted estimates. *from chi2 test. CI= confidential interval, BMI=body mass index, WC= waist circumference, Tc=total cholesterol, TG=triglyceride, Hdl=high density lipoprotein cholesterol.

Supplementary Table 4. Associations of the individual SNPs from our study, with fasting glucose, *n* = 1,642.

SNP (locus)	Effect size	95% CI	P-value
rs4607517 (GCK)	-0.02	-0.06 ; 0.02	0.33
rs340874 (PROX1)	0.02	-0.01 ; 0.04	0.23
rs11920090 (SLC2A2)	0.04	0.01 ; 0.08	0.02
rs11605924 (CRY2)	0.00	-0.02 ; 0.03	0.87
rs560887 (G6PC2)	0.09	0.06 ; 0.11	<0.0001
rs10885122 (ADRA2A)	0.03	0.00 ; 0.07	0.08
rs2191349 (DGKB-TMEM195)	0.00	-0.02 ; 0.03	0.71
rs7944584 (MADD)	0.04	0.01 ; 0.07	0.01
rs7034200 (GLIS3)	0.01	-0.02 ; 0.03	0.56
rs13266634 (SLC30A8)	0.04	0.01 ; 0.06	0.01
rs11708067 (ADCY5)	0.03	0.00 ; 0.06	<0.05
rs174550 (FADS1)	0.00	-0.02 ; 0.03	0.85
rs11071657 (C2CD4B)	0.01	-0.01 ; 0.04	0.35
rs7903146 (TCF7L2)	-0.01	-0.04 ; 0.02	0.33

Country, age, age group and gender adjusted. CI= confidential interval.