
Weight Loss Decreases Excess Pancreatic Triacylglycerol Specifically in Type 2 Diabetes.

Diabetes Care 2016, 39(1), 158-165.

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DOI link to article:

http://dx.doi.org/10.2337/dc15-0750

Date deposited:

19/04/2016
Weight loss decreases excess pancreatic triacylglycerol specifically in type 2 diabetes

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Abbreviated title: Weight loss and normalisation of pancreatic triacylglycerol content

Clinical trial number ISRCTN11969319
Key words: VLCD, bariatric surgery, intra-organ fat, metabolism

Word counts: Abstract 245; Manuscript: 3994
Number of Figures 3; Tables 1; Supplementary Figure 1

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Abstract

Objective: To determine whether the decrease in pancreatic triacylglycerol during weight loss in type 2 diabetes is simply reflective of whole body fat, or specific to diabetes and associated with the simultaneous recovery of insulin secretory function.

Research Design and Methods: Individuals listed for gastric bypass surgery who had either type 2 diabetes or normal glucose tolerance matched for age, weight and gender were studied before and 8 weeks after surgery. Pancreas and liver triacylglycerol were quantified using in-phase, out-of-phase magnetic resonance imaging. First phase insulin response to a stepped intravenous glucose infusion, hepatic insulin sensitivity and glycemic and incretin responses to a semi-solid test meal were also measured.

Results: Weight loss after surgery was similar (NGT: 12.8±0.8% and T2DM: 13.6±0.7%) as was change in fat mass (56.7±3.3 to 45.4±2.3 vs. 56.6±2.4 to 43.0±2.4kg). Pancreatic triacylglycerol did not change in NGT (5.1±0.2 to 5.5±0.4%) but decreased in the T2DM group (6.6±0.5 to 5.4±0.4%; p=0.007). First phase insulin response to a stepped intravenous glucose infusion did not change in NGT (0.24 (0.13-0.46) to 0.23 (0.19-0.37) nmol min\(^{-1}\) m\(^{-2}\)) but normalised in T2DM (0.08 (-0.01-0.10) to 0.22 (0.07-0.30) nmol min\(^{-1}\) m\(^{-2}\) at week 8 (p=0.005). No differential effect of incretin secretion was observed post-gastric bypass, with more rapid glucose absorption bringing about equivalently enhanced GLP-1 secretion in the two groups.

Conclusions: The fall in intra-pancreatic triacylglycerol in type 2 diabetes which occurs during weight loss is associated with the condition itself rather than decreased total body fat.
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 condition is widely recognised to be caused by a combination of insulin resistance and insulin secretory failure. However, insulin resistance alone does not cause blood glucose to rise [2] and type 2 diabetes occurs only when the acute insulin response of pancreatic beta cells becomes inadequate to control blood glucose [3; 4]. The etiologic process underlying this is still uncertain. Inhibition by excess intracellular fatty acids or their metabolites is a potential mechanism [5-7]. We have previously demonstrated in people with T2DM that weight loss over 8 weeks can normalize both the acute insulin response and intrapancreatic triacylglycerol concentration [8]. The resulting normoglycemia persists providing that weight regain is avoided [9]. These observations have confirmed some aspects of the twin cycle hypothesis of etiology of T2DM [10].

It remains uncertain whether the change in intra-pancreatic triacylglycerol is specific to the diabetes itself or whether it simply reflects decrease in whole body fat content and would occur during any substantial weight loss. Comparison of changes in T2DM and normal glucose tolerance (NGT) during weight loss could define those changes specific to the recovery of insulin secretory capacity. Achieving equivalent dietary weight loss in NGT individuals who do not have the motivation of potentially reversing their diabetes to normal would be challenging. Gastric bypass surgery for obesity produces reliable weight loss and permits detailed comparison of the pathophysiologic changes in T2DM and NGT groups.

Major changes in incretin hormones occur after RYGB which could potentially contribute to the observed increase in meal related insulin secretion [11]. Given the subnormal glucagon-like peptide 1 (GLP-1) response to food ingestion in T2DM [12],
RYGB may exert a specific effect in T2DM which differs from that in non-diabetic subjects. Some studies have supported this concept [13] although similar restoration of normoglycemia has been observed after calorie restriction alone [14]. Few studies have compared the effect of gastric bypass in T2DM and NGT both on physiologic incretin function and incretin-independent, intravenous glucose mediated insulin secretion.

