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.

Running title: Insulin sensitivity and beta-cell function after Roux-en-Y Gastric Bypass

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ABSTRACT
Roux-en-Y gastric bypass (RYGB) improves glycemic control within days after surgery, and changes in insulin sensitivity and beta-cell function are likely to be involved. We studied 10 obese patients with type 2 diabetes and 10 obese glucose tolerant subjects before, 1 week, 3 months and 1 year after RYGB. Participants were included after a preoperative diet induced total weight loss of −9.2±1.2%. Hepatic and peripheral insulin sensitivity were assessed using the hyperinsulinemc euglycemic clamp combined with glucose tracer technique and beta-cell function evaluated in response to an intravenous glucose-glucagon challenge as well as an oral glucose load. Already within 1 week, RYGB reduced basal glucose production, improved basal hepatic insulin sensitivity and increased insulin clearance highlighting the liver as an important organ responsible for the early effects on glucose metabolism after surgery. Insulin mediated glucose disposal and suppression of fatty acids did not improve immediately after surgery but increased at 3 months and 1 year likely related to the reduction in body weight. Insulin secretion increased after RYGB, but only in patients with type 2 diabetes and only in response to oral glucose, underscoring the importance of the changed gut anatomy.

KEYWORDS
Endogenous glucose production
Glucose disposal
Insulin clearance
Beta-cell function
Type 2 diabetes
Glucagon-like peptide-1
Glucagon
Glucose-dependent insulinotropic polypeptide
Fatty acids
Hyperinsulinemic euglycemic clamp
Glucose tracer technique
ABBREVIATIONS

AIR=Acute insulin response
CI=Clearance of insulin
DEXA=Dual energy x-ray absorptiometry
DI=Disposition index
FA=Fatty acids
FFM=Fat free mass
GIP=Glucose-dependent insulinotropic polypeptide
GLP-1=Glucagon-Like-peptide 1
HISI=Hepatic insulin sensitivity index
HOMA-IR=Homeostatic model assessment of insulin resistance
iAUC=Incremental area-under-the-curve
IGI=Insulinogenic index
IQR=Interquartile range
ISR=Insulin secretion rate
IV=Intravenous
NGT=Normal glucose tolerance
OGTT=Oral glucose tolerance test
Ra=Rate of appearance
Rd=Rate of disappearance
RER=Respiratory exchange ratio
RYGB=Roux-en-Y gastric bypass
TTR=Tracer-to-tracee ratio
Roux-en-Y gastric bypass (RYGB) surgery induces weight loss and improves metabolic abnormalities in severely obese patients (1). In patients with type 2 diabetes, the glucose-lowering effect of RYGB is superior to conventional antidiabetic therapy (2,3) and often occurs within days after surgery (4). Insulin resistance of liver, skeletal muscle and fat tissue is associated with the obese state, while patients with type 2 diabetes additionally suffer from beta-cell dysfunction and impairments of the incretin system (5).

Insulin sensitivity improves with weight loss, but whether increased insulin sensitivity plays a role in the early improvement of glycemic control after RYGB is not clarified (6). Reductions in homeostatic model assessment of insulin resistance (HOMA-IR) have been demonstrated within 1 week (7–11), but assessment using the hyperinsulinemic clamp has not been performed at this early time-point. At 2-3 weeks postoperatively, clamp studies have found no changes in peripheral insulin sensitivity (12,13), but improvements have been reported after 4 weeks (14) and later when weight loss is pronounced (12,13,15). As HOMA-IR primarily reflects hepatic insulin resistance while the hyperinsulinemic clamp estimates peripheral insulin sensitivity (16), this could indicate a differential effect of RYGB on hepatic and peripheral insulin sensitivity (6). Endogenous glucose production has not been assessed immediately after RYGB, but one study showed reductions after 1 month while another did not detect changes at 2 weeks (12,17).

