

**Increased GLP-1 at 7 days following RYGB does not translate into improved insulin secretion rates
or glucose control compared to 7 days of VLCD**

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Clinical trial number ISRCTN11969319

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Word count:

Running head: Increased GLP-1 following RYGB does not augment glucose metabolism compared to VLCD

The study was funded by an EFSD/Lilly European Diabetes Research Programme Grant.

The authors have no conflicts of interest to declare.

Novelty statement:

- The increased weight loss at 7 days following RYGB compared to 7 days of VLCD is due to greater loss of lean and water mass
- After RYGB, the early and enhanced post-meal rise in glucose and insulin is due to rapid absorption through the gastroenterostomy
- The increased post-meal GLP-1 secretion specific to RYGB is not accompanied by an improvement in insulin or glucose AUC when compared to VLCD
- The early improvement in glucose control after bariatric surgery is not explained by improved beta cell function
- The relationship between weight loss and loss of liver fat at 7 days following bariatric surgery and VLCD appears different

Abstract

It remains unclear whether the mechanism underlying the early improvement in glucose metabolism following gastric bypass surgery differs from that seen in the early phase of very low calorie diet (VLCD). Specifically, whether the markedly increased GLP-1 secretion following surgery is of primary importance has been controversial. This study aimed to compare directly the impact of GLP-1 secretion on glucose metabolism in individuals with type 2 diabetes listed for gastric bypass, randomised to be studied before and at 7 days following bariatric surgery or VLCD. A semi-solid meal test was used to investigate glucose, insulin and GLP-1 response. Insulin secretion to IV stimulus was also measured. Intra-organ fat content was measured using an MR method. Decrease in fat mass was almost identical after surgery or VLCD (3.0 ± 0.3 and 3.0 ± 0.7 kg respectively). The early plasma glucose rise and acute insulin secretion were greater following surgery compared to VLCD. However, the early GLP-1 rise was disproportionately greater (65 fold) after surgery than after VLCD. This did not translate into a greater improvement in fasting glucose or glucose AUC. The reduction in liver fat content (29.8 ± 3.7 vs. 18.6 ± 4.0 %) was greater after surgery and the relationship between weight loss and reduction in liver fat content differed following surgery or VLCD. In conclusion, this study demonstrates a gastroenterostomy increases the rate of nutrient absorption and a commensurately rapid rise insulin occurs. However, there was no relationship with the large post-meal rise in plasma GLP-1 and post-meal glucose homeostasis was similar after surgery or VLCD.

Introduction

It has been recognised since the 1950's that the metabolic derangements in type 2 diabetes can be ameliorated by bariatric surgery (1, 2). This was considered to be a direct effect of weight loss. However, following biliopancreatic diversion it was found that the major improvement in metabolism occurred within one week of surgery (3). As this occurred prior to any significant weight loss it was postulated that the major changes in incretin hormone secretion which occur after Roux-en-Y gastric bypass surgery (RYGB) could explain an increase in meal related insulin secretion (4). Many studies have confirmed the post-RYGB increase in plasma glucagon-like peptide-1 (GLP-1) following oral glucose administration (5, 6).

Following RYGB there is a profound decrease in calorie intake, the substrate flow into storage is reversed and lipid metabolites are rapidly removed from the cytoplasm of all cells. As the subjects in

the study by Guidone *et al.* had a mean body weight of 152kg, before the bypass surgery they would require to consume approximately 3,500 kcal/day to maintain stable weight at rest (3). The Twin Cycle Hypothesis was postulated to explain both the aetiology and reversibility of type 2 diabetes on the basis of calorie balance (7). The hypothesis was tested in a moderately obese group of individuals with type 2 diabetes and normalisation of fasting blood glucose was observed within 7 days (8). This has subsequently been confirmed by others (9, 10). However, associations between the GLP-1 response after gastric bypass surgery and improvement in fasting blood glucose continue to be reported (11-13) and there is widespread acceptance that the two are causally related (14-17). However, an unphysiological liquid glucose challenge has previously been used to test insulin secretion together with indirect measurements of insulin secretion and insulin sensitivity (10, 18, 19). Clarification of the role of GLP-1 in the immediate post-surgery period is required.

The aim of this study was to compare directly the impact of GLP-1 secretion following a semi-solid meal 7 days after bariatric surgery with that after 7 days of a very low calorie diet (VLCD). To ensure comparability of the groups, subjects listed for bariatric surgery were randomised to each group. The study design thus permitted direct comparison of insulin secretion and GLP-1 secretion after bariatric surgery or diet alone under conditions of similar further calorie reduction. Insulin secretion in response to IV stimulus, hepatic triglyceride content, and pancreatic triglyceride content were also quantified.

Methods

Participants

Individuals with type 2 diabetes listed for laparoscopic Roux-en-Y gastric bypass surgery (RYGB) were identified from two regional bariatric surgery centres. Inclusion criteria were: diabetes duration <15 yr; aged 25-65 yr; BMI up to 45 kg/m²; HbA1c <86 mmol/mol (10%) and no significant renal or hepatic dysfunction (creatinine <150 µmol/l; ALT <2.5-fold above the upper limit of normal). Exclusion criteria were: contraindication to MR scanning; alcohol consumption >14 units/wk; previous bowel surgery; or treatment with steroids, thiazolidinediones or GLP-1 analogues. The study protocol was approved by the Newcastle upon Tyne 1 Research Ethics Committee. All participants provided written informed consent. Participants were asked to stop metformin and/or sulphonylureas at least 72 hours prior to the first study or insulin at least 24 hours prior. Individuals were asked to avoid intensive physical activity/alcohol/caffeine intake in the 48 hours prior to each study. All metabolic studies were performed after an overnight fast.