The aims of this study were to test the hypotheses that the restoration of first phase insulin secretion after Roux-en-Y gastric bypass surgery (RYGB) would be accompanied by decrease in pancreatic triacylglycerol specifically in T2DM, and that the post-surgery change in incretin hormone responses would not differ in T2DM and NGT. As change in pancreas triacylglycerol must reflect export of very low density lipoprotein (VLDL) triacylglycerol from the liver, hepatic triacylglycerol content and hepatic insulin sensitivity were also assessed.

**Research Design and Methods**

**Participants**

Individuals listed for laparoscopic RYGB were identified from two regional bariatric surgery centres. Individuals with T2DM (n=18) were recruited with diabetes duration<15 yr, aged 25-65 yr, BMI up to 45 kg/m² (due to scanner constraints), HbA1c<10% (86 mmol/mol) and no significant renal or hepatic dysfunction (creatinine<150 µmol/l; alanine aminotransferase (ALT) <2.5-fold above upper limit of normal). Exclusion criteria were: contraindication to magnetic resonance (MR) scanning; alcohol consumption >14 units/wk; previous bowel surgery; or treatment with steroids, thiazolidinediones or GLP-1 analogues. The T2DM (n=18) and NGT individuals (n=9) were group matched for age and weight (49.1±1.6 vs. 46.3±2.1yr, 121.0±3.0 vs. 114.5±5.0 kg, 11F:7M, 7F:2M respectively) and NGT confirmed by 75g oral glucose tolerance test. Impaired glucose tolerance was found in 2 subjects and
screening was continued until the planned group size of 9 NGT was achieved. In the T2DM group, there were 3 individuals on insulin and 9 individuals on SU at recruitment. The study protocol was approved by the Newcastle upon Tyne 1 Research Ethics Committee. All participants provided written informed consent. One individual did not undergo surgery following baseline studies due to the diagnosis of an unrelated medical problem.

**Experimental Protocol**

The T2DM and NGT participants were studied just before surgery and at 8 weeks post-operatively. Pre-operatively all participants were asked to follow a hypocaloric (approximately 1200kcal) diet for 7-10 days. At each time point, metabolic and incretin responses to a standard meal test, first phase and maximal insulin secretion and pancreas and liver triacylglycerol content by MR scanning were measured. Participants were asked to stop metformin and/or sulfonylureas at least 72 hours prior to the first study, or to stop insulin at least 24 hours prior and all remained off hypoglycemic agents thereafter. Intensive physical activity/alcohol/caffeine intake were avoided 48 hours prior to each study. All metabolic studies were performed after a 10h overnight fast.

**Surgery**

RYGB was performed laparoscopically in all patients. A biliopancreatic limb of 50-70cms from the duodenoejejunal flexure was anastomosed to the 30-50ml gastric pouch. An alimentary limb of 100-150 cms was then measured and a side to side antimesenteric jejuno-jejunostomy carried out. At the time of operation, 2 patients with type 2 diabetes underwent sleeve gastrectomy instead of RYGB due to the
presence of significant intra-abdominal adhesions. These 2 patients have been excluded from the incretin analyses.

**Body composition and anthropometry**

Body composition was determined using a Bodystat®1500 (Bodystat Ltd, Isle of Man, UK). Waist and hip circumferences were measured using a standard non-distensible tape measure and height by stadiometer by one observer (SS).

**Meal test**

Each test was performed with the participant semi-reclined at a 45° angle in bed to avoid positional change affecting gastric emptying. Baseline blood samples were taken at -10 and 0 min. Subjects were then asked to consume a semi-solid meal within 3 minutes (10g Mornflake Instant Porridge Oats, 64g whole milk and 6g acacia honey: 100 kcal; 57% carbohydrate; 28% fat; 13% protein), designed in accordance with the expected volume and consistency of diet consumed one week following RYGB. Samples for glucagon, GLP-1 and gastrointestinal peptide (GIP) were taken into chilled EDTA tubes containing trasylol. All samples were immediately centrifuged at 4°C and the plasma separated into aliquots and frozen at -40°C until analysis. Samples were taken every 10 min for the first 30 min of the test, then every 30 min until 2 hours.

