Steven S, Hollingsworth KG, Al-Mrabeh A, Avery L, Aribisala B, Caslake M, Taylor R. [Very-Low-Calorie Diet and 6 Months of Weight Stability in Type 2 Diabetes: Pathophysiologic Changes in Responders and Nonresponders](http://dx.doi.org/10.2337/dc15-1942). *Diabetes Care* 2016, 1-8.

DOI link to article:

http://dx.doi.org/10.2337/dc15-1942

Date deposited:

20/04/2016

Very low calorie diet and 6 months of weight stability in type 2 diabetes: Pathophysiologic changes in responders and non-responders

Sarah Steven MB ChB[1], Kieren G Hollingsworth PhD[1], Ahmad Al-Mrabeh PhD[1], Leah Avery PhD[2], Benjamin Aribisala PhD[3], Muriel Caslake PhD[4], Roy Taylor MD[1]

1. Magnetic Resonance Centre, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK
2. MoveLab, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK
3. Computer Science Department, Faculty of Science, Lagos State University, Lagos, Nigeria
4. Institute of Cardiovascular & Medical Sciences , University of Glasgow, UK

Abbreviated title: **Very low calorie diet in Type 2 Diabetes**

Corresponding author and person to who reprint requests should be addressed:

Professor Roy Taylor, Magnetic Resonance Centre, Campus for Ageing & Vitality, Newcastle University, Newcastle upon Tyne NE4 5PL, UK.

roy.taylor@ncl.ac.uk Tel 0191 208 1172 ; Fax 0191 208 1151

Abstract

Objective: Type 2 diabetes mellitus (T2DM) is generally regarded as an irreversible chronic condition. As a very low calorie diet (VLCD) can bring about acute return to normal glucose control in some people with T2DM, this study tested the potential durability of this normalization. The underlying mechanisms were defined.

Research Design and Methods: People with T2DM duration 0.5-23 years (n=30) followed a VLCD for 8 weeks. All oral agents or insulins were stopped at baseline. Following stepped return to isocaloric diet, a structured, individualized program of weight maintenance was provided. Glucose control, insulin sensitivity, insulin secretion, hepatic and pancreas fat content were quantified at baseline, after return to isocaloric diet and after 6 months to permit the primary comparison of change between post-weight loss and 6 months in responders. Responders were defined as achieving fasting blood glucose <7mmol/l after return to isocaloric diet.

Results: Weight fell (98.0±2.6 to 83.8±2.4 kg) and remained stable over 6 months (84.7±2.5 kg). 12/30 achieved fasting plasma glucose <7mmol/l following return to isocaloric diet (responders), and 13/30 after 6 months. Responders had shorter duration diabetes and higher initial fasting plasma insulin level. HbA1c fell from 7.1±0.3 to 5.8±0.2% (55±4 to 40±2mmol/mol) in responders (p<0.001), and from 8.4±0.3 to 8.0±0.5% (68±3 to 64±5mmol/mol) in non-responders, remaining constant at 6 months (5.9±0.2 and 7.8±0.3%; 41±2 and 62±3 mmol/mol respectively). The responders were characterized by return of first phase insulin response.

Conclusions: A robust and sustainable weight loss program achieved continuing remission of diabetes for at least 6 months in the 40% who responded to a VLCD by achieving fasting plasma glucose of <7mmol/l. T2DM is a potentially reversible condition.

Introduction

Type 2 diabetes (T2DM) has reached epidemic proportions, affecting 9.2% of the US population and costing the country $322 billion in 2012 [1]. The personal cost is enormous in terms of visual loss, amputation and premature cardiovascular disease. The inevitably progressive nature of the disease has appeared beyond question as successive large studies have confirmed clinical experience of inexorably worsening glucose control [2; 3]. At diagnosis, patients are advised to get used to having a lifelong disease and to accept this in order to cope with the disease [4]. Sequential addition of therapies is required and within 10 years of diagnosis, 50% of individuals are on insulin therapy [3].

However, restoration of normal glucose control is possible following weight loss in some individuals with T2DM [5-8]. Although most commonly seen after bariatric surgery, reversal of diabetes can occur after any sharp decrease in calorie intake [9; 10]. In short duration T2DM, fasting plasma glucose becomes normal within days on a very low calorie diet (VLCD) diet due to rapid decrease in liver fat and return of normal hepatic insulin sensitivity, and normal beta cell function returns over 8 weeks [11]. If the demonstrated normalization of hepatic insulin sensitivity and beta cell function could be maintained in the longer term this could change the entire approach to management of T2DM.

The Counterbalance study (COUNTERacting BetA cell failure by Long term Action to Normalize Calorie intakE) was designed to test the hypothesis that individuals who achieve non-diabetic fasting blood glucose after VLCD would remain normoglycemic during weight stability. The pathophysiologic mechanisms underlying blood glucose control over 6 months were defined.

Methods

Study design

This prospective, longitudinal, single center study comprised 3 phases: very low calorie diet (VLCD) for 8 weeks; a stepped return to isocaloric intake of normal food over 2 weeks; and a structured, individualized weight maintenance program over 6 months. Assessments were carried out before the VLCD, after return to isocaloric eating and at the end of the 6 month follow up. The primary outcome measure was the fasting blood glucose at 6 months in the group achieving non-diabetic levels following VLCD and return to normal eating and the primary comparison was of change between post-weight loss and 6 months in responders. The study evaluates the pathophysiological response to dietary change rather than a comparative clinical study of treatment. In this study responders were defined as achieving fasting blood glucose <7mmol/l after return to isocaloric diet. Immediately after the 8 week VLCD 87% of short and 50% of long duration diabetes groups achieved non-diabetic fasting plasma glucose levels [12]. The study was designed to define the durability over 6 months of the clinical and pathophysiologic changes after VLCD and return to isocaloric eating and did not include a control group maintained on usual therapy.

Participants

Thirty individuals with T2DM were recruited by advertisement. The study was rapidly over-subscribed, and of the 48 individuals screened 18 were excluded due to duration of diabetes or HbA1c. To maximize detection of an effect of duration of T2DM, individuals with either short (<4 years) or long (>8years) duration disease were studied. Inclusion criteria were age ≥25-80 years and BMI 27-45 kg/m^2. Exclusion criteria were recent weight loss of >5kg, treatment with thiazolidinediones, GLP-1 agonists, steroids or atypical anti-psychotics, serum creatinine >150µmol/l or alcohol >3 units per day (women) or >4 units per day (men). Participants discontinued all anti-diabetic therapy immediately prior to the baseline study but remained on their usual lipid lowering treatment. Anti-hypertensive medications were decreased as necessary throughout the study. The study protocol was approved by Newcastle and North Tyneside 2 Ethics Committee (REC 12/NE/0208) and all participants gave informed written consent.