Experimental Protocol

The participants were studied at baseline and then at 7 days of intervention; randomised using online software (www.sealedenvelope.com) to either: Surgery only (studied before and then 7 days after surgery) or VLCD (studied before and after a 7 day VLCD). At each time point, a standard meal test was used to assess metabolic and incretin responses, hepatic and peripheral insulin sensitivity were assessed using an isoglycaemic hyperinsulinaemic clamp, first phase and maximal insulin secretion were measured using a gold standard test and hepatic and pancreatic triglyceride content was quantified by magnetic resonance scanning. Studies took place over 1 full day (assessment of insulin sensitivity and insulin secretion) and one half day (assessment of body composition and meal test). Participants were asked to continue their advised pre-operative diet until the start of the study, thus all were in modest negative calorie balance at the time of the first study. The post-operative diet was liquids on day 1 then a semi-solid diet for the rest of the first week.

Surgery

RYGB was performed laparoscopically in all patients. A gastric pouch (30-50ml) was fashioned on the lesser curve of stomach using an ETS 45 mm linear stapler firing blue (6R45B) cartridges (Ethicon Endosurgery, Cincinnati, OH). A biliopancreatic limb of 50-70 cm from the duodenojejunal flexure was anastomosed to the gastric pouch. An alimentary limb of 100-150 cm was then measured and a side to side antimesenteric jejuno-jejunostomy carried out. The roux construction was completed by dividing the omega loop close to the gastrojejunostomy. At the time of operation, 2 patients 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 (20).

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 (glucose, insulin, C-peptide, glucagon, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP)). 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 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. At all times during processing samples were kept on ice. Samples were taken every 10 min for the first 30 min of the test, then every 30 min until 2 hours.

Assessment of body composition and intra-organ triglyceride content

Body composition was determined using a Bodystat®1500 (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 (21) 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°. 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 fat and water contributions of the MRI signal were separated using an in-house programme written in MATLAB, with the triglyceride content in the images expressed as a percentage of the total signal from fat and water in each pixel. The intra-organ 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 subject status and timepoint. This method has previously been shown to have a repeatability co-efficient of 0.5% for liver and 0.9% for pancreas (8).

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 arterialised blood sampling. [6'-2H] glucose (98% enriched; Cambridge Isotope Laboratories, MA, USA) was used to determine hepatic glucose production (22, 23). Basal rates were calculated during the last 30 min of the 150 min basal period. Pre-infusion enrichment of isotope was insignificant throughout. An isoglycaemic–hyperinsulinaemic clamp (insulin infusion rate 40 mU m² min⁻¹) was initiated at 0 min. Each participant was clamped at the glucose level observed at the end of the basal period. Isoglycaemia 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 hyperinsulinaemic glucose clamp as whole-body glucose disposal per kg of fat free mass corrected for glucose space and urinary loss (24). In order to correct for the difference in fasting glucose levels during the course of the study, 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 hyperglycaemia (2.8 and 5.6 mmol/l above baseline) were achieved by priming glucose doses followed by variable 20% glucose infusion (25). 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 hyperglycaemia, followed by sampling every 2 min for 10 min. Insulin secretion rate was calculated using a computerised program implementing a regularisation method of deconvolution (26) and using a population model of C-peptide kinetics (27).

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). Plasma glucagon concentration was measured by radioimmunoassay (Millipore Corporation, Billerica, MA, USA). β -Hydroxybutyrate levels were measured 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 quadrupole mass spectrometer connected to a ThermoFinnigan Trace 2000 gas chromatograph (Thermo Scientific, Waltham, MA, USA). HbA1c, LFTs, 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 (AlpcoDiagnostics; 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 (28).

Statistical Analysis

Data are presented as mean \pm 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 T-

test, paired t-test, Mann U Whitney, Wilcoxon Rank and Spearman Rank correlations coefficient as appropriate using Minitab 16 statistical program (www.minitab.com).

Results

Weight loss

Baseline weight and BMI did not differ between the surgery and VLCD groups (120.8±5.0 vs. 121.4±3.7 kg; $p=0.932$ and 43.0±1.1 vs. 42.3±0.9 kg/m²; $p=0.577$ respectively). Weight loss was greater 7 days following surgery compared to after 7 days of VLCD (5.1±0.5 vs. 3.5±0.4 %; $p=0.03$). The components of weight loss were notably different in the surgery vs. VLCD groups (Fig. 1), with the additional weight loss in the surgery group consisting of lean mass (-2.5±0.3 vs. -1.3±0.6 kg; $p=0.108$) and body water (-2.4±0.3 vs. -1.6±0.5 L; $p=0.195$). Decrease in fat mass was almost identical after surgery or VLCD (3.0±0.3 and 3.0±0.7 kg respectively).

Plasma glucose, insulin, glucagon and metabolites

Fasting plasma glucose levels fell modestly and similarly in both groups (VLCD 1.2±0.6 mmol/l and surgery 0.9±0.5 mmol/l; $p=0.739$). Fasting ketones (β -hydroxybutyrate), ALT and NEFA increased in both groups after 7 days (Table 1). There was a modest change in fasting serum triglycerides after surgery (-0.3 (-0.8-0.3) mmol/l; $p=0.042$) but not after VLCD (0.1 (-0.2-2.4) mmol/l).

