

Calorie restriction and not GLP-1 explains the acute improvement in glucose control after gastric bypass in type 2 diabetes

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Novelty statement:

- The increased weight loss at 7 days following RYGB compared to 7 days of VLCD in T2DM 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 in T2DM 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

Aims

It remains unclear whether the mechanism underlying the early improvement in glucose metabolism in type 2 diabetes following roux-en-Y gastric bypass surgery (RYGB) differs from that seen with a very low calorie diet (VLCD). Specifically, whether the markedly increased GLP-1 secretion following surgery is of primary importance has been controversial. This study compared directly the impact of GLP-1 secretion on glucose metabolism in individuals with type 2 diabetes listed for RYGB, randomised to be studied before and at 7 days following RYGB or VLCD.

Methods

A semi-solid meal test was used to investigate glucose, insulin and GLP-1 response. Insulin secretion to intravenous glucose and arginine stimulus was measured. Hepatic and pancreatic fat content was quantified using a magnetic resonance (MR) method.

Results

Decrease in fat mass was almost identical after surgery or VLCD (3.0 ± 0.3 and 3.0 ± 0.7 kg). The early plasma glucose rise and acute insulin secretion were greater following surgery than VLCD. However, the early GLP-1 rise was disproportionately greater (7-fold) after surgery than VLCD. This did not translate into a greater improvement in fasting glucose or glucose area under the curve. The reduction in liver fat was greater after surgery (29.8 ± 3.7 vs. $18.6\pm 4.0\%$) and the relationship between weight loss and reduction in liver fat differed following surgery or VLCD.

Conclusions

In conclusion, this study demonstrates that gastroenterostomy increases the rate of nutrient absorption, bringing about a commensurately rapid rise insulin. However, there was no relationship with the large post-meal rise in 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, and considered to be a direct effect of weight loss (1). However, the major improvement in metabolism occurs within one week of surgery (2). As this happens 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 (3). The post-RYGB increase in plasma glucagon-like peptide-1 (GLP-1) following oral glucose administration is clear (4, 5).

Following RYGB there is a profound decrease in energy 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 (2). Calorie balance can explain both the aetiology and reversibility of type 2 diabetes by the Twin Cycle Hypothesis (6). Testing of this hypothesis in a moderately obese group of individuals with type 2 diabetes confirmed normalisation of fasting blood glucose within 7 days (7). However, associations between the GLP-1 response after gastric bypass surgery and improvement in glucose continue to be reported (8-10) and there is widespread acceptance that the two are causally related (11, 12). There are a number of methodological problems in the reported work, including unphysiological liquid glucose challenges to test insulin secretion together with indirect measurements of insulin secretion and insulin sensitivity (13-15). Studies on the role of RYGB on glucose-dependent insulinotropic polypeptide (GIP), secreted by direct contact of nutrients with duodenal K-cells, have shown inconsistent results (3, 16). 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 early impact of bariatric surgery with that of very low calorie diet on circulating GLP-1 levels following a semi-solid meal. To ensure comparability of the groups, subjects listed for bariatric surgery were randomised. 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, body weight and composition, hepatic triglyceride content, and pancreatic triglyceride content were also quantified.

Methods

Participants

Individuals with type 2 diabetes listed for laparoscopic 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%); creatinine <150 µmol/l; ALT <2.5-fold above ULN. 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 medications prior to the first study: metformin and/or sulphonylureas for at least 72 hours, DPP-4 inhibitors for 1 month and insulin for at least 24 hours. All metabolic studies were performed after an overnight fast.

Experimental Protocol

To compare the metabolic effects of RYGB surgery with those of a VLCD participants were studied at baseline and then at 7 days of intervention. They were randomised using online software (www.sealedenvelope.com) to either: RYGB only (n=9; studied before and then 7 days after surgery) or VLCD (n=9; studied before and after a 7 day VLCD (7)). 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 (approximately 600 kcal/day) for the rest of the first week. The VLCD provided an average of 700kcal/day. Those randomised to VLCD proceeded to their planned RYGB surgery after the research tests were completed. At each time point, a standard meal test was used to assess metabolic and incretin responses, and hepatic and peripheral insulin sensitivity, first phase and maximal insulin secretion, and pancreatic triglyceride content were quantified.

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, 1 patient from each group 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 (17). All subjects underwent all measurements except that MR scans were carried out on all but 1 subject (surgery group).