Experimental protocol

The VLCD consisted of a liquid diet formula (43% carbohydrate, 34% protein and 19.5% fat; 2·6 MJ/day [624 kcal/day]; Optifast; Nestlé Nutrition, Croydon, UK) taken as 3 shakes per day. In addition, up to 240g of non-starchy vegetables was consumed, making total energy intake 624-700 kcal/day. Participants were encouraged to drink at least two liters of calorie-free beverages per day and to maintain their habitual level of physical activity. To maximize adherence, one-to-one support was provided weekly by telephone, e-mail, text message or face-to-face contact (SS). During stepped food reintroduction shakes were gradually replaced by solid food over 7 days; with one meal replacing a shake every 3 days. Isocaloric intake was determined from resting energy expenditure measured by indirect calorimetry using an open circuit calorimeter (Quark RMR; COSMED, Rome, Italy) and a canopy hood. Studies were conducted a minimum of 6 days after full return to solid foods. The standard threshold for remission of diabetes (fasting plasma glucose level <7mmol/l) was used to define the group of responders [13]. In contrast to the initial study [11], the criterion was applied after return to isocaloric eating in order to avoid an acute dietary effect.

During the 6 month weight maintenance phase, participants were supported by a structured individualized program based on goal setting, action planning and barrier identification with monthly reviews [14]. The primary goal of this phase was to prevent weight regain by individualized dietary advice guided by weight trajectory. Physical activity was encouraged but food behaviors were the priority. If fasting plasma glucose exceeded 10mmol/l on 2 occasions, hypoglycemic agents were recommenced.

As in an earlier study, participants were excluded if they were unable to achieve weight loss targets of 3·8 % body weight at week one of the VLCD [11]. Only one participant did not meet the weight loss target and left the study after week one; therefore 29/30 participants completed the VLCD and 6 month weight maintenance phase. All 29 completed data collection at each time point.

Hepatic glucose production and insulin sensitivity

After an overnight fast, cannulae were inserted into an antecubital vein for infusion and the contralateral wrist vein for arterialized blood sampling. [6'6'-2H] glucose (98% enriched; Cambridge Isotope Laboratories, MA, USA) was used to determine hepatic glucose production [11]. Basal rates were calculated during the last 30 min of the 150 min basal period. An isoglycemic–hyperinsulinemic clamp (insulin infusion rate 40 mU m^2 min^1) was initiated at 0 min with isoglycemia selected to ensure that the true metabolic condition of each participant could be observed at each study time point. Whole-body insulin sensitivity was determined during the last 30 min of the 120 min hyperinsulinemic glucose clamp per kg of fat free mass corrected for glucose space and urinary loss [11]. Muscle insulin sensitivity was calculated as the sum of M value and basal hepatic glucose production minus the urinary glucose loss. Hepatic insulin resistance index was calculated as the product of basal hepatic glucose production and fasting insulin levels [15].

Measurement of hepatic VLDL-triglyceride production rates

VLDL$_1$-triglyceride production rate was measured by accumulation of plasma VLDL$_1$-triglyceride during competitive blockade of tissue uptake by excess Intralipid [16; 17]. After an overnight fast, 20% Intralipid (0.1g/kg body mass) was injected as a bolus followed by continuous infusion of 10% Intralipid at 0.1g/kg/hr. Plasma samples were collected at 8 points over 75 minutes. After centrifugation and ultracentrifugation to separate plasma, remove chylomicrons, Intralipid and to isolate VLDL$_1$, the triglyceride concentration of VLDL$_1$ was measured. VLDL$_1$-triglyceride production rates were calculated from the gradient of the linear increase in concentration over time.

Assessment of beta cell function

At least sixty minutes after the clamp test, when glucose levels had stabilized at fasting levels, two consecutive 30 min square-wave steps of hyperglycemia (2.8 and 5.6 mmol/l above baseline) were achieved by priming glucose doses followed by variable 20% glucose infusion [18]. Blood samples for determination of C-peptide concentrations were obtained every 2 min for the first 10 min then every 5 min for each step. An arginine bolus was administered during the second step of hyperglycemia to

assess maximal insulin secretory capacity, followed by sampling every 2 min for 10 min. Insulin secretion rate was calculated using a computerized program implementing a regularization method of deconvolution and using a population model of C-peptide kinetics as previously described [11].

Body composition and intra-organ triglyceride content

Body composition was determined using a Bodystat1500 (Bodystat Ltd, Isle of Man, UK). Magnetic resonance (MR) data were acquired using a 3 Tesla Philips Achieva scanner (Philips, Best, The Netherlands) with either a 6 channel cardiac array (Philips), or four large surface coils (large and medium flex coils, Philips) if required due to body habitus. Data were acquired using a three point Dixon method with gradient-echo scans acquired during four 17 second breath holds as previously described [11]. The intraorgan triglyceride percentage was evaluated from regions of interest on two image slices of pancreas and five image slices of liver, defined and averaged by one observer (SS). The pancreas triglyceride analysis was carried out blinded to the participants' details and time point.

Analytical procedures

Plasma hormones and metabolites were measured at a Clinical Pathology Accredited laboratory (Newcastle upon Tyne Hospital NHS Foundation Trust, Department of Clinical Biochemistry). VLDL$_1$-triglyceride was analyzed at the Institute of Cardiovascular & Medical Sciences, University of Glasgow using standard methods (Roche Diagnostics, West Sussex, UK). Immediate measurement of β- hydroxybutyrate levels to test dietary compliance was carried out using test strips (Abbott Diabetes Care, Oxfordshire, UK).

Statistical Analysis

Data are presented as mean ± SEM for parametric and median (range) for non-parametric data. Statistical analysis used Student's paired and 2-sample t-test, Mann Whitney U, Wilcoxon Rank and Spearman Rank correlation as appropriate using Minitab 16 statistical program (Minitab Inc, State College, PA).

Results

In the whole group, weight fell from 98.0±2.6 at baseline to 83.8±2.4 kg during the VLCD (p<0.001) and remained at 84.7±2.5 kg after 6 months. After return to isocaloric eating, 40% (12/30) achieved a fasting glucose of <7.0mmol/l (responders). After 6 months of weight loss maintenance, 43% (13/30) had a fasting plasma glucose <7 mmol/l off all oral hypoglycemic agents or insulin.

In the responders, fasting plasma glucose fell from 8.9±0.7 to 6.2±0.1 mmol/l (p=0.002) on isocaloric diet post-VLCD and remained constant at 6.2±0.3 mmol/l on no hypoglycemic agents (Figure 1A). In the non-responders, fasting plasma glucose fell from 13.2±0.6 to 10.9±1.1 mmol/l (p=0.016) post-VLCD and remained constant at 9.4±0.7 mmol/l. The rise in plasma glucose over the 2 weeks from the end of the VLCD to establishment on an isocaloric diet was significantly greater in non-responders (Figure 1A, shaded bar) and 6 individuals in this group restarted medication during the 6 month weight loss maintenance period (2 metformin only, 3 metformin and sulfonylurea and 1 insulin). HbA1c remained stable throughout the 6 month period in both groups (responders: 5.8±0.2 to 5.9±0.2 % (40±2 to 41±2 mmol/mol); p=0.540; 5 individuals <5.7% or 39mmol/l) and non-responders: 8.0±0.5 to 7.8±0.3 % (64±5 to 62±3 mmol/mol); p=0.481) (Figure 1B). The major improvement in blood pressure, triglyceride and non-HDL cholesterol following the VLCD in both responders and non-responders was maintained over the 6 month weight maintenance period (Table 1). At baseline 17 individuals were taking antihypertensive agents and these were decreased in dose or stopped in 8 individuals; bumetanide (n=1); nifedipine (n=1); amlodipine (n=1); doxazosin (n=2); ACEi (n=2); β-blocker (n=1).

