



Weight Loss Decreases Excess Pancreatic Triacylglycerol Specifically in Type 2 Diabetes

Diabetes Care 2016;39:158–165 | DOI: 10.2337/dc15-0750

Sarah Steven,¹ Kieren G. Hollingsworth,^{1,2}
Peter K. Small,³ Sean A. Woodcock,⁴
Andrea Pucci,⁵ Benjamin Aribisala,⁶
Ahmad Al-Mrabeh,² Ann K. Daly,²
Rachel L. Batterham,⁵ and Roy Taylor^{1,2}

OBJECTIVE

This study determined whether the decrease in pancreatic triacylglycerol during weight loss in type 2 diabetes mellitus (T2DM) 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 T2DM or normal glucose tolerance (NGT) matched for age, weight, and sex were studied before and 8 weeks after surgery. Pancreas and liver triacylglycerol were quantified using in-phase, out-of-phase MRI. Also measured were the first-phase insulin response to a stepped intravenous glucose infusion, hepatic insulin sensitivity, and glycemic and incretin responses to a semisolid test meal.

RESULTS

Weight loss after surgery was similar (NGT: $12.8 \pm 0.8\%$ and T2DM: $13.6 \pm 0.7\%$) as was the change in fat mass (56.7 ± 3.3 to 45.4 ± 2.3 vs. 56.6 ± 2.4 to 43.0 ± 2.4 kg). Pancreatic triacylglycerol did not change in NGT (5.1 ± 0.2 to $5.5 \pm 0.4\%$) but decreased in the group with T2DM (6.6 ± 0.5 to $5.4 \pm 0.4\%$; $P = 0.007$). 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 \cdot min⁻¹ \cdot m⁻²) but normalized in T2DM ($0.08 [-0.01$ to $-0.10]$ to $0.22 [0.07–0.30]$) nmol \cdot min⁻¹ \cdot m⁻² at week 8 ($P = 0.005$). No differential effect of incretin secretion was observed after gastric bypass, with more rapid glucose absorption bringing about equivalently enhanced glucagon-like peptide 1 secretion in the two groups.

CONCLUSIONS

The fall in intrapancreatic triacylglycerol in T2DM, which occurs during weight loss, is associated with the condition itself rather than decreased total body fat.

Type 2 diabetes mellitus (T2DM) has reached epidemic proportions, affecting 9.2% of the U.S. population and costing the country \$322 billion in 2012 (1). The condition is widely recognized 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 T2DM occurs only when the acute insulin response of pancreatic β -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 the acute insulin response and

¹Magnetic Resonance Centre, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, U.K.

²Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, U.K.

³Department of Surgery, Sunderland Royal Hospital, Sunderland, U.K.

⁴Department of Surgery, North Tyneside General Hospital, North Shields, U.K.

⁵Centre for Obesity Research, University College London, London, U.K.

⁶Computer Science Department, Faculty of Science, Lagos State University, Lagos, Nigeria

Corresponding author: Roy Taylor, roy.taylor@ncl.ac.uk.

Received 10 April 2015 and accepted 22 July 2015.

Clinical trial reg. no. ISRCTN11969319, www.isrctn.org.

This article contains Supplementary Data online at <http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc15-0750/-/DC1>.

© 2016 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered.

the 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 the etiology of T2DM (10).

It remains uncertain whether the change in intrapancreatic triacylglycerol is specific to the diabetes itself or simply reflects the 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 groups with T2DM and NGT.

Major changes in incretin hormones occur after Roux-en-Y gastric bypass (RYGB) surgery that 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 subjects without diabetes. Some studies have supported this concept (13), although a 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 on physiologic incretin function and on 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 RYGB surgery would be accompanied by a decrease in pancreatic triacylglycerol specifically in T2DM and that the post-surgery change in incretin hormone responses would not differ in T2DM and NGT. Because change in pancreas triacylglycerol must reflect export of 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 centers. Individuals with T2DM ($n = 18$) were recruited with diabetes duration <15 years, aged 25–65 years, BMI up to 45 kg/m² (due to scanner constraints), HbA_{1c} $<10\%$ (86 mmol/mol), and no significant renal or hepatic dysfunction (creatinine <150 μmol/L; alanine aminotransferase [ALT] <2.5 -fold above the upper limit of normal). Exclusion criteria were contraindication to MRI scanning, alcohol consumption >14 units/week, previous bowel surgery, or treatment with steroids, thiazolidinediones, or GLP-1 analogs. The 18 individuals with T2DM and the 9 NGT individuals were group matched for age and weight (49.1 ± 1.6 vs. 46.3 ± 2.1 year, 121.0 ± 3.0 vs. 114.5 ± 5.0 kg; 11 women, 7 men vs. 7 women, 2 men, respectively). NGT was confirmed by 75-g oral glucose tolerance test. Impaired glucose tolerance was found in two subjects, and screening was continued until the planned group size of 9 NGT was achieved. In the group with T2DM, three individuals were maintained with insulin therapy and nine with sulfonylurea 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 after the baseline studies due to the diagnosis of an unrelated medical problem.