**Measurement of intra-organ triacylglycerol content**

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 [15] with gradient-echo scans acquired during four 17 second breath holds (repetition time (TR)/echo time /averages/flip angle = 50ms/3.45, 4.60, 5.75ms/1/5°. Critically, participant cooperation was maximised by careful explanation from research radiographers. A matrix size of 160×109 and with a field view of 400-480 mm was used according to volunteer size. The liver data were acquired with slice thickness 10mm and the pancreas data with slice thickness 5mm. The triacylglycerol and water contributions of the MRI signal were separated by mathematical modelling of their known chemical shifts using an in-house programme written in MATLAB, with the triacylglycerol content in the images expressed as a percentage of the total signal in each pixel. The intraorgan triacylglycerol 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). As the analysis is image-based, selection of regions of interest ensures no inclusion of visceral adipose tissue and the measurement is specifically taken from the parenchyma of the pancreas avoiding any incursion of adipose tissue. The pancreas triacylglycerol analysis was carried out blinded to subject status and timepoint.

Hepatic glucose production and insulin sensitivity

[6′6′-2H] glucose (98% enriched; Cambridge Isotope Laboratories, MA, USA) was used to determine hepatic glucose production [16]. Basal rates were calculated during the last 30 min of the 150 min basal period. The hepatic insulin resistance index was derived from the product of fasting plasma insulin and fasting hepatic glucose production [17]. An isoglycemic–hyperinsulinemic clamp (insulin infusion rate 40 mU m⁻² min⁻¹) was initiated at 0 min. Each participant was clamped at the glucose level
observed at the end of the basal period. Isoglycemia was used 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 as whole-body glucose disposal per kg of fat free mass corrected for glucose space and urinary loss [18]. In order to correct for the difference in fasting glucose levels during the course of the study, whole body insulin sensitivity was expressed as glucose metabolic clearance by dividing the whole-body glucose disposal rate by steady-state plasma glucose.

Stepped insulin secretion test with arginine (SISTA)

Sixty minutes after the clamp test, when glucose levels had stabilised 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 [19]. Blood samples for determination of plasma glucose, insulin and 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, followed by sampling every 2 min for 10 min. Insulin secretion rate was calculated using a computerised program implementing a regularisation method of deconvolution and using a population model of C-peptide kinetics as previously described [8].

Analytical procedures

Plasma glucose was measured by the glucose oxidase method (YSI glucose analyser, Yellow Springs, OH). Serum insulin was measured using ELISA kits (DAKO; Ely, Cambridge, UK). Serum C-peptide was measured using ELISA kits (DAKO; Ely,
Cambridge, UK or Mercodia; Uppsala, Sweden with correction factor to ensure comparability). Plasma NEFA concentration was measured using a FLUOstar Omega microplate reader (BMG labtech; Ortenberg, Germany) by a commercially available enzymatic calorimetric kit (NEFA HR Reagent 1 and 2; Alpha laboratories, Eastleigh, Hampshire, UK). β-Hydroxybutyrate levels were measured to confirm dietary compliance using the Optium Exceed ketone meter (Abbott Diabetes Care, Oxfordshire, UK). [6′-2H] glucose was measured using Gas Chromatography Mass Spectrometry (GC/MS) technique on a Thermo ‘Voyager’ single quadruple mass spectrometer connected to a Thermo Finnigan Trace 2000 gas chromatograph (Thermo Scientific, Waltham, MA, USA). HbA1c, LFTs, gamma glutamyl transferase (GGT), and lipids were measured at a Clinical Pathology Accredited laboratory (Newcastle upon Tyne Hospital NHS Foundation Trust, Department of Clinical Biochemistry). Human Total GLP-1 (7-36, 9-36) was measured using ELISA kits (Alpco Diagnositics; Salem, NH, USA). Human Total GIP was measured using ELISA kits (Merck Millipore, Watford, UK). PNPLA3 genotyping was performed on DNA extracted from white blood cells. 10 ml of whole blood was collected in EDTA and after thorough mixing was then stored at -40°C. DNA was isolated and genotyping performed (blinded to the clinical parameters) using TaqMan SNP Genotyping Analysis (Applied Biosystems, USA) as described previously [20].

Statistical Analysis

Data are presented as mean ± SEM for parametric and median (range) for non-parametric data. Insulin secretion rates are given as median with 25th and 75th percentile. Statistical analysis used Student’s paired and unpaired t-test, Mann
Whitney U, Wilcoxon Rank and Spearman Rank as appropriate using Minitab 16 statistical program (www.minitab.com).