Meal test

Each test was performed with the participant semi-reclined at a 45° angle in bed. Baseline blood samples were taken and subjects then consumed a semi-solid meal within 3min (10g Mornflake Instant Porridge Oats, 64g whole milk and 6g acacia honey: 100 kcal; 57% carbohydrate; 28% fat; 13% protein), designed for volume and consistency of diet consumed one week following RYGB.

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). Data were acquired using a three point Dixon method as previously described (7). 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 (7).

PNPLA3 Genotyping

As the PNPLA3 C to G genotype causes increased hepatic triglyceride levels which do not bring about any metabolic consequence, this was tested to assist interpretation of the data. PNPLA3 genotyping was performed on DNA extracted from white blood cells. DNA was isolated and genotyping performed using TaqMan SNP Genotyping Analysis (Applied Biosystems, Carlsbad, CA), as described previously (20).

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 (18). Basal rates were calculated during the last 30min of the 150min basal period. An isoglycaemic–hyperinsulinaemic clamp (insulin infusion rate $40\text{mU}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$) was initiated at 0min. Isoglycaemia (end of the basal period) was used to ensure that the true metabolic condition of each participant could be observed at each time point. Whole-body insulin sensitivity was determined during the last 30min of the clamp as whole-body glucose disposal per kg of fat free mass corrected for glucose space and urinary loss (19). 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 30min 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 (20). Blood samples for

determination of plasma glucose, insulin and C-peptide concentrations were obtained every 2 min for the first 10min then every 5min 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 and using a population model of C-peptide kinetics (21).

Analytical procedures

Hormones and metabolites were measured as previous reported (7). Human total GLP-1 (7-36, 9-36) (AlpcoDiagnostics; Salem, NH, USA) and total GIP was measured (Merck Millipore, Watford, UK) using ELISA kits. PNPLA3 genotyping was performed on DNA extracted from white blood cells. DNA was isolated and genotyping performed using TaqMan SNP Genotyping Analysis (Applied Biosystems, USA) as described previously (22).

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 (Minitab Inc.; State College, PA: www.minitab.com).

Results

Weight loss

Baseline weight and BMI did not differ between the surgery and VLCD groups (120.8 \pm 5.0 vs. 121.4 \pm 3.7 kg; $p=0.932$ and 43.0 \pm 1.1 vs. 42.3 \pm 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 \pm 0.5 vs. 3.5 \pm 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 \pm 0.3 vs. -1.3 \pm 0.6 kg; $p=0.108$) and body water (-2.4 \pm 0.3 vs. -1.6 \pm 0.5 L; $p=0.195$). Decrease in fat mass was almost identical after surgery or VLCD (3.0 \pm 0.3 and 3.0 \pm 0.7 kg respectively).

HbA1c, Plasma glucose and metabolites

HbA1c at baseline was 56 \pm 6 mmol/mol (7.3 \pm 2.7%) and 62 \pm 6 mmol/mol (7.8 \pm 2.7%) in the surgery and VLCD groups respectively. Fasting plasma glucose levels fell modestly and similarly in both groups (surgery 0.9 \pm 0.5 mmol/l and VLCD 1.2 \pm 0.6 mmol/l; $p=0.739$; Table 1). 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). Fasting glucagon decreased in both groups after 7 days but the changes were not significant.

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, with 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. **Peak insulin levels were similar at baseline and at 7 days (32.1±7.5 to 37.2±7.8 and 37.7±6.7 to 37.4±5.6 pmol/l for surgery and VLCD groups respectively). The early GLP-1 rise was disproportionately greater (7-fold) after surgery than VLCD and did not translate into a greater improvement in fasting glucose or glucose area under the curve. In order to ensure that there was no effect of the modestly but non-significantly greater fasting plasma glucose in the VLCD group (Table 1), sub-groups matched for fasting plasma glucose (n=7 each; VLCD 8.9 ±0.8 mmol vs. Surgery 9.1±1.2 mmol/l) were compared. The 0-20min rise in GLP-1 was 7 fold greater, exactly as for the whole groups and neither peak nor AUC plasma glucose in the subgroup was improved to a greater extent after surgery (peak: 9.9±0.5 to 9.4±0.3 vs. 10.0±0.8 to 9.3±0.6 mmol/l; AUC 87.0±21.1 to 92.0±15.4 vs. 116.7±19.5 to 132.4±19.5 mmol.l⁻¹.min for VLCD vs. surgery respectively).**

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. In the VLCD group, plasma glucagon levels (0-20min) rose by 8.5±5.8ng/l at baseline but only 3.2±3.8ng/l at day 7 (p=0.127). In the surgery group the rise in glucagon was greater at day 7 compared to baseline (13.4±3.6 vs. 3.5±5.8 ng/l; p=0.164).