Experimental Protocol

The participants with T2DM and the NGT participants were studied just before surgery and at 8 weeks postoperatively. Preoperatively, all participants were asked to follow a hypocaloric ($\sim 1,200$ kcal) 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 MRI scanning were measured. Participants were asked to stop metformin and/or sulfonylureas at least 72 h before the first study or to stop insulin at least 24 h before, and all remained off hypoglycemic agents thereafter. Intensive physical activity and alcohol/caffeine intake were avoided 48 h before each study. All metabolic studies were performed after a 10-h overnight fast.

Surgery

RYGB was performed laparoscopically. A biliopancreatic limb of 50–70 cm from the duodenojejunal flexure was anastomosed to the 30–50 mL gastric pouch. An alimentary limb of 100–150 cm was then measured, and a side-to-side antimesenteric jejunojejunostomy was performed. Two patients with T2DM underwent sleeve gastrectomy instead of RYGB due to the presence of significant intra-abdominal adhesions and were excluded from the incretin analyses.

Body Composition and Anthropometry

Body composition was determined using a Bodystat1500 (Bodystat Ltd, Isle of Man, U.K.). Waist and hip circumferences were measured using a standard nondistensible tape measure, and height was measured by stadiometer by one observer (S.S.).

Meal Test

Each test was performed with the participant semireclined 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 semisolid meal within 3 min (10 g Mornflake Instant Porridge Oats, 64 g whole milk, and 6 g acacia honey: 100 kcal, 57% carbohydrate, 28% fat, 13% protein), designed in accordance with the expected volume and consistency of the diet consumed 1 week after 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 was 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 h.

Measurement of Intraorgan Triacylglycerol Content

MRI data were acquired using a 3-Tesla Philips Achieva scanner (Philips Healthcare, Best, the Netherlands) with a six-channel cardiac array (Philips Healthcare) or four large and medium flex surface coils (Philips Healthcare) if required due to body habitus. Data were acquired using a 3-point Dixon method (15), with gradient-echo scans acquired during four 17-s breath holds (repetition time/echo times/averages/flip angle =

50 ms/3.45, 4.60, 5.75 ms/1/5°). Critically, participant cooperation was maximized 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 a slice thickness of 10 mm and the pancreas data with a slice thickness 5 mm. The triacylglycerol and water contributions of the MRI signal were separated by mathematical modeling of their known chemical shifts using an in-house program 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 the pancreas and five image slices of the liver, defined and averaged by one observer (S.S.). Because 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 blinded to subject status and time point.

Hepatic Glucose Production and Insulin Sensitivity

[6'-6'-2H] glucose (98% enriched; Cambridge Isotope Laboratories, Andover, MA) 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 $40 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) 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 kilogram of fat-free mass (kg_{ffm}) corrected for glucose space and urinary loss (18). 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

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 a 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 and 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. The 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 (8).

Analytical Procedures

Plasma glucose was measured by the glucose oxidase method (YSI glucose analyzer; Yellow Springs Instrument Company, Yellow Springs, OH). Serum insulin was measured using ELISA kits (DAKO, Ely, U.K.). Serum C-peptide was measured using ELISA kits (DAKO or Mercodia, Uppsala, Sweden, with correction factor to ensure comparability). Plasma nonesterified fatty acid (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, U.K.). β -Hydroxybutyrate levels were measured to confirm dietary compliance using the Optium Exceed ketone meter (Abbott Diabetes Care, Oxfordshire, U.K.). [6'-6'-2H] glucose was measured using gas chromatography/mass spectrometry (GC/MS) technique on a Thermo Voyager single quadrupole MS connected to a Thermo Finnigan Trace 2000 GS (Thermo Scientific, Waltham, MA). HbA_{1c} , liver function tests, γ -glutamyl transferase (GGT), and lipids were measured at a Clinical Pathology Accredited laboratory (Newcastle upon

Tyne Hospital National Health Service Foundation Trust, Department of Clinical Biochemistry). Human total GLP-1 (7-36, 9-36) was measured using ELISA kits (Alpco, Salem, NH). Human total GIP was measured using ELISA kits (Merck Millipore, Watford, U.K.).

PNPLA3 genotyping was performed on DNA extracted from white blood cells. Whole blood (10 mL) was collected in EDTA and, after thorough mixing, was stored at -40°C . DNA was isolated and genotyping performed (blinded to the clinical parameters) using TaqMan SNP Genotyping Analysis (Applied Biosystems, Carlsbad, CA), as described previously (20).

Statistical Analysis

Data are presented as mean \pm SEM for parametric data and median (range) for nonparametric data. Insulin secretion rates are given as the median with the 25th and 75th percentile. Statistical analysis used the Student paired and unpaired *t* tests, Mann Whitney *U* test, Wilcoxon rank sum test, and Spearman rank correlation test, as appropriate, using the Minitab 16 statistical program (www.minitab.com).