Clinical features of responders compared with non-responders

Achieved weight loss after VLCD was similar between the responders and non-responders (15.8±0.5% vs. 13.6±0.7%; p=0.06). Weight remained constant over 6 months in both groups (Table 1 and Figure 1C). The responders (n=12; 8M:4F) had a shorter diabetes duration (3.8±1.0 vs. 9.8±1.6

yr; p=0.007) and were younger (52.0±2.9 vs. 59.9±2.1 yr; p=0.032) compared to the non-responders (n=17; 7M:10F). Responders comprised 9/15 of the short duration and 3/14 of the long duration groups. At baseline, responders had lower fasting glucose (8.9±0.7 vs. 13.2±0.6 mmol/l; p<0.001) and HbA1c (7.1±0.3 vs. 8.4±0.3 % [55±4 vs. 68±3 mmol/mol]; p=0.01). Achieved fasting glucose level after VLCD was positively correlated with diabetes duration (Rs 0.59; p=0.001). Although there was no difference between non-responders and responders in terms of initial weight or BMI, the total fat mass was higher in non-responders at baseline (p=0.04; Table 1). There was no difference in the achieved fasting plasma glucose after the VLCD in individuals with BMI more or less than 35 kg/m^2 at baseline (8.2 vs. 7.1 mmol/l; p=0.84) with 3/10 vs. 9/19 responders respectively. Prior to the study, the responders were on less treatment for diabetes compared to non-responders: diet control (5 vs. 2); metformin only (6 vs. 4); metformin and sulfonylurea (1 vs. 7), metformin, sulfonylurea and insulin (0 vs. 2); metformin, sulfonylurea and thiazolidinedione (0 vs. 1); insulin only (0 vs. 1).

Change in plasma hormones and metabolites

Responders were characterized by higher baseline serum insulin levels compared to non-responders (20.4 (5.7-48.1) vs. 9.3 (3.9-48.9) mU/l; p=0.005). This state of relative insulin deficiency in the non-responders was reflected in higher baseline fasting ketones (0.20 (0.10-1.10) vs. 0.10 (0.10-0.30) mmol/l; p=0.02) and FFA (0.69±0.04 vs. 0.51±0.05 mmol/l; p=0.01). Plasma insulin levels fell in both groups following the VLCD and remained stable throughout the weight maintenance phase (Table 1). Fasting serum triglyceride levels fell by 32±7% in responders and 18±9% in non-responders. After 6 months, HDL cholesterol was raised by 27% in responders.

Liver

Hepatic insulin resistance improved similarly in both groups following the VLCD: 2.15 (0.82-5.95) to 0.85 (0.30-1.80) mmol.min^{-1}.kg$_{ffm}$$^{-1}$.pmol.l^{-1} (p=0.003) in responders and 1.24 (4.25-6.60) to 0.77 (0.16-2.20) mmol.min^{-1}.kg$_{ffm}$$^{-1}$.pmol.l^{-1} (p=0.001) in non-responders (Figure 2B). There was no significant change in hepatic insulin resistance index following weight maintenance in either group

(0.75 (0.31-3.72) and 0.76 (0.17-2.40) mmol.min^{-1}.kg$_{ffm}$$^{-1}$.pmol.l^{-1} respectively). At baseline, the responders tended to have greater hepatic insulin resistance (Hepatic IR index: 2.15 (0.82-5.95) vs. 1.24 (0.42-6.60) mmol.min^{-1}.kg$_{ffm}$$^{-1}$.pmol.l^{-1}; p=0.060) (Figure 2B).

Marked normalization in hepatic triglyceride content was seen in both the responders (12.8±2.7 to 2.2±0.2 %; p=0.002) and non-responders (8.2±1.1 to 2.2±0.1 %; p<0.001) following the VLCD. Serum ALT levels decreased only in the responders (Table 1). There was no re-accumulation of hepatic triglyceride during the 6 month weight maintenance period in either responders or non-responders: 2.1±0.3 vs. 2.3±0.2 % respectively (Figure 2A). Responders had higher alanine transaminase (ALT) levels (43.0 (11.0-151.0) vs. 22.0 (12.0-61.0) U/l; p=0.02) and this was accompanied by a tendency to higher hepatic triglyceride content at baseline compared to non-responders (12.8±2.7 vs. 8.2±1.1%; p=0.09) (Figure 2A).

Hepatic VLDL$_1$-triglyceride production rate was similar in the two groups at baseline (responders: 125.3±22.9 vs. non-responders: 150.6±13.2 mg/kg/day; p=0.31). It fell by approximately 20% after VLCD in both groups, remaining stable during weight maintenance (Figure 2C).

Pancreas

First phase insulin response was markedly reduced at baseline in non-responders compared to responders (0.01±0.00 vs. 0.12±0.04 nmol min^{-1} m^{-2}; p=0.002). Similarly, maximal insulin secretory capacity (baseline to peak insulin secretion rate) was significantly impaired (0.21±0.03 vs. 1.06±0.35 nmol min^{-1} m^{-2}; p=0.008).

First phase insulin response improved in the responders: 0.12±0.04 to 0.26±0.07 nmol min^{-1} m^{-2}; p=0.03. There was a small increase in the non-responders: 0.01±0.00 to 0.03±0.01 nmol min^{-1} m^{-2}; p=0.04) (Figure 3A). There was no change in maximal insulin secretory capacity in either group (responders: 1.06±0.35 to 0.81±0.16 nmol min^{-1} m^{-2}; p=0.363 and non-responders: 0.21±0.03 to 0.25±0.05 nmol min^{-1} m^{-2}; p=0.30).

First phase insulin secretion did not change over the weight maintenance period in either responders or non-responders (0.26 ± 0.07 to 0.24 ± 0.05 vs. 0.03 ± 0.01 to 0.03 ± 0.01 nmol min^{-1} m^{-2}) (Figure 3) and there was no change in maximal insulin secretory capacity.

At baseline, pancreas fat levels were similar in responders and non-responders (5.3 ± 0.4 vs. 5.9 ± 0.7 %; p=0.49). Following the VLCD, there was a significant decrease in pancreas fat content in both groups (responders: 5.3 ± 0.4 to 4.5 ± 0.3 % (p=0.039) and non-responders: 5.9 ± 0.7 to 5.3 ± 0.5 % (p=0.004); Figure 3B). Pancreas fat content remained stable during weight maintenance (4.4 ± 0.3% and 5.0 ± 0.5%).