**Results**

**Weight loss**

Pre-operative weight did not differ between the T2DM and NGT groups (121.1±3.0 vs. 114.5±5.0 kg; p=0.244). At 8 post-operative weeks weight loss was similar in the two groups (13.6±0.7 % and 12.8±0.8 % respectively; p=0.286) as was change in total body fat content (Table 1).

**Plasma glucose, insulin and metabolites**

Fasting plasma glucose decreased from 9.4±0.8 pre-surgery to 6.4±0.4 mmol/l at week 8 in the T2DM group (p<0.001) and from 5.2±0.2 to 4.9±0.1 mmol/l in the NGT group (p=0.196). HbA1c decreased from 7.6±0.4 to 6.2±0.2 % (59±4 to 44±2mmol/mol) in the T2DM group (p<0.001) compared to 5.4±0.1 to 5.2±0.1 % (36±1 to 33±1mmol/mol) in the NGT group (p=0.01).

Fasting insulin levels fell in both groups (T2DM: 15.3 (4.3-61.2) to 11.3 (2.9-27.0) mU/l (p<0.001); NGT: 10.7±1.4 to 6.7±0.7 mU/l (p<0.01). There were significant decreases in fasting triacylglycerol, ALT and GGT in the T2DM but not the NGT group (Table 1). Fasting beta-hydroxybutyrate increased from 0.20 (0.00-0.70) to 0.30 (0.10-1.00) in the T2DM group (p=0.011) and from 0.33±0.08 to 0.55±0.15 in NGT (p=0.07). There was no difference in change from pre-surgery to week 8 in beta-hydroxybutyrate between the 2 groups ( -0.2 (-0.6-0.5) NGT -0.1 (-1-0); p=0.98).
**Pancreas triacylglycerol content**

Pancreatic triacylglycerol content was higher pre-surgery in the T2DM group compared to the NGT group (6.6±0.5 vs. 5.1±0.2 %; p=0.009). By week 8, pancreatic triacylglycerol content had decreased in the T2DM group to levels similar to the NGT group (6.6±0.5 to 5.4±0.4 %; p=0.007) but with no change in the NGT group (5.1±0.2 to 5.5±0.4 %; p=0.437) (Figure 1B) despite comparable decrease in whole body fat mass (Table 1).

**Change in insulin secretion**

Pre-surgery, the first phase insulin response (baseline to 6 min insulin secretion rate) in T2DM was severely impaired compared to the NGT group (0.08 (-0.01-0.10) vs. 0.24 (0.13-0.46); p=0.011; Figures 1A & 2B). There was marked restoration of the first phase insulin response in T2DM post-surgery: increasing to 0.22 (0.07-0.30) nmol min⁻¹ m² at week 8 in the T2DM group (p=0.005; Figures 1A & 2C). There was no change in first phase insulin response in the NGT group: 0.24 (0.13-0.46) at baseline and 0.23 (0.19-0.37) nmol min⁻¹ m² at week 8 (p=0.464; Figures 1A, 2B & 2C). Arginine-induced insulin response in the T2DM group was 0.80 (0.70-0.90) at baseline and 0.71 (0.50-1.15) at week 8 (p=0.567), and in the NGT group was 0.85 (0.71-1.21) at baseline and 0.62 (0.55-1.28) at week 8 (p=0.896).

**Hepatic triacylglycerol content and liver enzymes**

Pre-surgery, hepatic triacylglycerol content was over 2-fold higher in the T2DM group compared to the NGT group (9.3±1.5 vs. 4.2±1.4 %; p=0.022; Supplemental Figure 1A). Post-surgery, it decreased to a greater extent in the T2DM group (T2DM:
9.3±1.5 to 5.2±0.8 % (p=0.018); NGT: 4.2±1.4 to 2.3±0.6 % (p=0.059). These changes were reflected in the fall in serum ALT and GGT following surgery in the T2DM group only (Table 1).