Change in meal tolerance test

Following surgery, the gastroenterostomy caused a significantly greater early rise in post-test meal plasma glucose (Fig. 2). This was associated with a greater early rise in serum insulin which correlated with the rise in plasma glucose (Spearman rank=0.867; $p=0.002$). The resulting greater early insulin secretion did not fully compensate as shown by the overall glucose AUC, and indeed there was a modest increase in glucose AUC in the surgery group (104.2±17.2 to 130.2±15.2 mmol.l⁻¹.min; $p=0.185$). There was no significant change in the insulin AUC in either group. If GLP-1 was driving the insulin response, then it would be expected to be directly proportionate to the change in the insulin/glucose ratio. No such relationship was observed (Spearman rank: Surgery: -0.214; $p=0.610$ and VLCD: -0.190; $p=0.651$; Whole group: 0.365; $p=0.165$). The extent and duration of the gastroenterostomy associated postprandial rise in GLP-1 can be appreciated from the plasma hormone profile (Fig. 3). No significant change was observed in post-test meal plasma GIP levels after either intervention.

Change in insulin secretion and insulin sensitivity

The first phase insulin response to an intravenous glucose challenge and the arginine induced maximal insulin secretion rates at day 7 were unchanged compared to baseline in both groups (Table 2). Concordant with the modest change in fasting plasma glucose levels as a result of the pre-operative liver reduction diet, neither basal hepatic glucose production nor hepatic insulin sensitivity was significantly changed in either group at day 7 (Table 2). There was no change in peripheral insulin sensitivity as measured by glucose metabolic clearance rate in either group within 7 days (Table 2).

Intra-organ triglyceride

Hepatic triglyceride content decreased in both groups (VLCD by 18.6 ± 4.0 % and Surgery by 29.8 ± 3.7 %; $p=0.06$). After VLCD, the fall in hepatic triglyceride was directly related to the extent of weight loss, whereas after surgery the greatest reduction in liver fat occurred in subjects with modest weight loss (Fig. 4). This effect was not explained by PNPLA3 genotype: the rs738409 C to G adiponutrin/PNPLA3 genotype (coding for I148M) was found in 4 individuals in the VLCD group (one homozygous and 3 heterozygous; change in hepatic triglyceride 4.1 to 3.2% and 13.9 ± 1.8 to 12.2 ± 1.7 % respectively) and 1 individual in the Surgery group (heterozygous; change in hepatic triglyceride 7.2 to 4.8%). There was no correlation between the reduction in hepatic triglyceride content and the reduction in lean mass in the Surgery group (Spearman rank= -0.094; $p=0.840$). There was no change in pancreatic triglyceride content after 7 days of either VLCD or Surgery (6.4 ± 0.6 to 6.5 ± 0.7 %; $p=0.781$ and 6.7 ± 0.7 to 6.7 ± 0.6 %; $p=0.885$, respectively).

Discussion

After RYGB, a greater early rise in plasma glucose after the test meal was associated with a greater 0-20 minute rise in both plasma insulin and GLP-1. The latter was 65 fold greater in the RYGB compared to the VLCD group and plasma levels remained higher throughout the test meal period. Despite this, the incremental AUCs for insulin and glucose were not different between the VLCD and surgery groups. There was no association between the extent of GLP-1 rise and insulin secretion independent of the glucose stimulation and the marked increase in GLP-1 did not confer a clear benefit on post-meal glucose levels or insulin secretion. There was no change in non-incretin dependent beta cell function as assessed by the stepped intravenous glucose challenge in either RYGB or VLCD groups. Differences were observed between the 7 day response to RYGB or VLCD in components of body weight change and also in the relationship between extent of weight loss and change in liver fat content.

The present findings do not accord with the widespread acceptance that GLP-1 has a determinant role in improving meal time insulin secretion post-RYGB (29-31). However, close examination of the literature reveals no hard evidence that the undoubtedly greater GLP-1 response after RYGB plays a major part in the improvement of glucose control. A previous comparison of a matched group of obese individuals with type 2 diabetes one month after RYGB or 10kg dietary weight loss demonstrated that stimulated GLP-1 and GIP levels increased markedly after surgery only (4). There was a similar reduction in fasting glucose, insulin, C-peptide and HOMA-IR in both the surgery and diet groups. Although the authors concluded that the incretin effect explained the improvement of glucose control after RYGB, the data show that peak plasma glucose was higher after surgery and the calculated incretin effect did not differ after surgery or diet.

In contrast to the lack of definitive data on GLP-1 effect post RYGB, several studies have demonstrated the greater improvement in fasting plasma glucose by calorie restriction alone (10, 18). The very early effects of RYGB on glucose metabolism (within 4 days) have been compared to calorie restriction using replication of the post-RYGB diet (18). The increase in meal stimulated GLP-1 levels noted in the surgery group only was not accompanied by any additional benefits over the diet group. Lingvay *et al.* compared VLCD and surgery in patients with type 2 diabetes due to have RYGB in a paired design using individuals as their own controls (10). After 10 days there was a significant improvement in fasting glucose, peak glucose and glucose AUC during a mixed meal challenge test after VLCD but not after RYGB despite a greater GLP-1 response after RYGB. These studies together with the present data indicate that the major mechanism responsible for changes in glycaemic in the early post-operative period is via the severely restricted oral caloric intake. This does not exclude an ongoing, cumulative effect of the marked post-prandial spikes in plasma GLP-1. The effect of this has yet to be defined, although it is notable that sleeve gastrectomy, which is not associated with such high post-prandial GLP-1 levels, produces similar improvements in HbA1c in type 2 diabetes compared with RYGB over 3 years of follow-up (32).