RESULTS

Weight Loss

Preoperative weight did not differ between the group with T2DM and the NGT group (121.1 ± 3.0 vs. 114.5 ± 5.0 kg; $P = 0.244$). At 8 postoperative weeks, weight loss was similar in the two groups ($13.6 \pm 0.7\%$ and $12.8 \pm 0.8\%$ respectively; $P = 0.286$), as was the change in total body fat content (Table 1).

Plasma Glucose, Insulin, and Metabolites

Fasting plasma glucose decreased from 9.4 ± 0.8 mmol/L presurgery to 6.4 ± 0.4 mmol/L at week 8 in the group with T2DM ($P < 0.001$) and from 5.2 ± 0.2 to 4.9 ± 0.1 mmol/L in the NGT group ($P = 0.196$). HbA_{1c} decreased from 7.6 ± 0.4 to $6.2 \pm 0.2\%$ (59 ± 4 to 44 ± 2 mmol/mol) in the group with T2DM ($P < 0.001$) compared with 5.4 ± 0.1 to $5.2 \pm 0.1\%$ (36 ± 1 to 33 ± 1 mmol/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

Table 1—Anthropometric and metabolic data before and at 8 weeks after surgery in the group with T2DM and the NGT group

	T2DM before surgery	T2DM after surgery	<i>P</i>	NGT before surgery	NGT after surgery	<i>P</i>
Weight (kg)	121.1 ± 3.0	104.5 ± 2.7	<0.001	114.5 ± 5.0	99.7 ± 4.6	<0.001
BMI (kg/m ²)	42.7 ± 0.7	36.9 ± 0.7	<0.001	41.3 ± 1.0	36.4 ± 0.8	<0.001
Fat mass (kg)	56.6 ± 2.4	43.0 ± 2.4	<0.001	56.7 ± 3.3	45.4 ± 2.3	<0.001
Waist-to-hip ratio	0.97 ± 0.02	0.94 ± 0.02	0.006	0.90 ± 0.03*	0.87 ± 0.03#	0.066
Plasma glucose						
Fasting (mmol/L)	9.2 ± 0.8	6.2 ± 0.3	<0.001	5.2 ± 0.1	4.9 ± 0.1	0.089
2 h (mmol/L)	9.4 ± 0.8	6.4 ± 0.3	<0.001	5.4 ± 0.2*	5.0 ± 0.0#	0.022
Serum insulin						
Fasting (mU/L)	15.3 (4.3–61.2)	11.3 (2.9–27.0)	<0.001	11.0 ± 1.6	6.7 ± 0.7#	0.008
2 h (mU/L)	18.4 (5.2–78.9)	11.2 (4.9–31.0)	0.001	12.4 (4.4–63.5)*	6.0 (5.0–7.4)#	0.042
Fasting						
Glucagon (ng/L)	74.6 ± 9.8	58.0 ± 8.3	0.001	49.4 ± 5.0	48.1 ± 5.0	0.865
Free fatty acid (mmol/L)	0.85 ± 0.08	0.77 ± 0.05	0.207	0.72 ± 0.09	0.80 ± 0.07	0.263
Triacylglycerol (mmol/L)	1.5 (0.6–3.7)	1.1 (0.5–2.2)	0.011	1.2 ± 0.2	1.1 ± 0.2	0.16
ALT (units/L)	37.7 ± 4.1	25.7 ± 2.4	0.009	24.3 ± 2.8	22.1 ± 3.7	0.542
GGT (units/L)	33 (13–148)	15 (7–69)	0.002	23 (8–39)	11 (5–122)	0.234

*Indicates a statistically significant difference between the two groups at baseline. #Indicates a statistically significant difference between the two groups at week 8.

the group with T2DM but not the NGT group (Table 1). Fasting β -hydroxybutyrate increased from 0.20 mmol/L (0.00–0.70) to 0.30 mmol/L (0.10–1.00) in the group with T2DM ($P = 0.011$) and from 0.33 ± 0.08 to 0.55 ± 0.15 mmol/L in the NGT group ($P = 0.07$). There was no difference in change from presurgery to week 8 in β -hydroxybutyrate between the two groups (T2DM: -0.2 [–0.6 to 0.5], NGT: -0.1 [–1 to 0]; $P = 0.98$).

Pancreas Triacylglycerol Content

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

Change in Insulin Secretion

Presurgery, the first-phase insulin response (baseline to 6 min insulin secretion rate) in the group with T2DM was severely impaired compared with the NGT group (0.08 [–0.01 to 0.10] vs. 0.24 [0.13–0.46]; $P = 0.011$; Fig. 1A and Fig. 2B). There was marked restoration of the first-phase insulin response in T2DM postsurgery, increasing to 0.22 (0.07–0.30) $\text{nmol} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$ at week 8 in the group with T2DM

($P = 0.005$; Fig. 1A and Fig. 2C). There was no change in the first-phase insulin response in the NGT group: 0.24 (0.13–0.46) at baseline and 0.23 (0.19–0.37) $\text{nmol} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$ at week 8 ($P = 0.464$; Fig. 1A and Fig. 2B and C). The arginine-induced insulin response in the group with T2DM 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

Presurgery, hepatic triacylglycerol content was more than twofold higher in the group with T2DM compared with the NGT group (9.3 ± 1.5 vs. $4.2 \pm 1.4\%$; $P = 0.022$; Supplementary Fig. 1A). Postsurgery, it decreased to a greater extent in the group with T2DM (9.3 ± 1.5 to $5.2 \pm 0.8\%$; $P = 0.018$) than in the NGT group (4.2 ± 1.4 to $2.3 \pm 0.6\%$; $P = 0.059$). These changes were reflected in the fall in serum ALT and GGT after surgery only in the group with T2DM (Table 1).