Hepatic insulin sensitivity

Basal hepatic glucose production in T2DM decreased post-surgery (3.60±0.24 to 2.69±0.12 mg/kg_{ffm}/min; p<0.001; Supplemental Figure 1B). There was no significant change in the NGT group (2.60±0.08 to 2.51±0.20 mg/kg_{ffm}/min; p=0.555). Hepatic insulin sensitivity improved in the T2DM group: hepatic IR index 2.76±0.41 to 1.33±0.23 mmol.min⁻¹.kg_{ffm}⁻¹.pmol.l⁻¹ (p=0.002); NGT group: 1.18±0.19 to 0.70±0.07 mmol.min⁻¹.kg_{ffm}⁻¹.pmol.l⁻¹ (p=0.062)(Supplemental Figure 1C). The insulin induced suppression of hepatic glucose production was greater in the T2DM group at 8 weeks post-RYGB: 67±4 to 85±3 %; p<0.001, with no change in the NGT group: 84±4 to 77±8 %; p=0.339.

Peripheral tissue insulin sensitivity

Insulin stimulated glucose metabolic clearance did not change in either group: 2.46 (0.86-8.80) to 2.69 (0.45-10.07) ml/kg_{ffm}/min in T2DM (p=0.223) and 4.51±0.63 to 4.79±0.70 ml/kg_{ffm}/min in NGT (p=0.572). Both before surgery (p=0.033) and after surgery (p=0.024) peripheral insulin sensitivity was significantly lower in the T2DM group.

Subcutaneous and visceral fat data

Pre-surgery, there was no difference between subcutaneous adipose tissue (SAT) area in T2DM compared to NGT (453.8±28.9 cm² vs. 496.4±16.0 cm²; p=0.318). Visceral
adipose tissue (VAT) area was 300.4±17.5 cm$^2$ in T2DM compared to 244.5±28.4 cm$^2$ in NGT (p=0.09). In T2DM, SAT decreased to 393.2±26.8 cm$^2$ at week 8 (p<0.001) and VAT to 241.3±11.0 cm$^2$ (p<0.001). In NGT, SAT decreased to 409.7±26.0 cm$^2$ (p=0.016) and VAT decreased to 187.9±28.3 cm$^2$ (p=0.01).

*Change in meal tolerance test*

As a result of the gastroenterostomy, the rise in plasma glucose over the first 20 min of the meal test was greater in both the T2DM and NGT groups (0.6±0.1 pre-operatively to 1.8±0.1 mmol/l post-operatively (p<0.001) and 0.5±0.1 to 1.7±0.2 mmol/l (p=0.004) respectively) (Figure 3A). There was a significant difference in decrease of peak glucose between T2DM and NGT (1.84 (-1.06-11.5) vs. -0.66 (-1.73-0.34); p<0.001). 2 hour post-meal glucose was lower in both groups: 9.4±0.8 to 6.4±0.3 mmol/l in the T2DM group (p<0.001) and 5.5±0.2 to 5.0±0.0mmol/l in the NGT group (p=0.022). The change in 2 hour post-meal glucose between T2DM and NGT was also significant: (1.87 (-0.02-11.49) vs. 0.41 (-0.26-0.91); p<0.001).

The incremental rise in plasma insulin over the first 20 minutes increased in both groups at 8 weeks post-RYGB, with a higher and earlier peak plasma insulin being achieved (T2DM pre-operatively: 35.2±4.9 mU/l at 60 (10-120) min vs. week 8: 47.7±5.8 mU/l at 20 (10-30) min; p=0.01; NGT pre-operatively: peak insulin 37.0±5.0 mU/l at 60 (30-120) min vs. week 8: 58.1±10.0 mU/l at 20 (20-30) min; p=0.032) (Figure 3B).

Peak GLP-1 levels during the meal test increased from 5.0±0.3 to 12.7±1.3 pmol/l in the T2DM group (p<0.001) and 5.1±0.6 to 12.9±1.2 pmol/l in the NGT group.
Peak GIP levels increased from 197.6±18.1 to 246.2±24.4 pg/ml in the T2DM group (p=0.051) but did not change in the NGT group: 206.7±28.6 to 231.2±32.6 pg/ml (p=0.482). There was an earlier rise in GIP in both groups (Figure 3D).

**PNPLA3**

In the whole group (n=26), the rs738409 C to G adiponutrin/PNPLA3 genotype (coding for I148M) was found in 9 individuals: 8 were heterozygous for the SNP: CG (148I/M) and 1 homozygous: GG (148M/M), 6 of whom had T2DM and 3 of whom were NGT. In the T2DM group, mean baseline liver triacylglycerol content was 8.7±1.9 vs 10.6±2.3 % (p=0.59) in those with CC vs. CG/GG. At 8 weeks post-operatively this was 3.9±0.5 and 8.2±1.9 % respectively (p=0.006). The GG homozygous T2DM individual had liver triacylglycerol of 4.1% pre-operatively and 6.1% postoperatively.