The present study was able to identify clear differences in the metabolic state 7 days after RYGB compared with that after VLCD. Surgery appears to bring about a decrease in lean body mass, presumably as a catabolic response to the intervention, although identical decreases in fat mass were seen in both groups. The difference in relationship of decrease in body weight to fall in liver fat between VLCD and bariatric surgery has not, to our knowledge, been demonstrated previously. The exacerbation of fatty liver disease, steatosis and rarely fulminant hepatic failure following jejuno-ileal bypass is well recognised (33) and it is possible that the underlying mechanism for this could disturb the relationship. A second possibility is that the GLP-1 peak after every meal might produce a

cumulative effect on liver fat content. Therapeutic use of GLP-1 agonist can decrease liver fat content, a phenomenon seen both with GLP-1 agonists and with DPP4 inhibitors (34, 35). In view of this a possible relationship between extent of post-meal GLP-1 elevation and fall in liver fat in the surgery group was examined. The strong correlation (Spearman rank 0.590; $P=0.016$) between peak GLP-1 and fall in liver fat content is suggestive of a causal relationship and this requires further examination.

Previously we have demonstrated that the improvement in first phase insulin response with VLCD changes very gradually over 8 weeks in step with the decrease in intra-pancreatic fat content with no meaningful change after 7 days (8). The present data confirm this for VLCD and demonstrate that the same is true after RYGB. Change in beta cell function does not explain the early improvement in glucose control after bariatric surgery. In contrast, both liver fat content and hepatic insulin sensitivity improves rapidly after calorie restriction. Fasting plasma glucose concentration is determined by the rate of hepatic glucose production (23) and elevated liver fat concentration is associated with decreased insulin sensitivity to suppression of hepatic glucose production (36, 37). While carbohydrate overfeeding can induce liver fat accumulation (38), weight loss brings about reduction in liver fat (23, 39). The design of this study, with pre-operative calorie restriction due to the surgical requirements, minimised change in hepatic insulin resistance although the fall in liver fat continues beyond the first week of calorie restriction, as demonstrated previously (8).

The limitations of the study must be discussed. The conclusions of the study are limited to the early post-RYGB phase and relate only to the early improvement of glucose control. The subjects studied had a wide range of diabetes duration and previous glucose lowering treatment, although this is representative of the heterogeneous population undergoing bariatric surgery in which improved glucose control is observed. Although relatively small numbers were studied, definitive results were obtained due to the very large difference in post-meal GLP-1 secretion. The study design differs from the majority of studies after RYGB in that aliquid glucose challenge was not used and this must be considered in comparing results from earlier studies. However, use of a semi-solid test meal allows a more physiological, everyday assessment of post-meal physiology following RYGB.

In conclusion, after RYGB a more rapid entry of food into the small intestine causes both a more rapid rise in plasma glucose and a more rapid rise in plasma insulin. No relationship with the large post-prandial rise in plasma GLP-1 after surgery could be detected and overall glucose homeostasis was similar after diet or surgery.

Funding

The study was funded by an EFSD/Lilly European Diabetes Research Programme Grant.

Conflicts of interest

The authors have none to declare.

Acknowledgements

We are very grateful to the participants for their enthusiastic contribution to the work. We thank L Hughes, A Burnett and T Dew for the laboratory work, and acknowledge the expertise of the radiographers L Ward, T Hodgson and T Gaudie.

SS performed the studies, analysed the data and wrote the manuscript, KGH edited the manuscript, PKS and SW designed the study and edited the manuscript, AP performed the incretin analysis, BA performed the mathematical modelling for insulin secretion data and edited the manuscript, AA performed GCMS on dideuterated glucose samples and edited the manuscript, RB edited the manuscript and RT designed the study and edited the manuscript. The guarantor for the study is RT.

Figure 1. Effect at day 7 of VLCD or Surgery on body weight, fat mass and lean body mass. * = $p < 0.05$ for between group difference.

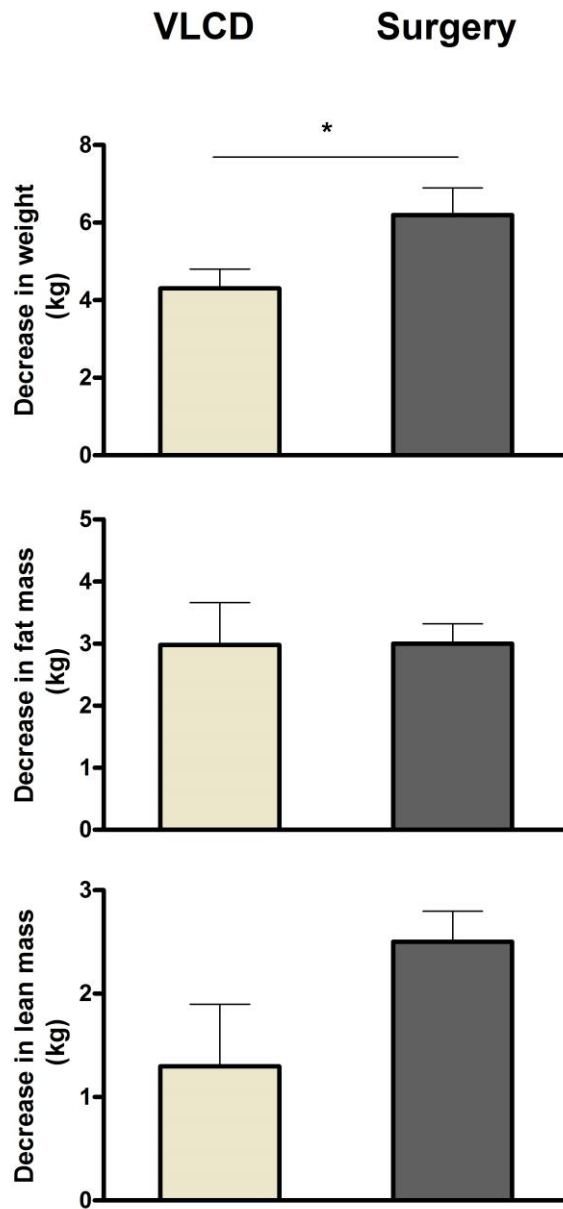


Figure 2. Incremental change in glucose, insulin and GLP-1 from fasting to 20 minutes (A) and change in positive incremental area under the curve (B) during the meal tolerance test before (pale) and 7 days (dark) after intervention (VLCD or Surgery). * = $p < 0.05$ for baseline to day 7 difference.