Hepatic Insulin Sensitivity

Basal hepatic glucose production in T2DM decreased postsurgery (3.60 ± 0.24 to 2.69 ± 0.12 $\text{mg}/\text{kg}_{\text{ffm}}/\text{min}$; $P < 0.001$) (Supplementary Fig. 1B). There was no significant change in the NGT group (2.60 ± 0.08 to 2.51 ± 0.20 $\text{mg}/\text{kg}_{\text{ffm}}/\text{min}$; $P = 0.555$). Hepatic insulin sensitivity improved in the group

with T2DM: hepatic insulin resistance index 2.76 ± 0.41 to 1.33 ± 0.23 $\text{mmol} \cdot \text{min}^{-1} \cdot \text{kg}_{\text{ffm}}^{-1} \cdot \text{pmol} \cdot \text{L}^{-1}$ ($P = 0.002$); NGT group: 1.18 ± 0.19 to 0.70 ± 0.07 $\text{mmol} \cdot \text{min}^{-1} \cdot \text{kg}_{\text{ffm}}^{-1} \cdot \text{pmol} \cdot \text{L}^{-1}$ ($P = 0.062$) (Supplementary Fig. 1C). The insulin-induced suppression of hepatic

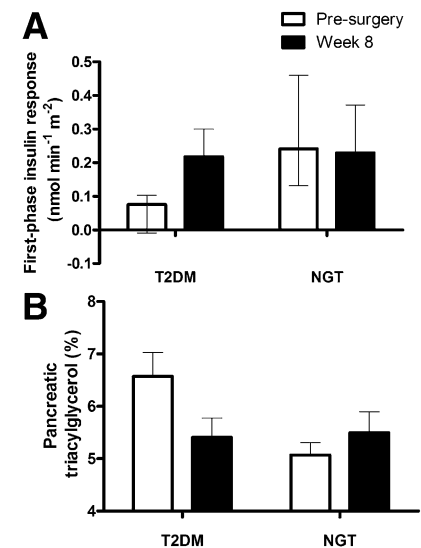


Figure 1—A: Change in first-phase insulin response (baseline to 6 min insulin secretion rate shown as median and 25th–75th percentile). Presurgery difference between groups: $P = 0.011$; presurgery to week 8 change: $P = 0.005$ for T2DM and $P = 0.9$ for NGT. **B:** Pancreatic triacylglycerol content (shown as mean and SEM) in the T2DM and NGT groups presurgery ($P < 0.01$) and then at 8 postoperative weeks. Presurgery to 8 week change: $P < 0.01$ for T2DM and $P = 0.44$ for NGT.