Postprandial insulin secretion increases already 1 week after RYGB (9,18) and is associated with exaggerated release of Glucagon-Like Peptide-1 (GLP-1) (19–21). In contrast, after intravenous (iv) challenges, insulin secretion increases more gradually after RYGB in patients with type 2 diabetes (14,22–24) and even declines in glucose tolerant subjects (24,25). Improved beta-cell function after RYGB may thus be linked to the oral rather than the iv route of administration (6). However, as insulin secretion adapts to changes in insulin sensitivity (26), assessment of beta-cell function also requires concomitant evaluation of insulin sensitivity. Evaluation of beta-cell function using oral and
iv tests as well as clamp-derived measures of insulin sensitivity has previously only been performed at 4 weeks after RYGB (14).

We studied patients with type 2 diabetes and obese glucose tolerant subjects before, 1 week, 3 months and 1 year after RYGB with assessment of hepatic and peripheral insulin sensitivity using the hyperinsulinemic clamp combined with glucose tracer technique. A secondary aim was to evaluate insulin secretion in response to both an iv glucose-glucagon challenge and an oral glucose load.

RESEARCH DESIGN AND METHODS

Subjects. We recruited 10 obese patients with type 2 diabetes (T2D group, age 43.6 ± 3.4 years, median diabetes duration 2.5 (range 1-11) years) and 10 obese normal glucose tolerant subjects (NGT group, age 40.1 ± 2.8 years) scheduled for laparoscopic RYGB at Hvidovre Hospital (Hvidovre, Denmark). Before enrollment in the study all participants fulfilled the inclusion criteria for bariatric surgery in Denmark and had completed a preoperative diet-induced total body weight loss of 8% required by health authorities. After the preoperative weight loss, all patients in the T2D-group had a 2-h P-glucose of ≥11.1 mmol/l, and diabetes was controlled with diet alone (n=2), metformin alone (n=4) or metformin combined with liraglutide (n=2) or NPH insulin (n=2). Liraglutide was discontinued ≥10 days and all other antidiabetic agents ≥3 days prior to experimental days, and discontinuation of antidiabetic medication was maintained after surgery. One patient with diabetes for 11 years required metformin at 4-11 months postoperatively. In the NGT-group, all had 2-h P-glucose <7.8 mmol/l and HbA1c <6% (42 mmol/mol). Written informed consent was obtained from all participants and the study was approved by the Municipal Ethical Committee of Copenhagen in accordance with the Helsinki II declaration and by the Danish Data Protection Agency and registered at www.ClinicalTrials.gov (NCT 01202526).
**Study Design.** On separate days before, 1 week, 3 months and 1 year after RYGB, we performed hyperinsulinemic euglycemic clamps and intravenous glucose-glucagon tests. Before, 3 months and 1 year after RYGB, oral glucose tolerance tests (OGTT) and whole-body dual energy x-ray absorptiometry (DEXA) scans were performed on an additional study day. All participants completed the preoperative and 3 months visits, while 4 subjects did not complete the 1 week visit due to postoperative complications and 2 did not wish to participate at the 1 year visit. Prior to experiments participants were instructed to refrain from strenuous physical activity and alcohol for 3 days and to fast overnight (10-12 h). On the day of the experiment, subjects were weighed and placed in a reclined position in a hospital bed allowing no physical activity.