Adipose tissue and muscle

There was no difference in either visceral adipose tissue or subcutaneous adipose tissue areas between responders and non-responders at baseline (287.0 ± 23.1 vs. 289.6 ± 5.7 cm^2; p=0.94 and 319.6 ± 31.0 vs. 285.4 ± 24.7 cm^2; p=0.40 respectively). Both visceral and subcutaneous adipose tissue areas decreased following the VLCD then remained constant during the 6 month follow up (responders: visceral: 287.0 ± 23.1 to 191.9 ± 18.9 (p<0.001) then 179.5 ± 22.3 cm^2 and subcutaneous: 319.6 ± 31.0 to 232.0 ± 23.1 (p<0.001) and then 238.6 ± 20.3 cm^2); non-responders: visceral: 289.6 ± 23.7 to 209.5 ± 22.1 (p<0.001) and then 198.9 ± 4.8 cm^2 and subcutaneous: 285.4 ± 24.7 to 223.3 ± 23.5 (p<0.001) and then 219.3 ± 22.8 cm^2). There was no significant improvement in muscle insulin sensitivity following the VLCD (responders: 5.9 ± 0.4 at baseline, 7.0 ± 0.6 after VLCD and 7.2 ± 0.8 mg/kg$_{ffm}$/min at month 6; non-responders: 8.9 ± 1.3, 9.0 ± 0.9 and 10.4 ± 1.2 mg/kg$_{ffm}$/min).

Discussion

We demonstrate that in the 40% of individuals who respond to a VLCD by achieving fasting plasma glucose of less than 7mmol/l, remission of T2DM lasts for at least 6 months. Return to non-diabetic blood glucose levels was characterized by improvement in acute insulin secretion and this was

sustained off all hypoglycemic agents. Hepatic insulin sensitivity improved in both responders and non-responders. The structured, individualized weight maintenance program was successful in preventing weight gain.

Weight loss brought about normalization of liver fat content and insulin sensitivity in both responders and non-responders. It is notable that there was no redistribution of fat to the liver from the subcutaneous or other deposits over 6 months of weight stability even though the individuals remained obese or overweight (Table 1). This supports the concept of a personal fat threshold above which adipose tissue cannot store the available triglyceride [19]. It has major implications for the management of NAFLD. Achievement of normal liver fat content was accompanied by a 20% decrease in rate of production of $VLDL_1$-triglyceride, the lipoprotein responsible for delivery of triglyceride to all extra-hepatic cells and tissues. The observed fall in pancreas fat is a secondary consequence of decreased tissue delivery of triglyceride.

The responders differed primarily in having higher baseline plasma insulin levels and a degree of beta cell response to intravenous glucose. Recovery of acute insulin secretory capacity to non-diabetic levels [20; 21] was seen in responders and not in non-responders. The constancy of the arginine-induced insulin response implies persistence of the insulin secretory mechanism in reversible type 2 diabetes despite loss of glucose responsiveness. This is consistent with type 2 diabetes being a condition of beta cell de-differentiation rather than beta cell loss. Non-responders were characterized by evidence of insulin deficiency at baseline and lack of ability to regenerate insulin secretion capacity. In the human pancreas the magnetic resonance technique detects the total intra- and extra-cellular triglyceride in exocrine and endocrine cells and this is decreased uniquely in T2DM following weight loss [20]. The importance of pancreas triglyceride in the pathogenesis of T2DM was initially shown in obese rodents with local lipolysis bringing about fatty acid-mediated inhibition of beta cell function [22]. Exposure to even modest concentrations of fatty acids causes marked triglyceride accumulation in human islets in vitro [23]. Chronic exposure of beta cells to triglyceride or fatty acids *in vitro* decreases beta cell capacity to respond to an acute increase

in glucose levels [24; 25] and if beta cell fatty acid receptors are knocked out insulin secretion returns to normal [26]. In the human pancreas, intracellular fat droplets are widely distributed within the exocrine pancreatic cells, in addition to widely scattered isolated adipocytes [27]. Local lipolysis will bring about interstitial and intracellular concentrations of fatty acids sufficient to inhibit beta cell function, and the data suggest that removal of the excess fat allows recovery of function. We hypothesize that in the responders, release from fat-mediated inhibition allowed expression of a remaining latent capacity for glucose responsive insulin secretion.

Current concepts of T2DM have been powerfully shaped by several large studies which have demonstrated a steadily increasing requirement for hypoglycemic agents over years [2; 28; 29]. In particular, the inexorable loss of beta cell function observed during the UK Prospective Diabetes Study reinforced the view of T2DM as irreversible and progressive [3; 30]. However, progressive weight gain over time occurred during these long term observations, and hence the published data show T2DM to be irreversible only during chronic positive calorie balance. The present demonstration of ongoing reversal of T2DM (in 41% of this cohort overall or 60% of individuals with short duration diabetes) is reflected in population data which indicate that T2DM is solely a response to overnutrition. Ready access to low cost food is uniformly accompanied by high rates of T2DM [31-33] and when food supply becomes limited for any reason, the prevalence of T2DM falls [34; 35].

The question of possible therapeutic application of VLCD for T2DM was raised immediately on publication of the Counterpoint study [36]. The present data confirm reversal of T2DM at least for 6 months in those who achieve non-diabetic plasma glucose levels after VLCD. However, the critical question for health care provision is whether truly long term reversal of T2DM can be achieved in Primary Care. To answer this question, a community based study (DiRECT: Diabetes REmission Clinical Trial) is now underway of 280 people with T2DM randomized to VLCD with structured individualized weight maintenance or to best possible guideline-based care. The overall effect of the alternative approaches will be assessed, as the impact of weight loss upon blood pressure and lipids is considerable even if plasma glucose levels do not normalize. Being able to stop taking multiple

tablets is important to people with T2DM, and the potential associated healthcare savings are very great indeed.

The likelihood of individuals who respond to a VLCD remaining free of diabetes indefinitely must be considered. Following media coverage of our earlier study, many people with T2DM reversed their own diabetes [37]. For such motivated individuals who avoid weight regain, maintenance of normoglycemia for up to 3 years has been reported [37; 38]. Follow up of the LookAhead Study with 8.6% weight loss achieved remission of diabetes in a small proportion of the total group, and this was observed over 4 years [39]. As progression of long term complications of diabetes relates to ambient blood glucose control, durable reversal of diabetes would be expected to be associated with long term health. The effect of a period of normoglycemia confers substantial benefits in decreasing risk of complications even if hyperglycemia recurs [40]. Whether or not blood glucose control normalized, major benefit in vascular risk was achieved in terms of reduction of blood pressure and blood lipids. Long term prospective study of VLCD followed by a weight maintenance program is now required to define overall benefit.

The intense motivation to return to normal health in a proportion of people with type 2 diabetes has not been widely recognized. Such individuals respond readily to simple, unambiguous advice to lose weight [37]. For those people who have repeatedly failed to lose weight over many years this approach is much less likely to succeed. Severely obese subjects are selected for bariatric surgery after all other methods to lose weight have failed, and this group are appropriately treated. However, around 50% of newly diagnosed individuals in the UK have a BMI under 30kg/m^2 and in the UK Prospective Diabetes Study 36% had a BMI of less than 25kg/m2 [19]. The overall proportion of people with type 2 diabetes who will be able to succeed in the significant long term lifestyle modification required for VLCD and subsequent weight maintenance with ongoing support remains to be determined.