**Conclusions**

Despite similar weight loss following bariatric surgery in groups of well-matched individuals with T2DM or NGT, intra-pancreatic triacylglycerol decreased uniquely in T2DM. This was associated with normalisation of first phase insulin secretion in the T2DM group. There was no change in intra-pancreatic triacylglycerol in the NGT group despite a 5 unit decrease in BMI. Hepatic insulin sensitivity both fasting and during insulin stimulation normalised in the T2DM group in step with a greater decrease in liver triacylglycerol compared to the NGT group. The meal-related rise in
plasma glucose was faster in both groups after RYGB and there was an equivalently enhanced GLP-1 response.

T2DM develops as a consequence of positive calorie balance over many years and ectopic fat storage appears to be central to the process [10]. The importance of pancreas triacylglycerol in the pathogenesis of T2DM was initially suggested by a study in obese rodents [21]. In humans with T2DM supranormal pancreas triacylglycerol content decreases as weight loss allows recovery of first phase insulin secretion [22]. Identification of the location of triacylglycerol within the pancreas has been hampered by rapid post-mortem autolysis, but study of pancreata retrieved and not used for pancreas transplantation has shown intracellular fat droplets widely distributed within the exocrine cells, in addition to widely scattered isolated adipocytes [23]. Exposure to even modest concentrations of fatty acids causes marked triacylglycerol accumulation in human islets in vitro [7]. It is likely that local lipolysis is likely to bring about interstitial and intracellular concentrations of fatty acids sufficient to inhibit beta cell function. Fatty acid receptors are expressed in mouse and human pancreatic beta cells and when knocked out allow recovery of insulin secretion [24]. However, the change in pancreas triacylglycerol content demonstrated during reversal of T2DM is small in absolute terms (~1% of the pancreas volume) and consistent with change in intracellular triacylglycerol content. Cross sectional studies are relatively insensitive and there are ethnic differences in intrapancreatic triacylglycerol [25; 26]. Decreased insulin secretion after oral glucose has been observed to reflect increased pancreas triacylglycerol in non-diabetic individuals [22].
The method used to quantify intra-pancreatic triacylglycerol must be considered. MR spectroscopy gathers chemical information from a pre-defined volume, and with careful application physiologically relevant data can be acquired [26]. If the volume selected is too large it is likely that visceral fat will be included in the measurement [27]. Use of the 3-point Dixon imaging method avoids this problem as chemical information is derived post-acquisition and placement of the volume of interest is guided by the image [8]. However, care is required in ensuring validity of the method as otherwise serious errors including negative numbers for percentage tissue triacylglycerol may be derived [27]. In the present study we have used a reproducible, robust method with blinded analysis. At 8 weeks post-surgery there was a 15% decrease in pancreas triacylglycerol levels in T2DM to the same level as NGT individuals, demonstrating that the increase in the fat content of the pancreas is specific to the condition rather than being a reflection of obesity per se. This weight loss associated decrease in pancreas triacylglycerol content occurred at the same time as the recovery in first phase insulin secretion, as previously observed following a very low calorie diet [8].

Fasting plasma glucose concentration is determined by the rate of hepatic glucose production [16] which in turn is controlled by insulin [28]. Hepatic insulin sensitivity is known to be impaired by increased liver triacylglycerol [17; 29]. Short term carbohydrate overfeeding can induce liver triacylglycerol accumulation [30] and furthermore, weight loss with consequent reduction in liver fat is associated with improvements in insulin sensitivity and fasting plasma glucose levels [16; 31]. The present study demonstrates a greater reduction in liver fat content post-RYGB in individuals with T2DM compared to NGT with normalisation of hepatic insulin sensitivity. The similarity in change in liver triacylglycerol and hepatic insulin
sensitivity in both groups was striking. In NGT, endogenous glucose production falls after bariatric surgery when baseline liver triacylglycerol is high [32]. Individual differences in susceptibility to the adverse metabolic effects of intra-hepatic fat are implied by the range of baseline liver triacylglycerol in the T2DM group. Data from the UKPDS on individuals with normal BMI supports the concept of a variable personal fat threshold of susceptibility to develop and reverse T2DM [33]. The PNPLA3 polymorphism, measured because this is a specific and known factor determining intra-hepatic triacylglycerol, blunted the weight-loss associated decrease in liver fat.