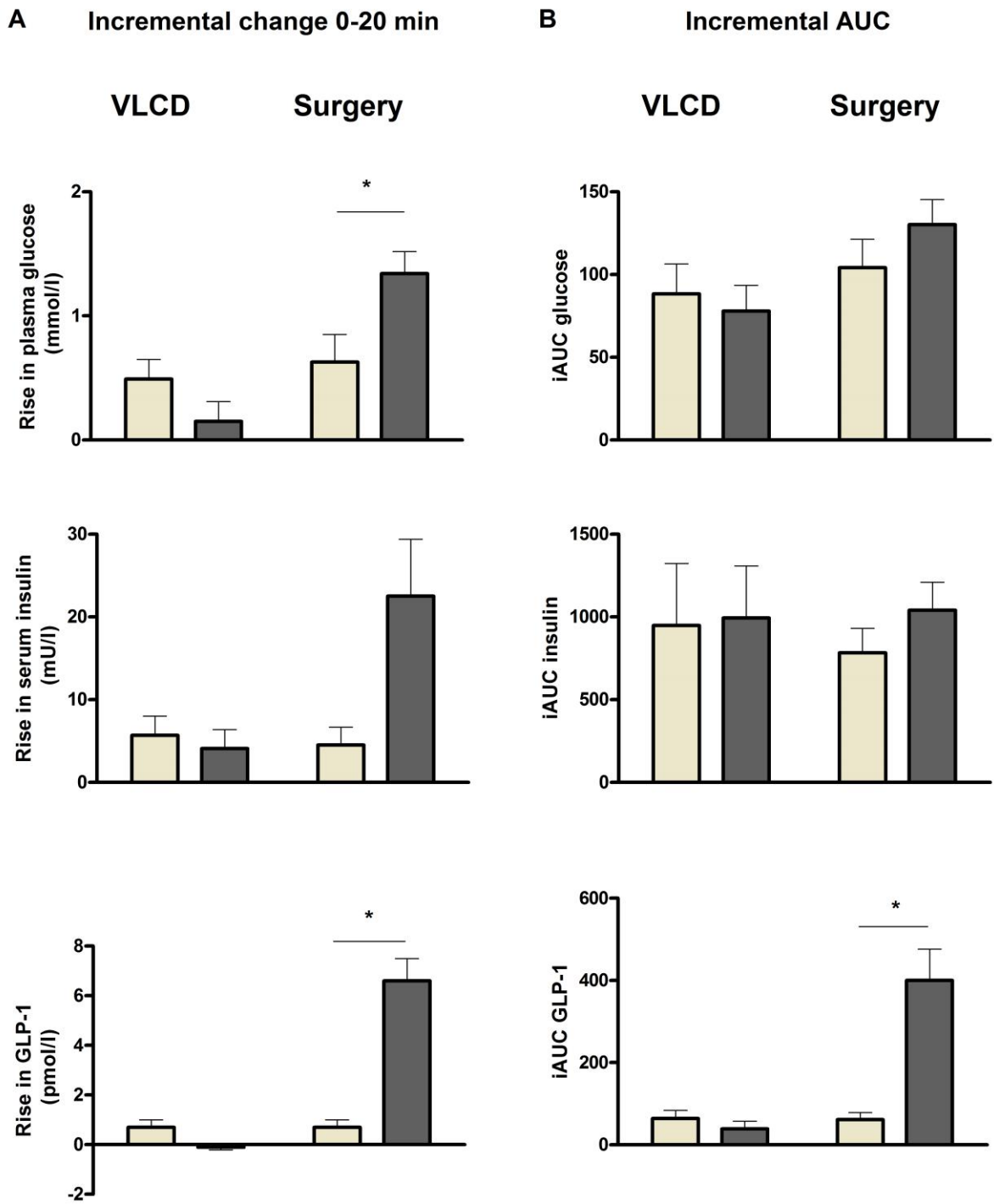


Figure 3. Total GLP-1 and total GIP levels (mean \pm SEM) during the 2 hour meal test in the VLCD and Surgery groups at baseline (squares) and at day 7 (triangles).