The VLCD was found to be acceptable as indicated by the low dropout rate in both this and the previous study [11]. The principal reason reported was the absence of hunger at this level of calorie intake. The main difficulty was re-adjusting to normal eating after the VLCD and this was mitigated by definitive prescription of food type and amount during food re-introduction and weight maintenance phase. Importantly, the need to become used to eating approximately one third less than previously had been explained in advance. The weight maintenance program, with clear focus upon calorie restriction, individual identification of potential barriers and regular contact was successful in avoiding weight gain during the 6 month follow up period. The separate effects of very low calorie intake itself and change in underlying pathophysiology were defined by the rise in plasma glucose before and after return to isocaloric eating (Figure 1).

The limitations of the study must be considered. Less than half of participants (12/30) were classified as responders on the basis of achieving a fasting glucose of <7 mmol/L on no antidiabetic medication treatment at the time point immediately after return to isocaloric diet. The study has a small sample size, although the effect size is larger than in pharmacological studies of one or more hypoglycemic agents [41], and the results are definitive. The group size was determined by our previous observations [11] in order to achieve the specific aims of the study to examine whether those who achieve non-diabetic fasting blood glucose after VLCD would remain normoglycemic during weight stability and to determine underlying mechanisms. This is not a primarily a treatment trial but rather a pathophysiologic study to achieve proof of concept. Six months follow up is sufficient to detect any redistribution of fat stores during isocaloric diet, although longer duration studies are required to define effectiveness as a routine clinical treatment. The group studied was representative of the wider T2DM population, predominantly Caucasian in the North East of England. Study of other ethnic groups is required. The heterogeneous group studied represents the spectrum of individuals with T2DM who may wish to undertake calorie restriction. A gold standard insulin secretion test was used rather than a test meal to define the acute insulin response because loss of this parameter is a characteristic of T2DM.

T2DM can now be understood to be a metabolic syndrome potentially reversible by substantial weight loss. This is an important paradigm shift. Not all people with T2DM will be willing to make the changes necessary, but for those who do, metabolic health may be regained and sustained in just under half. The observations carry profound implications for the health of individuals and for the economics of future healthcare.

Funding

Author Contributions

SS performed the clinical and metabolic studies and co-wrote the manuscript, KGH developed the magnetic resonance methodology and co-wrote the manuscript, LA delivered behavioral intervention during weight maintenance and edited the manuscript, AAM performed GC-MS analyses and edited the manuscript, BSA analysed data and edited the manuscript, MC analyzed $VLDL_1$-triglyceride data and edited the manuscript and RT designed the study and co-wrote the manuscript. The guarantor for the study is RT.

Acknowledgements

Disclosure statement

RT has received lecture fees from Novartis, NovoNordisk and Lilly, and contribution to running an EASD Workshop from Nestle Ltd. All other authors have no conflicts of interest.

Legends to Figures

Figure 1. Change in fasting plasma glucose (A), HbA1c (B) and weight (C) over the study in responders (circles) and non-responders (triangles). The grey band represents the stepped transition from VLCD to isocaloric eating of solid foods. Results are shown as mean ± SEM.

Figure 2. Hepatic triglyceride content (A), hepatic insulin resistance index (B) and hepatic VLDL-triglyceride production (C) in responders and non-responders at baseline (hatched), after VLCD (chequered) and after 6 months weight maintenance (stripes). * denotes $p<0.05$ for baseline to post-VLCD difference and # denotes $p<0.05$ for baseline to month 6 difference.

Figure 3. Change in first phase insulin response (A) and pancreas triglyceride content (B) in responders and non-responders at baseline (hatched), after VLCD (chequered) and after 6 months weight maintenance (stripes). * denotes $p<0.05$ for baseline to post-VLCD difference and # denotes $p<0.05$ for baseline to month 6 difference.

Table 1. *Fasting anthropometric and metabolic data in responders and non-responders at baseline, after the VLCD and return to isocaloric eating, and then after a 6 month weight maintenance period (* = p<0.05 for baseline to after VLCD difference; # = p<0.05 for baseline to month 6 difference and ° = p<0.05 for baseline difference between groups). HGP=hepatic glucose production; IR= insulin resistance; IS = insulin sensitivity; VAT=visceral adipose tissue; SAT=subcutaneous adipose tissue; BP= blood pressure.*

	Responders (n=12)			Non-Responders (n=17)		
	Baseline	*After VLCD*	*After 6 months*	*Baseline*	*After VLCD*	*After 6 months*
Weight (kg)	99.8±3.2	84.1±3.1	84.4±3.2	96.7±3.9	83.6±3.5	84.8±3.7
BMI (kg/m²)	34.0±0.8	28.6±0.8 *	28.7±0.7 #	34.4±1.1	29.8±1.1 *	30.2±1.1 #
Waist:hip ratio	0.97±0.02	0.93±0.02 *	0.93±0.02 #	0.96±0.02	0.91±0.01 *	0.92±0.01 #
Fat mass (%)	36.2±1.9	30.1±2.0 *	31.5±1.9 #	42.6±2.2 °	37.2±2.0 *	40.8±2.5
Serum insulin (mU/l)	20.4 (5.7-48.1)	7.9 (3.4-16.6) *	7.6 (3.1-31.6) #	9.3 (3.9-48.9) °	5.5 (1.4-22.9) *	5.9 (1.2-14.9) #
Serum ALT (U/l)	43 (11-151)	26 (18-42) *	21 (7-27) #	22 (12-61) °	19 (13-47)	18 (9-33) #
Triglycerides (mmol/l)	1.97±0.32	1.25±0.16 *	1.15±0.12 #	1.30 (0.50-8.10)	1.00 (0.60-2.00) *	1.20 (0.50-3.10) #
Non-HDL cholesterol (mmol/l)	3.6±0.3	2.8±0.3 *	2.8±0.3 #	3.3±0.3	2.7±0.3 *	2.7±0.2 #
HDL cholesterol (mmol/l)	1.1±0.1	1.1±0.1	1.4±0.1 #	1.3±0.1 °	1.3±0.1	1.5±0.1
Basal HGP (mg/kg$_{ffm}$/min)	2.6 (2.2-4.0)	2.4 (2.1-3.5)	2.7 (2.4-3.1)	3.3 (2.6-8.1)	3.0 (2.4-4.5) *	3.2 (2.2-8.6)
Hepatic IR index (µmol.min⁻¹.kg$_{ffm}$⁻¹.pmol.l⁻¹)	2153 (822-5947)	851 (305-1798)	750 (310-3723)	1237 (425-6602)	773 (156-2201)	755 (166-2397)
Muscle IS (mg/kg$_{ffm}$/min)	5.9±0.4	7.0±0.6	7.2±0.8	8.9±1.3	9.0±0.9	10.4±1.2 #
VAT area (cm²)	287.0±23.1	191.9±18.9 *	179.5 ±22.3 #	289.6±23.7	209.5±22.1 *	198.9±4.8 #
SAT area (cm²)	319.6±31.0	232.0±23.1 *	238.6±20.3 #	285.4±24.7	223.3±23.5 *	219.3±22.8 #
Systolic BP (mmHg)	142±5	129±7 *	128±5 #	159±6	139±5 *	143±6 #
Diastolic BP (mmHg)	91±2	84±4 *	82±2 #	90±2	84±2 *	85±2 #