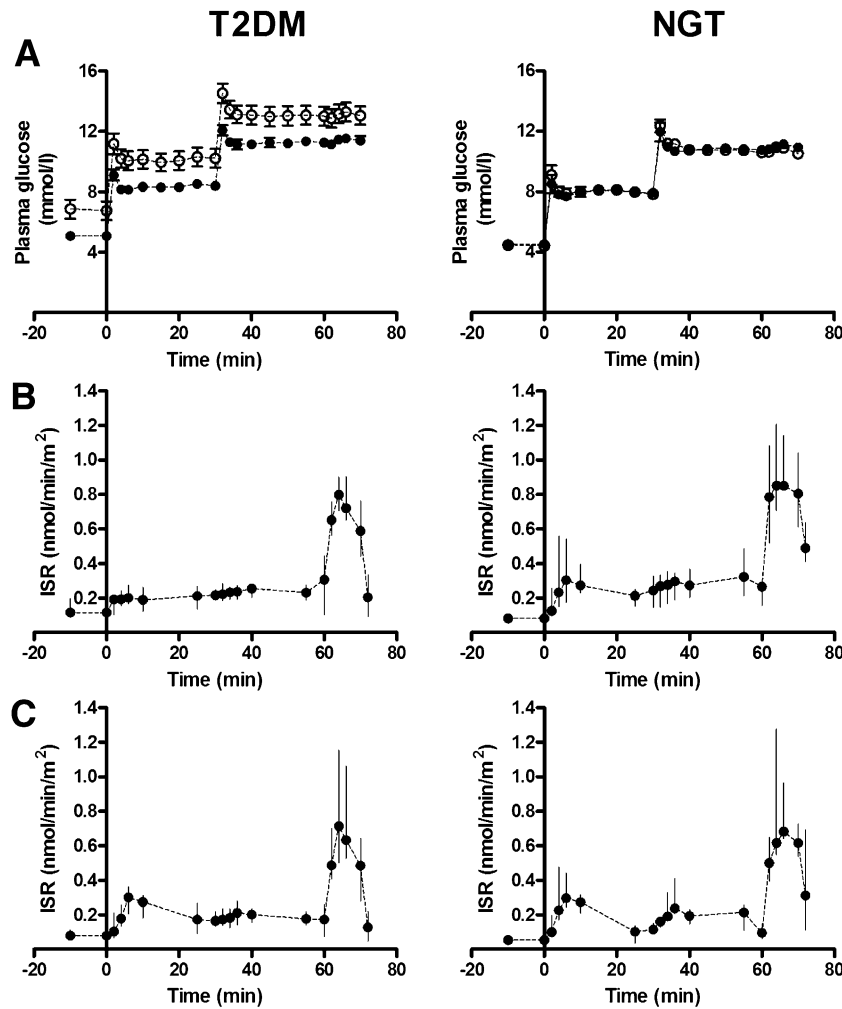


Figure 2—Stepped insulin secretion test with arginine shows the induced change in plasma glucose presurgery (○) and week 8 (●) (A panels), presurgery insulin secretion rates (ISR; median and 25th–75th percentile) (B panels), and postsurgery insulin secretion rates (median and 25th–75th percentile) (C panels) for the group with T2DM and the NGT group.

glucose production was greater in the group with T2DM at 8 weeks post-RYGB (67 ± 4 to $85 \pm 3\%$; $P < 0.001$), with no change in the NGT group (84 ± 4 to $77 \pm 8\%$; $P = 0.339$).

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) $\text{mL/kg}_{\text{ffm}}/\text{min}$ in T2DM ($P = 0.223$) and 4.51 ± 0.63 to 4.79 ± 0.70 $\text{mL/kg}_{\text{ffm}}/\text{min}$ in NGT ($P = 0.572$). Peripheral insulin sensitivity was significantly lower in the T2DM group before ($P = 0.033$) and after surgery ($P = 0.024$).

Subcutaneous and Visceral Fat Data

Presurgery, there was no difference between subcutaneous adipose tissue (SAT) area in T2DM compared with

NGT (453.8 ± 28.9 cm^2 vs. 496.4 ± 16.0 cm^2 ; $P = 0.318$). Visceral adipose tissue (VAT) area was 300.4 ± 17.5 cm^2 in T2DM compared with 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 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 the group with T2DM and the NGT group, from 0.6 ± 0.1 preoperatively to 1.8 ± 0.1 mmol/L postoperatively ($P < 0.001$) in T2DM and from 0.5 ± 0.1 to 1.7 ± 0.2 mmol/L ($P = 0.004$) in NGT (Fig. 3A). There was a significant

difference in decrease of peak glucose between T2DM and NGT (1.84 [-1.06 to 11.5] vs. -0.66 [-1.73 to 0.34]; $P < 0.001$). The 2-h postmeal glucose was lower in both groups: 9.4 ± 0.8 to 6.4 ± 0.3 mmol/L in the group with T2DM ($P < 0.001$) and 5.5 ± 0.2 to 5.0 ± 0.0 mmol/L in the NGT group ($P = 0.022$). The change in the 2-h postmeal glucose between T2DM and NGT was also significant (1.87 [-0.02 to 11.49] vs. 0.41 [-0.26 to 0.91]; $P < 0.001$).

The incremental rise in plasma insulin over the first 20 min increased in both groups at 8 weeks post-RYGB, with a higher and earlier peak plasma insulin being achieved in T2DM preoperatively (35.2 ± 4.9 mU/L at 60 [10 – 120] min) versus week 8 (47.7 ± 5.8 mU/L at 20 [10 – 30] min; $P = 0.01$) and in NGT preoperatively (37.0 ± 5.0 mU/L at 60 [30 – 120] min) versus week 8 (58.1 ± 10.0 mU/L at 20 [20 – 30] min; $P = 0.032$) (Fig. 3B).

Peak GLP-1 levels during the meal test increased from 5.0 ± 0.3 to 12.7 ± 1.3 pmol/L in the group with T2DM ($P < 0.001$) and from 5.1 ± 0.6 to 12.9 ± 1.2 pmol/L in the NGT group ($P = 0.001$) (Fig. 3C). Peak GIP levels increased from 197.6 ± 18.1 to 246.2 ± 24.4 pg/mL in the group with T2DM ($P = 0.051$) 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 (Fig. 3D).