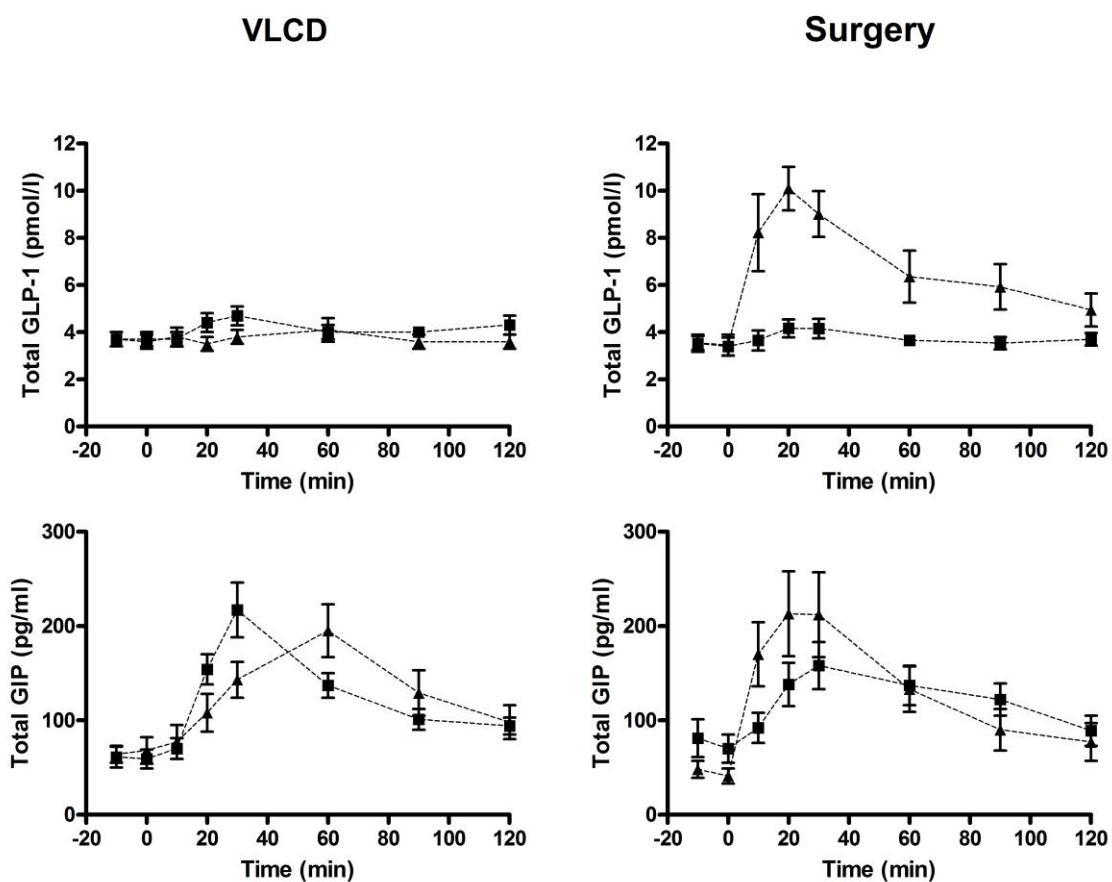
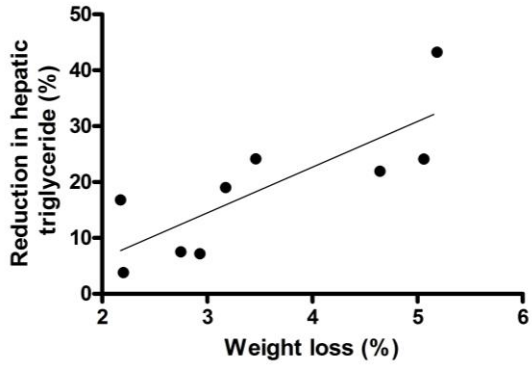


Figure 4. Relationship between achieved weight loss and reduction in hepatic triglyceride content at day 7 in the VLCD group (A; Pearson correlation coefficient 0.80, $p < 0.01$) and Surgery group (B; Pearson correlation coefficient -0.88, $p < 0.004$). [TAKE Pearson off Graphs]

A

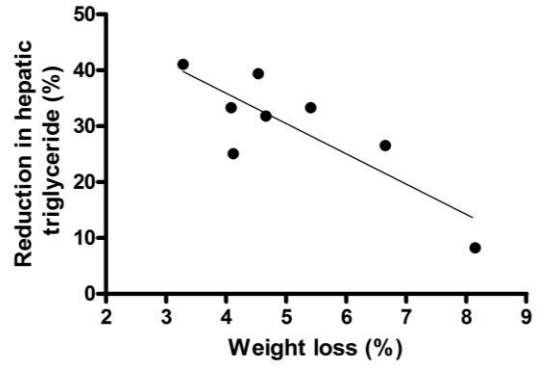
VLCD



Pearson correlation = 0.798; p=0.010

B

Surgery



Pearson correlation = -0.880; p=0.004

Table 1. Metabolic response at day 7 in the VLCD and Surgery groups

| | <i>Before VLCD</i> | <i>7 days after VLCD</i> | <i>p</i> | <i>Before Surgery</i> | <i>7 days after Surgery</i> | <i>p</i> |
|--|--------------------|--------------------------|----------|-----------------------|-----------------------------|----------|
| Fasting serum insulin (mU/l) | 18.5 (13.5-44.6) | 14.8 (10.6-38.8) | 0.813 | 13.5 (4.3-61.2) | 9.8 (5.2-23.2) | 0.076 |
| 2hr serum insulin (mU/l) | 22.2 (18.3-78.9) | 19.5 (10.1-76.2) | 0.363 | 14.4 (5.2-70.5) | 11.0 (5.6-28.3) | 0.124 |
| Fasting β-hydroxybutyrate (mmol/l) | 0.22 \pm 0.07 | 0.63 \pm 0.17 | 0.005 | 0.29 \pm 0.06 | 0.86 \pm 0.18 | 0.019 |
| Fasting NEFA (mmol/l) | 0.85 \pm 0.13 | 0.91 \pm 0.08 | 0.402 | 0.85 \pm 0.11 | 0.96 \pm 0.08 | 0.087 |
| Fasting glucagon (ng/l) | 69.7 \pm 10.8 | 66.0 \pm 11.4 | 0.511 | 79.5 \pm 16.8 | 63.0 \pm 8.4 | 0.120 |
| Fasting triglycerides (mmol/l) | 1.7 \pm 0.3 | 1.4 \pm 0.2 | 0.217 | 1.4 \pm 0.1 | 1.7 \pm 0.1 | 0.090 |
| Fasting ALT (U/l) | 34.7 \pm 6.4 | 39.9 \pm 7.2 | 0.020 | 40.8 \pm 5.2 | 61.4 \pm 9.9 | 0.108 |
| Fasting GGT (U/l) | 33 (20-113) | 27 (15-81) | 0.014 | 39.0 (13.0-148.0) | 40.0 (29.0-241.0) | 0.155 |