References

1. Dall TM, Yang W, Halder P, Pang B, Massoudi M, Wintfeld N, Semilla AP, Franz J, Hogan PF: The economic burden of elevated blood glucose levels in 2012: diagnosed and undiagnosed diabetes, gestational diabetes mellitus, and prediabetes. Diabetes Care 2014;37:3172-3179

2. Hayward RA, Reaven PD, Wiitala WL, Bahn GD, Reda DJ, Ge L, McCarren M, Duckworth WC, Emanuele NV, Investigators V: Follow-up of glycemic control and cardiovascular outcomes in type 2 diabetes. The New England journal of medicine 2015;372:2197-2206

3. Turner RC, Cull CA, Frighi V, Holman RR: Glycemic control with diet, sulfonylurea, metformin, or insulin in patients with type 2 diabetes mellitus: progressive requirement for multiple therapies (UKPDS 49). UK Prospective Diabetes Study (UKPDS) Group. JAMA : the journal of the American Medical Association 1999;281:2005-2012

4. Delahanty LM, Grant RW, Wittenberg E, Bosch JL, Wexler DJ, Cagliero E, Meigs JB: Association of diabetes-related emotional distress with diabetes treatment in primary care patients with Type 2 diabetes. Diabetic medicine : a journal of the British Diabetic Association 2007;24:48-54

5. Henry RR, Schaeffer L, Olefsky JM: Glycaemic effects of intensive caloric restriction and siocaloric refeeding in non-insulin dependent diabetes mellitus. Journal of Clinical Endocrinology and Metabolism 1985;61:917-925

6. UKPDS: UK Prospective Diabetes Study 7: Response of fasting plasma glucose to diet therapy in newly presenting type II diabetic paitents. Metabolism: clinical and experimental 1990;39:905-912

7. Wing RR, Phelan S: Long-term weight loss maintenance. Am J Clin Nutr 2005;82:222S-225S

8. Gregg EW, Chen H, Wagenknecht LE, Clark JM, Delahanty LM, Bantle J, Pownall HJ, Johnson KC, Safford MM, Kitabchi AE, Pi-Sunyer FX, Wing RR, Bertoni AG, Look ARG: Association of an intensive lifestyle intervention with remission of type 2 diabetes. JAMA : the journal of the American Medical Association 2012;308:2489-2496

9. Lingvay I, Guth E, Islam A, Livingston E: Rapid improvement in diabetes after gastric bypass surgery: is it the diet or surgery? Diabetes Care 2013;36:2741-2747

10. Jackness C, Karmally W, Febres G, Conwell IM, Ahmed L, Bessler M, McMahon DJ, Korner J: Very low-calorie diet mimics the early beneficial effect of Roux-en-Y gastric bypass on insulin sensitivity and beta-cell Function in type 2 diabetic patients. Diabetes 2013;62:3027-3032

11. Lim EL, Hollingsworth KG, Aribisala BS, Chen MJ, Mathers JC, Taylor R: Reversal of type 2 diabetes: normalisation of beta cell function in association with decreased pancreas and liver triacylglycerol. Diabetologia 2011;54:2506-2514

12. Steven S, Taylor R: Restoring normoglycaemia by use of a very low calorie diet in long versus short duration type 2 diabetes. Diabetic medicine : a journal of the British Diabetic Association 2015;32:In press

13. Buse JB, Caprio S, Cefalu WT, Ceriello A, Del Prato S, Inzucchi SE, McLaughlin S, Phillips GL, 2nd, Robertson RP, Rubino F, Kahn R, Kirkman MS: How do we define cure of diabetes? Diabetes Care 2009;32:2133-2135

14. Michie S, Ashford S, Sniehotta FF, Dombrowski SU, Bishop A, French DP: A refined taxonomy of behaviour change techniques to help people change their physical activity and healthy eating behaviours: The CALO-RE taxonomy. Psychol Health 2011;

15. Groop LC, Bonadonna RC, DelPrato S, Ratheiser K, Zyck K, Ferrannini E, DeFronzo RA: Glucose and free fatty acid metabolism in non-insulin dependent diabetes mellitus: evidence for multiple sites of insulin resistance. Journal of Clinical Investigation 1989;84:205-213

16. Bjorkegren J, Packard CJ, Hamsten A, Bedford D, Caslake M, Foster L, Shepherd J, Stewart P, Karpe F: Accumulation of large very low density lipoprotein in plasma during intravenous infusion of a chylomicron-like triglyceride emulsion reflects competition for a common lipolytic pathway. J Lipid Res 1996;37:76-86

17. Al-Shayji IA, Gill JM, Cooney J, Siddiqui S, Caslake MJ: Development of a novel method to determine very low density lipoprotein kinetics. J Lipid Res 2007;48:2086-2095

18. Toschi E, Camastra S, Sironi AM, Masoni A, Gastaldelli A, Mari A, Ferrannini E, Natali A: Effect of acute hyperglycemia on insulin secretion in humans. Diabetes 2002;51 Suppl 1:S130-133

19. Taylor R, Holman R: Normal weight individuals who develop Type 2 diabetes: the personal fat threshold. Clinical Science 2015;128:405-410

20. Steven S, Hollingsworth KG, Small P, Woodcock S, Pucci A, Aribisala BS, Al-Mrabeh A, Daly AK, Batterham RL, Taylor R: Weight loss decreases excess pancreatic triacylglycerol specifically in type 2 diabetes. Diabetes Care 2015;In press

21. Hollingsworth KG, Al-Mrabeh A, Steven S, Taylor R: Pancreatic triacylglycerol distribution in type 2 diabetes. Diabetologia 2015;In press

22. Lee Y, Lingvay I, Szczepaniak LS, Ravazzola M, Orci L, Unger RH: Pancreatic steatosis: harbinger of type 2 diabetes in obese rodents. Int J Obes 2009;34:396-400

23. Lalloyer F, Vandewalle B, Percevault F, Torpier G, Kerr-Conte J, Oosterveer M, Paumelle R, Fruchart JC, Kuipers F, Pattou F, Fievet C, Staels B: Peroxisome proliferator-activated receptor alpha improves pancreatic adaptation to insulin resistance in obese mice and reduces lipotoxicity in human islets. Diabetes 2006;55:1605-1613

24. Tushuizen ME, Bunck MC, Pouwels PJ, Bontemps S, van Waesberghe JH, Schindhelm RK, Mari A, Heine RJ, Diamant M: Pancreatic fat content and beta-cell function in men with and without type 2 diabetes. Diabetes Care 2007;30:2916-2921