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 (surgery by 29.8 ± 3.7 % and VLCD by 18.6 ± 4.0 %; $p=0.06$). 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 1 individual in the Surgery group (heterozygous; change in hepatic triglyceride 7.2 to 4.8%) and 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). **However, the preponderance of the G allele of PNPLA3 in the VLCD group was associated with a modest but not significantly higher hepatic triglyceride level (Table 1). As this allele determines an increase in triglyceride in a non-metabolically active manner no effect would be expected upon the metabolic outcomes reported.** 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 correlation between the change in hepatic triglyceride content and GLP-1 AUC in either group. There was no change in pancreatic triglyceride content after 7 days of either surgery or VLCD (6.7 ± 0.7 to 6.7 ± 0.6 %; $p=0.885$ and 6.4 ± 0.6 to 6.5 ± 0.7 %; $p=0.781$, 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 change in the latter was 7-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. Non-incretin dependent beta cell function as assessed by the stepped intravenous glucose challenge remained unchanged in both RYGB and 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 (23, 24). 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 (3) despite a similar reduction in fasting glucose, insulin, C-peptide and HOMA-IR in both 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 greater improvement in fasting plasma glucose by calorie restriction alone (13, 14). The increase in meal stimulated GLP-1 levels noted in after surgery 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 using individuals as their own controls (13). 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 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, with identical decreases in fat mass. 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. Exacerbation of fatty liver disease following jejunio-ileal bypass is well recognised (25) and the underlying mechanism for this might 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 or DPP4 inhibitors can decrease liver fat content (26, 27). A possible relationship between extent of post-meal GLP-1 elevation and fall in liver fat in the surgery group was therefore 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 (7). The present data confirm this for VLCD and 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 improve rapidly after calorie restriction. Fasting plasma glucose concentration is determined by the rate of hepatic glucose production (18) and elevated liver fat content is associated with decreased insulin sensitivity to suppression of hepatic glucose production (28). While carbohydrate overfeeding can induce liver fat accumulation (29), weight loss brings about reduction in liver fat (18, 30). 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 (7). **The continuing calorie restriction after RYGB causes hepatic triglyceride to decrease to an average of 2% (31), and it is likely that there is a personal fat threshold effect for bringing about metabolic improvement (32).**

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. Although relatively small numbers were studied, definitive results were obtained due to the very large difference in post-meal total GLP-1 secretion. The study design differs from the majority of studies after RYGB in that a liquid 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.

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Conflicts of interest

The very low calorie liquid diet product was provided on request by Nestle. The company had no input into the study at any stage.

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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 and RLB 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, RLB edited the manuscript and RT designed the study and edited the manuscript. The guarantor for the study is RT.