Table 2. Change in insulin secretion and insulin sensitivity at day 7 in the VLCD and Surgery groups. ISR=insulin secretion rate; HGP=hepatic glucose production; IR= insulin resistance

| | <i>Before VLCD</i> | <i>7 days after VLCD</i> | <i>p</i> | <i>Before Surgery</i> | <i>7 days after Surgery</i> | <i>p</i> |
|---|--------------------|--------------------------|----------|-----------------------|-----------------------------|----------|
| First phase insulin response (nmol.min⁻¹.m⁻²) | 0.04 (-0.03-0.10) | 0.10 (0.08-0.14) | 0.155 | 0.08 (0.07-0.10) | 0.06 (0.00-0.13) | 0.722 |
| Peak arginine induced ISR (nmol.min⁻¹.m⁻²) | 0.67 (0.53-0.70) | 0.54 (0.43-0.72) | 0.528 | 0.80 (0.63-0.84) | 0.67 (0.54-1.46) | 0.354 |
| Basal HGP (mg/kg_{ffm}/min) | 3.48±0.32 | 3.43±0.63 | 0.906 | 3.71±0.36 | 3.16±0.33 | 0.083 |
| Hepatic IR index (mmol.min⁻¹.kg_{ffm}⁻¹.pmol.l⁻¹) | 2.38 (1.07-4.62) | 1.97 (0.60-6.98) | 0.427 | 2.15 (0.61-8.02) | 1.25 (0.20-3.14) | 0.077 |
| Suppression of HGP by insulin (%) | 62±6 | 73±6 | 0.173 | 73±5 | 80±8 | 0.357 |
| Metabolic clearance rate (ml/kg_{ffm}/min) | 2.21 (0.91-8.80) | 1.83 (1.10-13.79) | 0.791 | 2.55 (0.86-5.15) | 2.40 (0.59-3.94) | 0.724 |

References

1. Buchwald H, Avidor Y, Braunwald E, Jensen MD, Pories W, Fahrbach K, et al. Bariatric surgery: a systematic review and meta-analysis. *JAMA*. 2004;292(14):1724-37.
2. Friedman MN, Sancetta AJ, Magovern GJ. The amelioration of diabetes mellitus following subtotal gastrectomy. *Surgery, gynecology & obstetrics*. 1955;100(2):201-4.
3. Guidone C, Manco M, Valera-Mora E, Iaconelli A, Gniuli D, Mari A, et al. Mechanisms of recovery from type 2 diabetes after malabsorptive bariatric surgery. *Diabetes*. 2006;55(7):2025-31.
4. Laferrere B, Teixeira J, McGinty J, Tran H, Egger JR, Colarusso A, et al. Effect of weight loss by gastric bypass surgery versus hypocaloric diet on glucose and incretin levels in patients with type 2 diabetes. *Journal of Clinical Endocrinology and Metabolism*. 2008;93(7):2479-85.
5. Roux CWI, Aylwin SJB, Batterham RL, Borg CM, Coyle F, Prasad V, et al. Gut Hormone Profiles Following Bariatric Surgery Favor an Anorectic State, Facilitate Weight Loss, and Improve Metabolic Parameters. *Annals of Surgery*. 2006;243(1):108-14.
6. Korner J, Bessler M, Inabnet W, Taveras C, Holst JJ. Exaggerated glucagon-like peptide-1 and blunted glucose-dependent insulinotropic peptide secretion are associated with Roux-en-Y gastric bypass but not adjustable gastric banding. *Surg Obes Relat Dis*. 2007;3(6):597-601.
7. Taylor R. Pathogenesis of Type 2 diabetes: Tracing the reverse route from cure to cause. *Diabetologia*. 2008;51:1781-9.
8. 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(10):2506-14.
9. Lips MA, de Groot GH, van Klinken JB, Aarts E, Berends FJ, Janssen IM, et al. Calorie restriction is a major determinant of the short-term metabolic effects of gastric bypass surgery in obese type 2 diabetic patients. *Clinical endocrinology*. 2014;80(6):834-42.
10. 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(9):2741-7.
11. Jørgensen NB, Dirksen C, Bojsen-Møller KN, Jacobsen SH, Worm D, Hansen DL, et al. Exaggerated Glucagon-Like Peptide 1 Response Is Important for Improved β -Cell Function and Glucose Tolerance After Roux-en-Y Gastric Bypass in Patients With Type 2 Diabetes. *Diabetes*. 2013;62(9):3044-52.
12. Laferrere B, Heshka S, Wang K, Khan Y, McGinty J, Teixeira J, et al. Incretin levels and effect are markedly enhanced 1 month after Roux-en-Y gastric bypass surgery in obese patients with type 2 diabetes. *Diabetes Care*. 2007;30(7):1709-16.
13. Salehi M, Prigeon RL, D'Alessio DA. Gastric bypass surgery enhances glucagon-like peptide 1-stimulated postprandial insulin secretion in humans. *Diabetes*. 2011;60(9):2308-14.
14. Salehi M, D'Alessio DA. Effects of glucagon like peptide-1 to mediate glycemic effects of weight loss surgery. *Reviews in Endocrine & Metabolic Disorders*. 2014;15(3):171-9.
15. Holst JJ. Enteroendocrine secretion of gut hormones in diabetes, obesity and after bariatric surgery. *Current Opinion in Pharmacology*. 2013;13(6):983-8.
16. Laferrère B. Diabetes remission after bariatric surgery: is it just the incretins? *International journal of obesity (2005)*. 2011;35(0 3):S22-S5.
17. Vella A. Does caloric restriction alone explain the effects of Roux-en-Y gastric bypass on glucose metabolism? Not by a long limb. *Diabetes*. 2013;62(9):3017-8.
18. Isbell J, Tamboli R, Hansen E, Saliba J, Dunn J, Phillips S, et al. The importance of caloric restriction in the early improvement in insulin sensitivity after Roux-en-Y gastric bypass surgery. *Diabetes Care*. 2010;33:1438-42.