25. Lee Y, Hirose H, Ohneda M, Johnson JH, McGarry JD, Unger RH: B-Cell lipotoxicity in the pathogenesis of non-insulin-dependent diabetes mellitus of obese rats: impairment in adipocyte-B-Cell relationships. Proceedings of the National Academy of Science of USA 1994;91:10878-10882

26. Tang C, Ahmed K, Gille A, Lu S, Grone HJ, Tunaru S, Offermanns S: Loss of FFA2 and FFA3 increases insulin secretion and improves glucose tolerance in type 2 diabetes. Nature medicine 2015;

27. Pinnick KE, Collins SC, Londos C, Gauguier D, Clark A, Fielding BA: Pancreatic ectopic fat is characterized by adipocyte infiltration and altered lipid composition. Obesity 2008;16:522-530

28. UKPDS: Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. Lancet 1998;352:854-865

29. Group AC, Patel A, MacMahon S, Chalmers J, Neal B, Billot L, Woodward M, Marre M, Cooper M, Glasziou P, Grobbee D, Hamet P, Harrap S, Heller S, Liu L, Mancia G, Mogensen CE, Pan C, Poulter N, Rodgers A, Williams B, Bompoint S, de Galan BE, Joshi R, Travert F: Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. The New England journal of medicine 2008;358:2560-2572

30. U.K. prospective diabetes study 16. Overview of 6 years' therapy of type II diabetes: a progressive disease. U.K. Prospective Diabetes Study Group. Diabetes 1995;44:1249-1258

31. Zimmet P, Alberti KG, Shaw J: Global and societal implications of the diabetes epidemic. Nature 2001;414:782-787

32. Knowler WC, Bennett PH, Hamman RF, Miller M: Diabetes incidence and prevalence in Pima Indians: a 19 fold greater incidence than in Rochester Minnesota. American Journal of Epidemiology 1978;108:497-505

33. Farrell GC, Wong VW, Chitturi S: NAFLD in Asia--as common and important as in the West. Nature reviews Gastroenterology & hepatology 2013;10:307-318

34. Himsworth HP: Diet in the aetiology of human diabetes. Proc Royal Soc Med 1949;42:323-326

35. Franco M, Bilal U, Ordunez P, Benet M, Morejon A, Caballero B, Kennelly JF, Cooper RS: Population-wide weight loss and regain in relation to diabetes burden and cardiovascular mortality in Cuba 1980-2010: repeated cross sectional surveys and ecological comparison of secular trends. Brit Med J 2013;346:f1515

36. Yki-Järvinen H: Type 2 diabetes: remission in just a week. Diabetologia 2011;54:2477-2479

37. Steven S, Lim E, Taylor R: Population response to information on reversibility of type 2 diabetes. Diabetic Medicine 2013;30:e135-138

38. Peters C, Steven S, Taylor R: Reversal of type 2 diabetes by weight loss despite presence of macro-and micro-vascular complications. In *Diabetes Case Studies: Real Problems, Practical Solutions* Draznin B, Ed. Alexandria, VA, American Diabetes Association, 2015

39. Ryan DH, Espeland MA, Foster GD, Haffner SM, Hubbard VS, Johnson KC, Kahn SE, Knowler WC, Yanovski SZ, Look ARG: Look AHEAD (Action for Health in Diabetes): design and methods for a clinical trial of weight loss for the prevention of cardiovascular disease in type 2 diabetes. Controlled clinical trials 2003;24:610-628

40. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA: 10-year follow-up of intensive glucose control in type 2 diabetes. The New England journal of medicine 2008;359:1577-1589