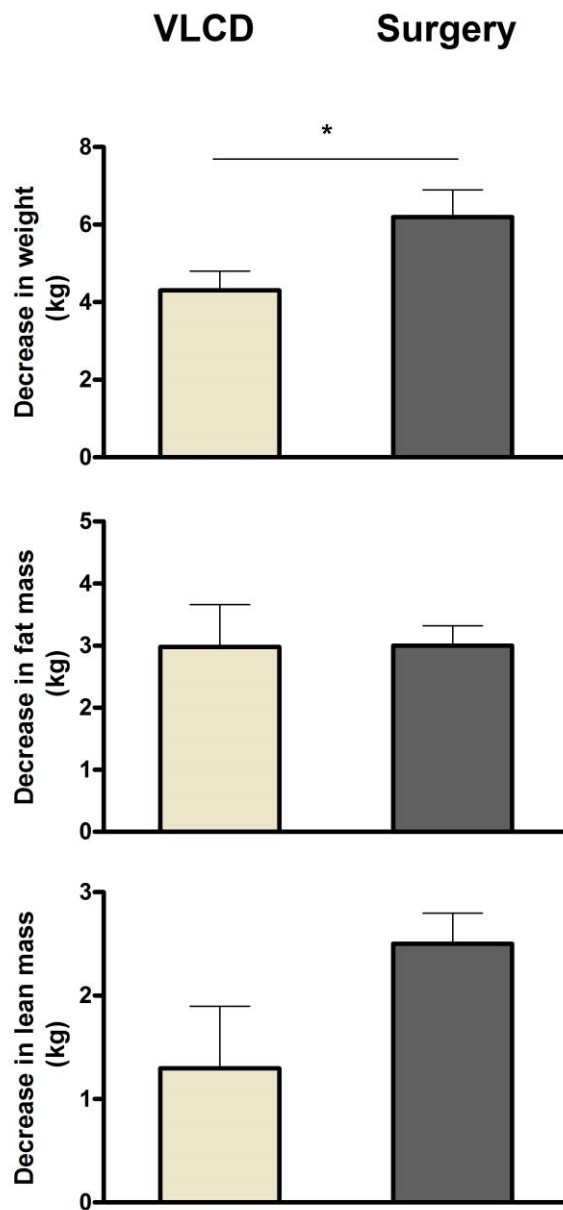


Figure 1. Difference between baseline and day 7 in body weight, fat mass and lean body mass in the VLCD and surgery groups. * = $p < 0.05$ for between group difference.