19. Jackness C, Karmally W, Febres G, Conwell IM, Ahmed L, Bessler M, et al. 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(9):3027-32.
20. Yousseif A, Emmanuel J, Karra E, Millet Q, Elkalaawy M, Jenkinson AD, et al. Differential effects of laparoscopic sleeve gastrectomy and laparoscopic gastric bypass on appetite, circulating acyl-ghrelin, peptide YY3-36 and active GLP-1 levels in non-diabetic humans. *Obes Surg*. 2014;24(2):241-52.
21. Glover GH, Schneider E. Three-point Dixon technique for true water/fat decomposition with B0 inhomogeneity correction. *Magn Reson Med*. 1991;18(2):371-83.
22. Hother-Nielsen O, Henriksen JE, Holst JJ, Beck-Nielsen H. Effects of insulin on glucose turnover rates in vivo: Isotope dilution versus constant specific activity technique. *Metabolism*. 1996;45(1):82-91.
23. Ravikumar B, Gerrard J, Dalla Man C, Firbank MJ, Lane A, English PT, et al. Pioglitazone decreases fasting and postprandial endogenous glucose production in proportion to decrease in hepatic triglyceride content. *Diabetes*. 2008;57:2288-95.
24. Rizza RA, Mandarino LJ, Gerich JE. Dose response characteristics for effects of insulin on production and utilisation of glucose in man. *Am J Physiol*. 1981;240:E630-E9.
25. Toschi E, Camastra S, Sironi AM, Masoni A, Gastaldelli A, Mari A, et al. Effect of acute hyperglycemia on insulin secretion in humans. *Diabetes*. 2002;51 Suppl 1:S130-3.
26. Hovorka R, Soons PA, Young MA. ISEC: a program to calculate insulin secretion. *Computer Methods and Programs in Biomedicine*. 1996;50(3):253-64.
27. Van Cauter E, Mestrez F, Sturis J, Polonsky KS. Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes*. 1992;41(3):368-77.
28. Liu YL, Patman GL, Leathart JB, Piguat AC, Burt AD, Dufour JF, 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(1):75-81.
29. Holst JJ. Postprandial Insulin Secretion After Gastric Bypass Surgery: The Role of Glucagon-Like Peptide 1. *Diabetes*. 2011;60(9):2203-5.
30. Naslund E, Hellstrom PM. Elucidating the mechanisms behind the restoration of euglycemia after gastric bypass surgery. *Diabetes*. 2013;62(4):1012-3.
31. Goldfine AB, Patti ME. Diabetes Improvement Following Roux-en-Y Gastric Bypass: Understanding Dynamic Changes in Insulin Secretion and Action. *Diabetes*. 2014;63(5):1454-6.
32. Schauer PR, Kashyap SR, Wolski K, Brethauer SA, Kirwan JP, Pothier CE, et al. Bariatric Surgery versus Intensive Medical Therapy in Obese Patients with Diabetes. *New England Journal of Medicine*. 2012;366(17):1567-76.
33. Peters RL, Gay T, Reynolds TB. Post-jejunoileal-bypass hepatic disease. Its similarity to alcoholic hepatic disease. *American journal of clinical pathology*. 1975;63(3):318-31.
34. Macauley M, Hollingsworth KG, Smith FE, Thelwall PE, Al-Mrabeh A, Schweizer A, et al. Effect of vildagliptin on hepatic steatosis. *The Journal of clinical endocrinology and metabolism*. 2015;100(4):1578-85.
35. Cuthbertson DJ, Irwin A, Gardner CJ, Daousi C, Purewal T, Furlong N, et al. Improved Glycaemia Correlates with Liver Fat Reduction in Obese, Type 2 Diabetes, Patients Given Glucagon-Like Peptide-1 (GLP-1) Receptor Agonists. *PLoS ONE*. 2012;7(12):e50117.
36. Seppala-Lindroos A, Vehkavaara S, Hakkinen A, Goto T, Westerbacka J, Sovijarvi A, 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. *Journal of Clinical Endocrinology and Metabolism*. 2002;87(7):3023-8.
37. Gastaldelli A, Cusi K, Pettiti M, Hardies J, Miyazaki Y, Berria R, et al. Relationship between hepatic/visceral fat and hepatic insulin resistance in nondiabetic and type 2 diabetic subjects. *Gastroenterology*. 2007;133(2):496-506.

38. Sevastianova K, Santos A, Kotronen A, Hakkarainen A, Makkonen J, Silander K, et al. Effect of short-term carbohydrate overfeeding and long-term weight loss on liver fat in overweight humans. *The American Journal of Clinical Nutrition*. 2012;96(4):727-34.
39. Petersen K, Dufour S, Befroy D, Lehrke M, Hendler R, Shulman G. Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. *Diabetes*. 2005;54(3):603-8.