41. Mingrone G, Panunzi S, De Gaetano A, Guidone C, Iaconelli A, Nanni G, Castagneto M, Bornstein S, Rubino F: Bariatric-metabolic surgery versus conventional medical treatment in obese patients with type 2 diabetes: 5 year follow-up of an open-label, single-centre, randomised controlled trial. Lancet 2015;386:964-973
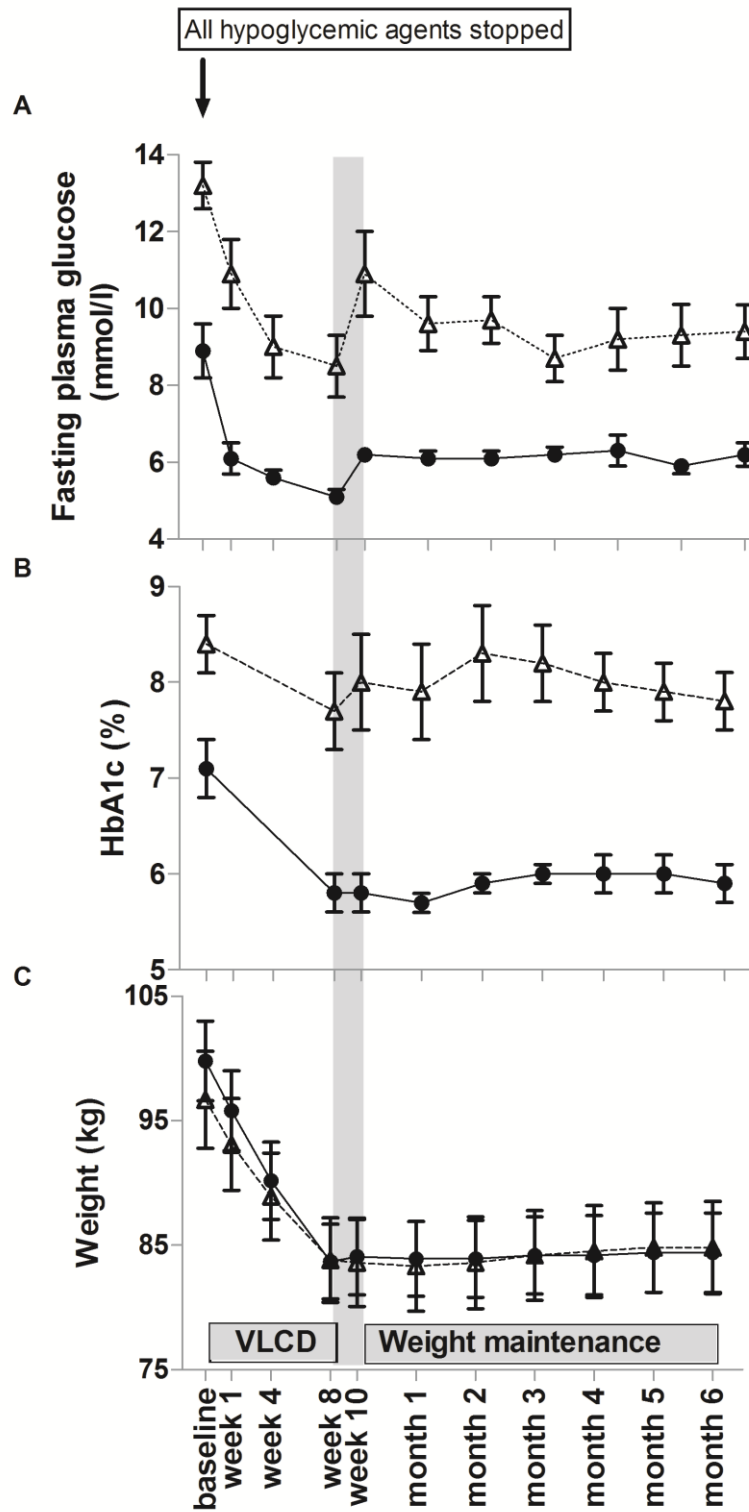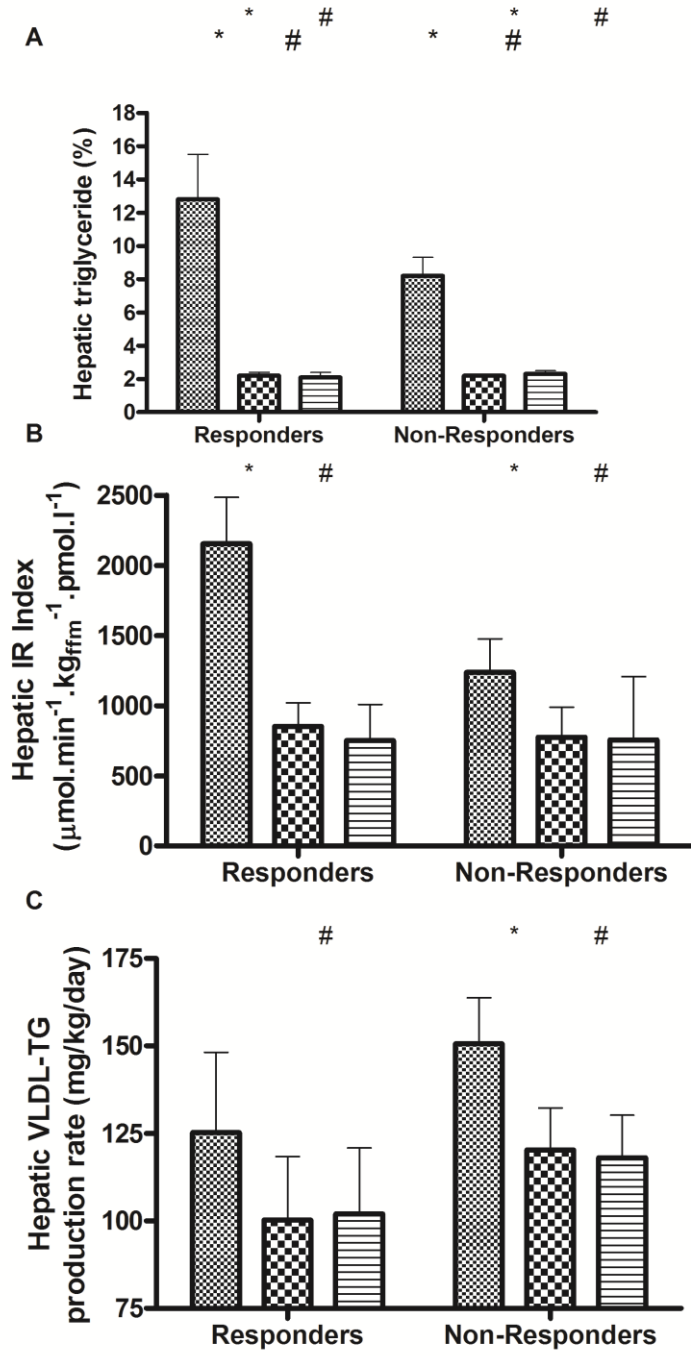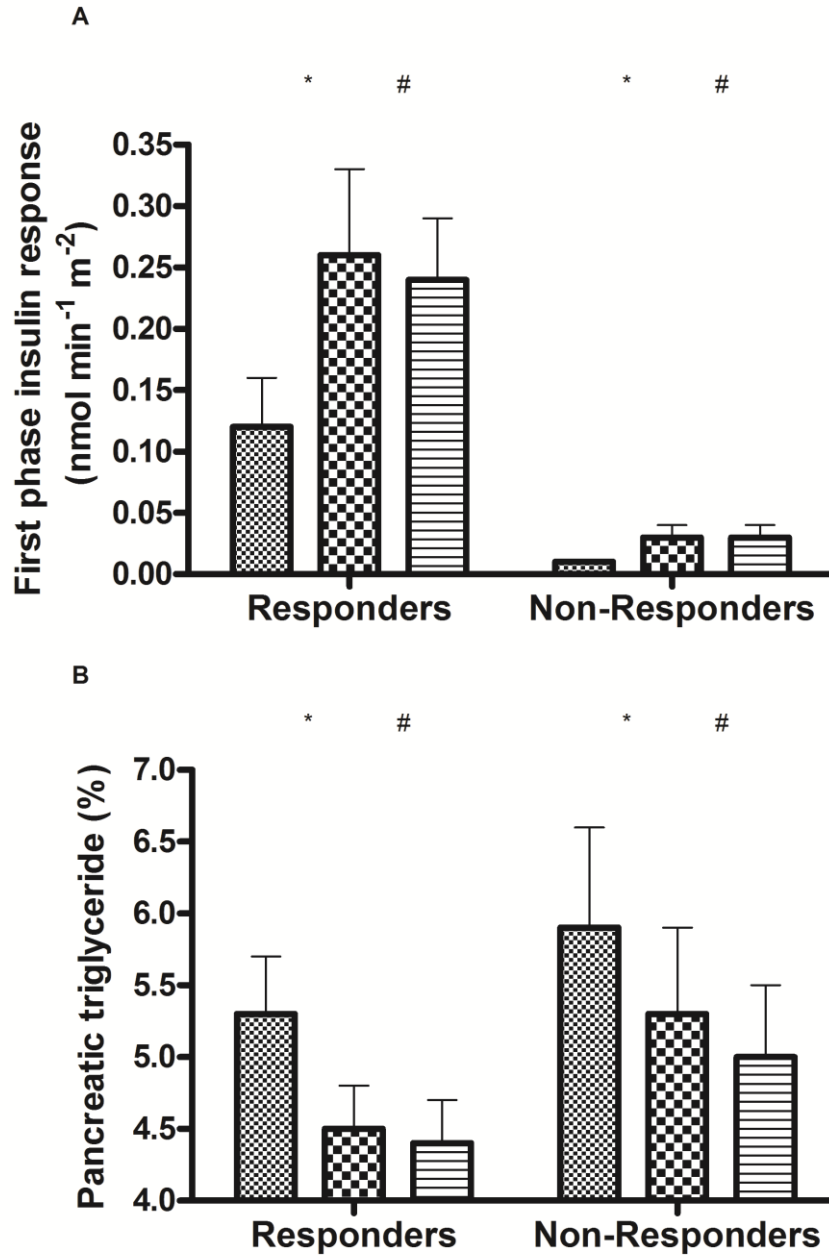
Figure 1

Figure 2

A

B

Figure 3