The markedly increased nutrient-stimulated secretion of GLP-1 after RYGB is well recognised, and the present observations confirm this. Normalisation of the first phase insulin response to intravenous infusion of glucose demonstrates the improvement to be independent of acute incretin stimulation. After RYGB, a 2.6-fold increase of insulin secretion assessed by IVGTT disposition index has been reported [34]. VLCD or RYGB result in similarly increased insulin secretion in both groups despite a marked increase in GLP-1 after RYGB only [35]. A specific GLP-1 receptor antagonist does not affect insulin secretion post RYGB [36; 37].

Nutrients pass rapidly into the mid-jejunum post RYBG [38]. Most studies have used an oral glucose challenge [11; 34] or liquid meal [39] with very rapid absorption and a non-physiological incretin stimulus [36]. The present study used a semi-solid meal to minimise the rapid nutrient entry into the jejunum, but even so a more rapid rise in glucose levels was observed after surgery in both T2DM and NGT. The GIP peak after test meal was earlier and greater in both groups post-RYGB. Overall, it appears
that acute post-meal enhanced incretin secretion does not explain the improved beta cell function in T2DM following bariatric surgery.

The limitations of the study must be discussed. The group sizes were sufficient to achieve clear statistical significance, and although smaller numbers of NGT were studied the range of responses within this group was small. Although the T2DM group were unselected in terms of diabetes duration and treatments, this is representative of the heterogeneous population undergoing bariatric surgery. We measured total rather than active GLP-1, although the responses of active and total GLP-1 are tightly correlated [40].

In summary, T2DM individuals exhibit an attenuated first phase insulin response and increased pancreatic triacylglycerol compared to BMI-matched NGT individuals. 8 weeks after bariatric surgery both first phase insulin response and pancreas triacylglycerol normalised uniquely in the T2DM group. GLP-1 response after a semi-solid meal improved equally in T2DM and NGT. These observations support the concept of intra-pancreatic triacylglycerol and metabolites being central to the etiology of T2DM. The understanding of T2DM as a disease of fat accumulation above a personal threshold lays the foundation both for more appropriate clinical management.

Author Contributions
SS performed the studies, analysed the data and wrote the manuscript. KGH, PKS and SW designed the study and edited the manuscript, AP performed the incretin analyses and edited the manuscript, BA performed the mathematical modelling of insulin secretion rates and edited the manuscript, AA performed GC-MS analyses and edited the manuscript, AKD oversaw PNPLA3 genotyping and edited the manuscript, RB edited the manuscript, and RT designed the study and edited the manuscript. No others assisted with writing or editing the manuscript. RT is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Acknowledgements

We are grateful to the participants for their enthusiastic contribution to the work, L Hughes, A Burnett and T Dew for hormone assay, and L Ward, T Hodgson and T Gaudi for expert radiography. The study was funded by an EFSD/Lilly European Diabetes Research Programme Grant.