PNPLA3

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

CONCLUSIONS

Despite similar weight loss after bariatric surgery in groups of well-matched

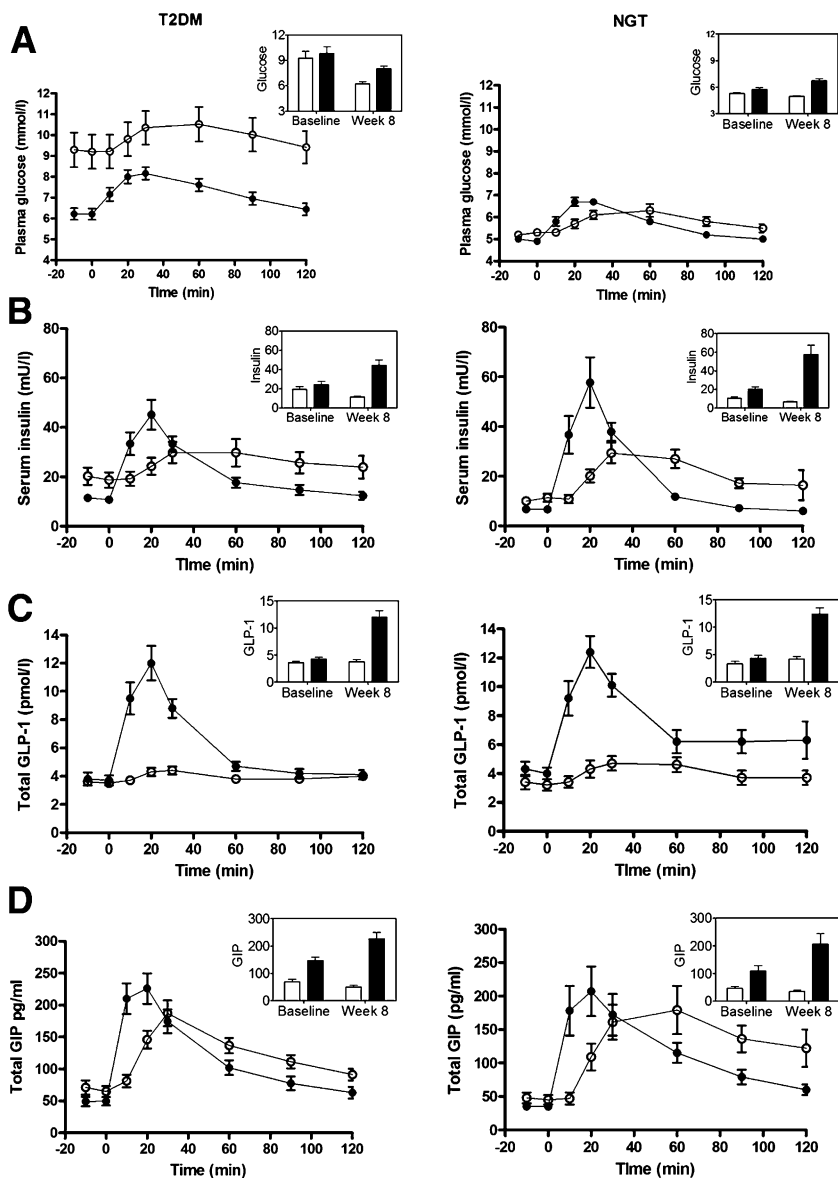


Figure 3—Glucose (A panels), insulin (B panels), total GLP-1 (C panels), and total GIP (D panels) levels (mean ± SEM) during the 2-h meal test in the group with T2DM and the NGT group presurgery (○) and at week 8 (●). Inset graph displays fasting (□) and 20-min (■) levels presurgery compared with week 8. The more rapid 0–20 min increase in postsurgery 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.

individuals with T2DM or NGT, intrapancreatic triacylglycerol decreased uniquely in T2DM. This was associated with normalization of first-phase insulin secretion in the T2DM group. No change occurred in intrapancreatic triacylglycerol in the NGT group despite a 5-unit decrease in BMI. Hepatic insulin sensitivity, both fasting and during insulin stimulation, normalized in the group with T2DM in step with a greater decrease in liver triacylglycerol compared with 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 postmortem autolysis, but a study of pancreata retrieved and not used for pancreas transplantation showed that intracellular fat droplets were 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). Local lipolysis is likely to bring about interstitial and intracellular concentrations of fatty acids sufficient to inhibit β -cell function. Fatty acid receptors are expressed in mouse and human pancreatic β -cells and allow recovery of insulin secretion when knocked out (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 the 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 individuals without diabetes (22).

The method used to quantify intrapancreatic triacylglycerol must be considered. MR spectroscopy gathers chemical information from a predefined volume, and with careful application, physiologically relevant data can be acquired (26). If the volume selected is too large, visceral fat will likely be included in the measurement (27). Use of the 3-point Dixon imaging method avoids this problem because chemical information is derived after acquisition, and placement of the volume of interest is guided by the image (8). However, care is required in ensuring validity of the method, because otherwise, serious errors, including negative numbers for percentage tissue triacylglycerol, may be derived (27). In the current study, we used a reproducible, robust method with blinded analysis. At 8 weeks postsurgery, there was a 15% decrease in pancreas triacylglycerol levels in individuals with 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 after 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 a consequent reduction in liver fat is associated with improvements in insulin sensitivity and fasting plasma glucose levels (16,31). The current study demonstrates a greater reduction in liver fat content post-RYGB in individuals with T2DM compared with NGT, with normalization 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 intrahepatic fat are implied by the range of baseline liver triacylglycerol in the group with T2DM. Data from the UK Prospective Diabetes Study 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 intrahepatic triacylglycerol, blunted the weight loss–associated decrease in liver fat.