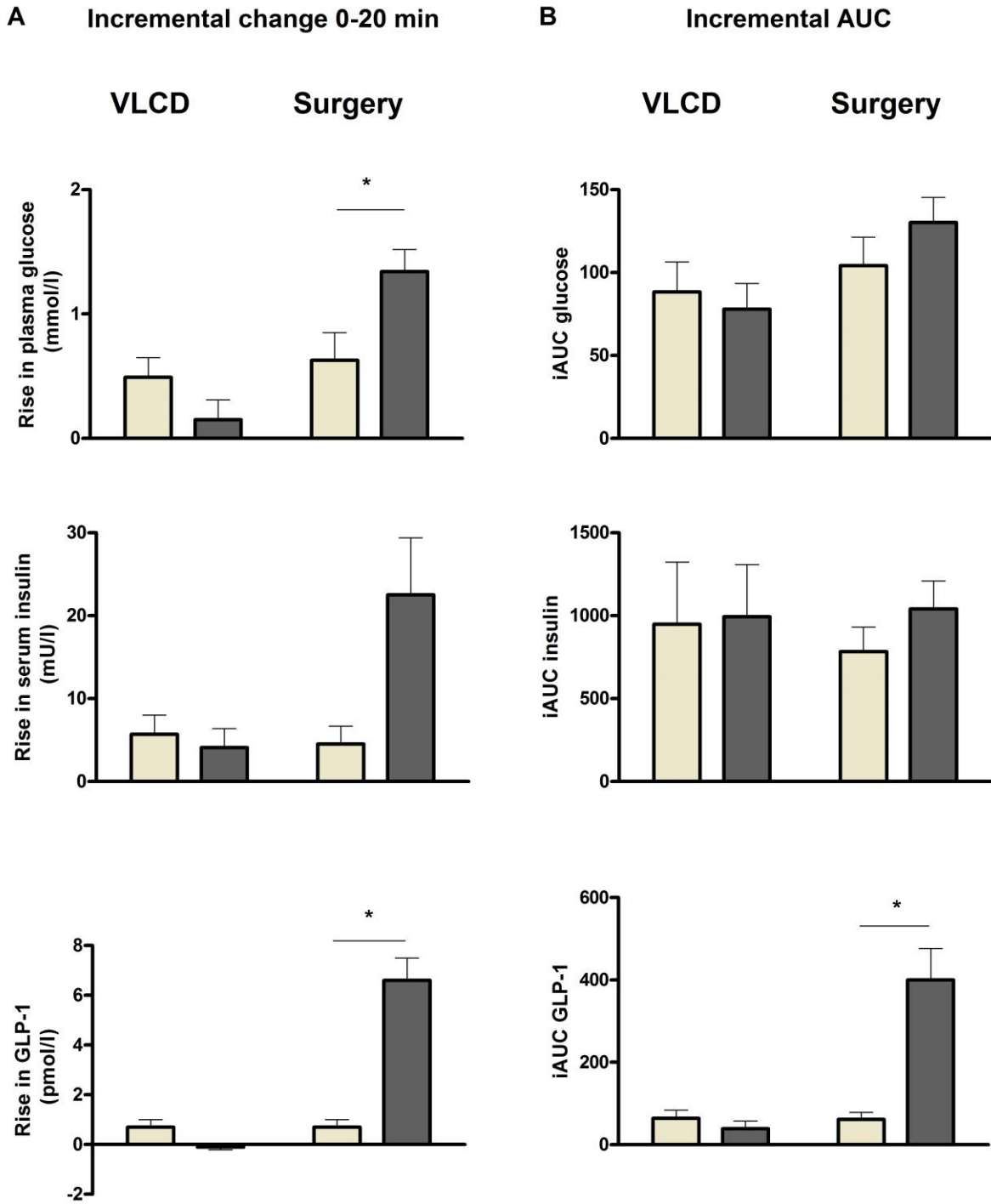


Figure 2. (A) Incremental change in glucose, insulin and GLP-1 from fasting (pale bar) to 20 minutes (dark bar) and (B) change in positive incremental area under the curve during the meal tolerance test before (pale) and 7 days (dark) after intervention (VLCD or Surgery). * = $p < 0.05$ for baseline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

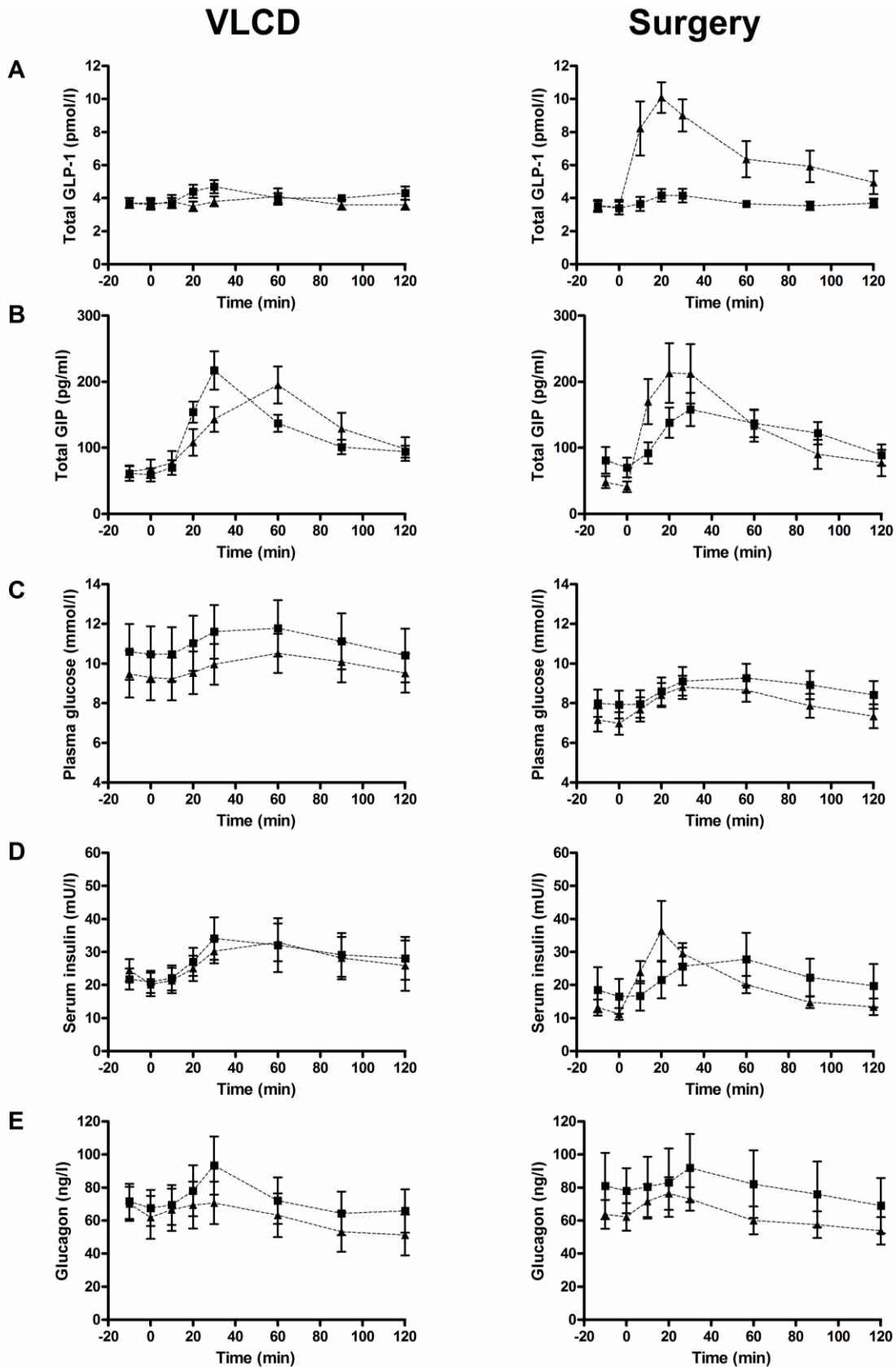


Figure 3. Total GLP-1 (A), total GIP (B), glucose (C), insulin (D) and glucagon (E) levels (mean \pm SEM) during the 2 hour meal test in the VLCD and Surgery groups at baseline (squares) and at day 7 (triangles).