Disclosure statement: the authors have no conflicts of interest.
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20. Liu YL, Patman GL, Leathart JB, Piguet AC, Burt AD, Dufour JF, Day CP, Daly AK, Reeves HL, Anstee QM: Carriage of the PNPLA3 rs738409 C >G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma. J Hepatol 2014;61:75-81
<table>
<thead>
<tr>
<th></th>
<th>T2DM Before surgery</th>
<th>T2DM After surgery</th>
<th>p</th>
<th>NGT Before Surgery</th>
<th>NGT After Surgery</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>121.1±3.0</td>
<td>104.5±2.7</td>
<td>&lt;0.001</td>
<td>114.5±5.0</td>
<td>99.7±4.6</td>
<td>&lt;0.001</td>
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<td>BMI (kg/m²)</td>
<td>42.7±0.7</td>
<td>36.9±0.7</td>
<td>&lt;0.001</td>
<td>41.3±1.0</td>
<td>36.4±0.8</td>
<td>&lt;0.001</td>
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<td>Fat mass (kg)</td>
<td>56.6±2.4</td>
<td>43.0±2.4</td>
<td>&lt;0.001</td>
<td>56.7±3.3</td>
<td>45.4±2.3</td>
<td>&lt;0.001</td>
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<tr>
<td>Waist:Hip ratio</td>
<td>0.97±0.02</td>
<td>0.94±0.02</td>
<td>0.006</td>
<td>0.90±0.03 *</td>
<td>0.87±0.03 #</td>
<td>0.066</td>
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<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>9.2±0.8</td>
<td>6.2±0.3</td>
<td>&lt;0.001</td>
<td>5.2±0.1</td>
<td>4.9±0.1</td>
<td>0.089</td>
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<tr>
<td>2hr plasma glucose (mmol/l)</td>
<td>9.4±0.8</td>
<td>6.4±0.3</td>
<td>&lt;0.001</td>
<td>5.4±0.2 *</td>
<td>5.0±0.0 #</td>
<td>0.022</td>
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<tr>
<td>Fasting serum insulin (mU/l)</td>
<td>15.3 (4.3-61.2)</td>
<td>11.3 (2.9-27.0)</td>
<td>&lt;0.001</td>
<td>11.0±1.6</td>
<td>6.7±0.7 #</td>
<td>0.008</td>
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<tr>
<td>2hr serum insulin (mU/l)</td>
<td>18.4 (5.2-78.9)</td>
<td>11.2 (4.9-31.0)</td>
<td>0.001</td>
<td>12.4 * (4.4-63.5)</td>
<td>6.0 # (5.0-7.4)</td>
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<td>Fasting glucagon (ng/l)</td>
<td>74.6±9.8</td>
<td>58.0±8.3</td>
<td>0.001</td>
<td>49.4±5.0</td>
<td>48.1±5.0</td>
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<td>Fasting FFA (mmol/l)</td>
<td>0.85±0.08</td>
<td>0.77±0.05</td>
<td>0.207</td>
<td>0.72±0.09</td>
<td>0.80±0.07</td>
<td>0.263</td>
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<td>Fasting triacylglycerol (mmol/l)</td>
<td>1.5 (0.6-3.7)</td>
<td>1.1 (0.5-2.2)</td>
<td>0.011</td>
<td>1.2±0.2</td>
<td>1.1±0.2</td>
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<td>Fasting ALT (U/l)</td>
<td>37.7±4.1</td>
<td>25.7±2.4</td>
<td>0.009</td>
<td>24.3±2.8</td>
<td>22.1±3.7</td>
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<tr>
<td>Fasting GGT (U/l)</td>
<td>33 (13-148)</td>
<td>15 (7-69)</td>
<td>0.002</td>
<td>23 (8-39)</td>
<td>11 (5-122)</td>
<td>0.234</td>
</tr>
</tbody>
</table>

Table 1. Anthropometric and metabolic data before and at 8 weeks post-operatively in the T2DM and NGT groups. * indicates a statistically significant difference between the 2 groups at baseline. # indicates a statistically significant difference between the 2 groups at week 8.
Legend to Figures

Figure 1. (A) Change in first phase insulin response (baseline to 6 min ISR shown as median and 25th-75th percentile)(pre-surgery difference between groups p=0.011; pre-surgery to week 8 change p=0.005 and p=0.9 for T2DM and NGT respectively). (B) Pancreatic triacylglycerol content (shown as mean and SEM) in the T2DM and NGT groups pre-surgery (p<0.01) and then at 8 post-operative weeks (p<0.01 and p=0.44 for pre-surgery to 8 week change for T2DM and NGT respectively).

Figure 2. Stepped insulin secretion test with arginine, showing (panels A) the induced change in plasma glucose pre-surgery (open circles) and week 8 (closed circles), (panels B) pre-surgery insulin secretion rates (median and 25th-75th percentile), and (panels C) post-surgery insulin secretion rates (median and 25th-75th percentile) for the T2DM and NGT groups.

Figure 3. Glucose (panels A), insulin (panels B), total GLP-1 (panels C) and total GIP (panels D) levels (mean ± SEM) during the 2 hour meal test in the T2DM and NGT groups pre-surgery (open circles) and week 8 (closed circles). Inset graph displays fasting (open bar) and 20 min (black bar) levels pre-surgery compared to week 8. The more rapid 0-20 min increase in post-surgery plasma glucose (p<0.001 and p<0.005), insulin (p<0.01 and p=0.03), GLP-1 (p<0.001 and p=0.002) and GIP (p=0.001 and p=0.016) was similar for T2DM and NGT respectively.