The markedly increased nutrient-stimulated secretion of GLP-1 after RYGB is well recognized, and the present observations confirm this. Normalization of the first-phase insulin response to an intravenous infusion of glucose demonstrates the improvement is independent of acute incretin stimulation. After RYGB, a 2.6-fold increase of insulin secretion assessed by the intravenous glucose tolerance test disposition index has been reported (34). A very low–calorie diet or RYGB results 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 after RYGB (36,37).

Nutrients pass rapidly into the mid-jejunum after RYGB (38). Most studies have used an oral glucose challenge (11,34) or liquid meal (39) with very rapid absorption and a nonphysiological incretin stimulus (36). The current study used a semisolid meal to minimize 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 the test meal was earlier and greater in both groups post-RYGB. Overall, it appears that acute postmeal-enhanced incretin secretion does not explain the improved β -cell function in T2DM after 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 individuals with T2DM were unselected in 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, individuals with T2DM exhibit an attenuated first-phase insulin response and increased pancreatic triacylglycerol compared with BMI-matched NGT individuals. At 8 weeks after bariatric surgery, the first-phase insulin response and pancreas triacylglycerol were both normalized uniquely in the group with T2DM. GLP-1 response after a semisolid meal improved equally in T2DM and NGT. These observations support the concept of intrapancreatic 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 for more appropriate clinical management.

Acknowledgments. The authors are grateful to the participants for their enthusiastic contribution to the work; L. Hughes, A. Burnett, and T. Dew of the Newcastle upon Tyne Hospitals Blood Laboratory for hormone assay; and L. Ward, T. Hodgson, and T. Gaudi of the Newcastle Magnetic Resonance Centre for expert radiography.

Funding. The study was funded by a European Foundation for the Study of Diabetes/Lilly European Diabetes Research Programme Grant (BH102090).

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. S.S. performed the studies, analyzed the data, and wrote the manuscript. K.G.H., P.K.S., S.A.W., and R.T. designed the study and edited the manuscript. A.P. performed the incretin analyses and edited the manuscript. B.A. performed the mathematical modelling of insulin secretion rates and edited the manuscript. A.A.-M. performed GC/MS analyses and edited the manuscript. A.K.D. oversaw PNPLA3 genotyping and edited the manuscript. R.L.B. edited the manuscript. No others assisted with writing or editing the manuscript. R.T. 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.

Prior Presentation. Parts of this study were presented at the International Diabetes Federation's 2015 World Diabetes Congress, Vancouver, Canada, 30 November–4 December 2015.

References

- Dall TM, Yang W, Halder P, et al. 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
- Taylor R. Insulin resistance and type 2 diabetes. *Diabetes* 2012;61:778–779
- Ferrannini E, Nannipieri M, Williams K, Gonzales C, Haffner SM, Stern MP. Mode of onset of type 2 diabetes from normal or impaired glucose tolerance. *Diabetes* 2004;53:160–165
- Festa A, Williams K, D'Agostino R Jr, Wagenknecht LE, Haffner SM. The natural course of beta-cell function in nondiabetic and diabetic individuals: the Insulin Resistance Atherosclerosis Study. *Diabetes* 2006;55:1114–1120
- Lee Y, Hirose H, Ohneda M, Johnson JH, McGarry JD, Unger RH. Beta-cell lipotoxicity in the pathogenesis of non-insulin-dependent diabetes mellitus of obese rats: impairment in adipocyte-beta-cell relationships. *Proc Natl Acad Sci U S A* 1994;91:10878–10882
- Diakogiannaki E, Dhayal S, Childs CE, Calder PC, Welters HJ, Morgan NG. Mechanisms involved in the cytotoxic and cytoprotective actions of saturated versus monounsaturated long-chain fatty acids in pancreatic beta-cells. *J Endocrinol* 2007;194:283–291
- Lalloyer F, Vandewalle B, Percevault F, et al. 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
- 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
- Steven S, Lim EL, Taylor R. Dietary reversal of type 2 diabetes motivated by research knowledge. *Diabet Med* 2010;27:724–725
- Taylor R. Type 2 diabetes: etiology and reversibility. *Diabetes Care* 2013;36:1047–1055
- Guidone C, Manco M, Valera-Mora E, et al. Mechanisms of recovery from type 2 diabetes