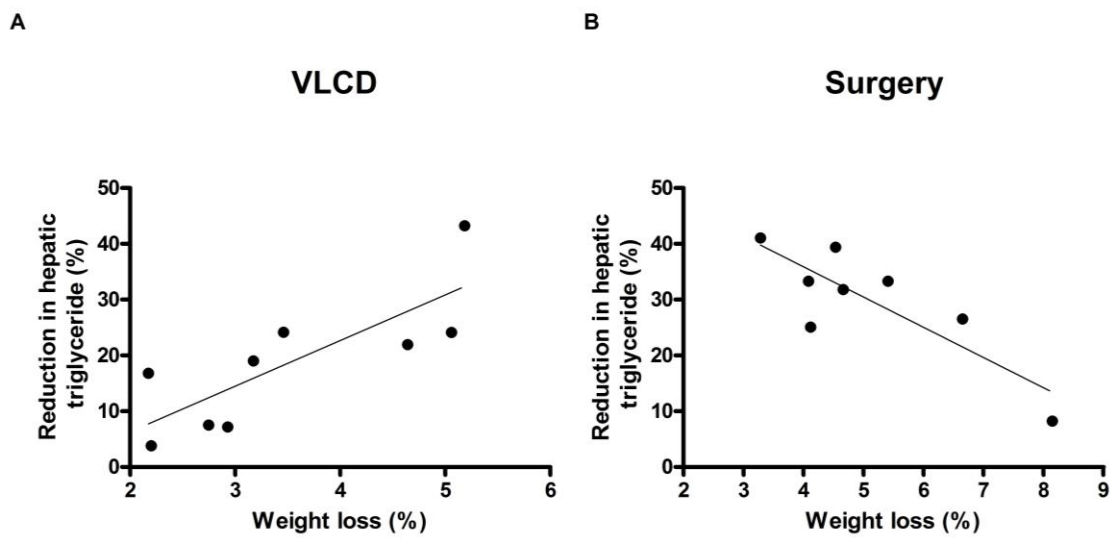


Figure 4. Relationship between achieved weight loss and reduction in hepatic triglyceride content at day 7 in the VLCD group (A; Pearson correlation coefficient 0.80, $p=0.01$) and Surgery group (B; Pearson correlation coefficient -0.88, $p<0.01$).

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 plasma glucose (mmol/l)	10.5 ± 1.4	9.4 ± 1.2	0.105	8.0 ± 0.7	7.1 ± 0.6	0.078
Fasting β-hydroxybutyrate (mmol/l)	0.22 ± 0.07	0.63 ± 0.17	0.005	0.29 ± 0.06	0.86 ± 0.18	0.019
Fasting NEFA (mmol/l)	0.85 ± 0.13	0.91 ± 0.08	0.402	0.85 ± 0.11	0.96 ± 0.08	0.087
Fasting glucagon (ng/l)	69.7 ± 10.8	66.0 ± 11.4	0.511	79.5 ± 16.8	63.0 ± 8.4	0.120
Fasting triglycerides (mmol/l)	1.7 ± 0.3	1.4 ± 0.2	0.217	1.4 ± 0.1	1.7 ± 0.1	0.090
Fasting ALT (U/l)	34.7 ± 6.4	39.9 ± 7.2	0.020	40.8 ± 5.2	61.4 ± 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
Hepatic triglyceride (%)	11.8 ± 2.2	9.8 ± 1.9	0.003	6.5 ± 1.6	4.5 ± 1.0	0.027

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}⁻¹.min⁻¹)	3.48±0.32	3.43±0.63	0.906	3.71±0.36	3.16±0.33	0.083
Basal HGP (mg.m⁻².min⁻¹)	97.5±5.8	94.2±14.2	0.776	104.7±13.8	86.2±8.9	0.100
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

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