- after malabsorptive bariatric surgery. *Diabetes* 2006;55:2025–2031
12. Vilsbøll T, Krarup T, Deacon CF, Madsbad S, Holst JJ. Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. *Diabetes* 2001;50:609–613
13. Manning S, Pucci A, Batterham RL. GLP-1: a mediator of the beneficial metabolic effects of bariatric surgery? *Physiology (Bethesda)* 2015;30:50–62
14. 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
15. Glover GH, Schneider E. Three-point Dixon technique for true water/fat decomposition with B0 inhomogeneity correction. *Magn Reson Med* 1991;18:371–383
16. Ravikumar B, Gerrard J, Dalla Man C, et al. Pioglitazone decreases fasting and postprandial endogenous glucose production in proportion to decrease in hepatic triglyceride content. *Diabetes* 2008;57:2288–2295
17. Gastaldelli A, Cusi K, Pettiti M, et al. Relationship between hepatic/visceral fat and hepatic insulin resistance in nondiabetic and type 2 diabetic subjects. *Gastroenterology* 2007;133:496–506
18. Rizza RA, Mandarino LJ, Gerich JE. Dose-response characteristics for effects of insulin on production and utilization of glucose in man. *Am J Physiol* 1981;240:E630–E639
19. Toschi E, Camastra S, Sironi AM, et al. Effect of acute hyperglycemia on insulin secretion in humans. *Diabetes* 2002;51(Suppl. 1):S130–S133
20. Liu YL, Patman GL, Leathart JB, et al. 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
21. 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* 2010;34:396–400
22. Tushuizen ME, Bunck MC, Pouwels PJ, et al. Pancreatic fat content and beta-cell function in men with and without type 2 diabetes. *Diabetes Care* 2007;30:2916–2921
23. 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 (Silver Spring)* 2008;16:522–530
24. Tang C, Ahmed K, Gille A, et al. Loss of FFA2 and FFA3 increases insulin secretion and improves glucose tolerance in type 2 diabetes. *Nat Med* 2015;21:173–177
25. van der Zijl NJ, Goossens GH, Moors CC, et al. Ectopic fat storage in the pancreas, liver, and abdominal fat depots: impact on β -cell function in individuals with impaired glucose metabolism. *J Clin Endocrinol Metab* 2011;96:459–467
26. Szczepaniak LS, Victor RG, Mathur R, et al. Pancreatic steatosis and its relationship to β -cell dysfunction in humans: racial and ethnic variations. *Diabetes Care* 2012;35:2377–2383
27. Begovatz P, Koliaki C, Weber K, et al. Pancreatic adipose tissue infiltration, parenchymal steatosis and beta cell function in humans. *Diabetologia* 2015;58:1646–1655
28. Prager R, Wallace P, Olefsky JM. In vivo kinetics of insulin action on peripheral glucose disposal and hepatic glucose output in normal and obese subjects. *J Clin Invest* 1986;78:472–481
29. Seppälä-Lindroos A, Vehkavaara S, Häkkinen AM, et al. Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. *J Clin Endocrinol Metab* 2002;87:3023–3028
30. Sevastianova K, Santos A, Kotronen A, et al. Effect of short-term carbohydrate overfeeding and long-term weight loss on liver fat in overweight humans. *Am J Clin Nutr* 2012;96:727–734
31. Petersen KF, Dufour S, Befroy D, Lehrke M, Hendler RE, Shulman GI. Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. *Diabetes* 2005;54:603–608
32. Immonen H, Hannukainen JC, Iozzo P, et al. Effect of bariatric surgery on liver glucose metabolism in morbidly obese diabetic and non-diabetic patients. *J Hepatol* 2014;60:377–383
33. Taylor R, Holman RR. Normal weight individuals who develop type 2 diabetes: the personal fat threshold. *Clin Sci (Lond)* 2015;128:405–410
34. Bojsen-Møller KN, Dirksen C, Jørgensen NB, et al. Early enhancements of hepatic and later of peripheral insulin sensitivity combined with increased postprandial insulin secretion contribute to improved glycemic control after Roux-en-Y gastric bypass. *Diabetes* 2014;63:1725–1737
35. Jackness C, Karmally W, Febres G, et al. Very low-calorie diet mimics the early beneficial effect of Roux-en-Y gastric bypass on insulin sensitivity and β -cell function in type 2 diabetic patients. *Diabetes* 2013;62:3027–3032
36. Salehi M, Prigeon RL, D'Alessio DA. Gastric bypass surgery enhances glucagon-like peptide 1-stimulated postprandial insulin secretion in humans. *Diabetes* 2011;60:2308–2314
37. Jiménez A, Casamitjana R, Viaplana-Masclans J, Lacy A, Vidal J. GLP-1 action and glucose tolerance in subjects with remission of type 2 diabetes after gastric bypass surgery. *Diabetes Care* 2013;36:2062–2069
38. Falkén Y, Hellström PM, Holst JJ, Näslund E. Changes in glucose homeostasis after Roux-en-Y gastric bypass surgery for obesity at day three, two months, and one year after surgery: role of gut peptides. *J Clin Endocrinol Metab* 2011;96:2227–2235
39. Isbell JM, Tamboli RA, Hansen EN, et al. The importance of caloric restriction in the early improvements in insulin sensitivity after Roux-en-Y gastric bypass surgery. *Diabetes Care* 2010;33:1438–1442
40. Heijboer AC, Frans A, Lomecky M, Blankenstein MA. Analysis of glucagon-like peptide 1; what to measure? *Clin Chim Acta* 2011;412:1191–1194