**Hyperinsulinemic euglycemic clamp.** Catheters were placed in an antecubital vein for infusion and in a dorsal hand vein for blood sampling with the hand placed in a heated box for arterialization. After collection of three fasting samples, a primed-continuous basal infusion (0.036 mg/kg/min) of [6,6-\(^2\)H\(_2\)]-glucose (99 atom percent enrichment, Cambridge Isotope Laboratories, Andover, MA, USA, sterilized and packed in vials at the Central Pharmacy of the Capital Region, Herlev, Denmark) was started and continued for 120 min using a precision infusion pump (P2000, IVAC medical systems, Hampshire, UK). Priming dose was adjusted for the ambient fasting P-glucose (3.6 mg/min×fasting P-glucose in mmol/l×1/5). Urine was sampled during the basal infusion period in the T2D group and tested for traces of glucose (Multistix7, Siemens, Berlin, Germany). After 120 min, the clamp was initiated (4 h primed-continuous insulin infusion, 40 mU/m\(^2\)/min, Actrapid, Novo Nordisk, Bagsværd, Denmark). Insulin was dissolved in saline to which was added blood from the participant. P-glucose was allowed to drop to 5.5 mmol/l before initiation of a variable infusion of 20% glucose (wt/vol) enriched with [6,6-\(^2\)H\(_2\)]-glucose (median enrichment: 2.16%, interquartile range (IQR): 2.10-2.24), while glucose infusion was initiated at clamp-start if P-glucose was ≤5.5 mmol/l. The basal infusion of [6,6-\(^2\)H\(_2\)]-glucose was decreased to 25% upon initiation of glucose-
infusion. Blood was sampled every 10 min during the last 30 min of the basal and clamp periods, and every 20 min during the remainder of the clamp, while P-glucose was assessed every 5 min. Stable tracer-to-tracee ratios (TTRs) were obtained in the basal and clamp periods with CVs of 4.8±2.2% and 2.9±2.1% (mean±SD), respectively, and P-glucose was kept stable for the last 30 min of the clamp (CV: 3.1±1.4%) without differences in CVs between pre- and postoperative days or between groups. Biopsies of abdominal subcutaneous fat and muscle from the vastus lateralis muscle were obtained during the basal period and after 4 h insulin infusion using a modified Bergström needle with suction under local anesthesia. Results from biopsies will be presented elsewhere.

**Glucose-glucagon test.** Catheters were placed in antecubital veins in both arms. At t=0 min, a bolus of 50% glucose (wt/vol) was injected over 1 min. The volume of the bolus was fixed for the individual patient throughout the study: (20 – fasting preoperative P-glucose [mmol/l]) × (height² [m²]) × (2.1 [ml/m²/mmol/l]). At t=2 min, a bolus of 1 mg glucagon (Novo Nordisk, Bagsværd, Denmark) was injected. Blood was sampled at t= -10, -5, 0, 2, 6, 8, 10, 12 min.

**Oral glucose tolerance test.** Participants ingested 75 g of glucose dissolved in 250 ml of water within 5 min. Blood samples were obtained from a catheter in an antecubital vein at t=0, 15, 30, 60, 90, 120 min. Two participants did not complete the postoperative OGTTs due to vomiting. Otherwise the test was well tolerated after surgery, except for mild degrees of nausea during the first hour.

**DEXA.** Body composition was assessed by DEXA-scanning (Discovery A, S/N 83487, Hologic Inc., Bedford, MA, USA) with software package Apex 2.3.

**Surgical Procedure.** Surgery was performed as previously described (9).

**Preoperative Weight Loss.** Data on the preoperative weight loss was collected retrospectively from patient records.

**Postoperative Diet.** From the day after the operation patients were on a liquid diet of approximately 1200 kcal/day until 14 days postoperatively where the diet gradually changed towards solid foods.
Analytic procedures. Blood collected in prechilled EDTA-tubes (for analysis of glucagon and fatty acids), EDTA-tubes added DPP-IV inhibitor (valine–pyrrolidide: final concentration 0.01 mmol/l, for GLP-1, GIP and glucagon from OGTTs) and heparin-tubes (for TTR) were immediately centrifuged while clot-activator tubes (for insulin and C-peptide) were left for 30 min before centrifugation. EDTA-Eppendorph tubes were immediately centrifuged and analyzed for P-glucose using YSI model 2300 STAT plus (YSI, Yellow Springs, OH, USA). Serum C-peptide and insulin were analyzed using AutoDELFIA fluoroimmunoassay (Wallac OY, Turku, Finland), HbA1c was measured using high pressure liquid chromatography (Tosoh Bioscience, Tokyo, Japan) and plasma fatty acids (FA) (NEFA C kit, Wako Chemicals GmbH) were measured using enzymatic colorimetric methods (Hitachi 912 automatic analyzer, Boehringer, Mannheim). Glucagon, total GLP-1 and GIP were analyzed as previously described (9). TTR was analyzed using mass spectrometry as previously described (27).

Calculations and statistical analysis. Rate of appearance (Ra) and disappearance (Rd) of glucose were calculated from the last 30 min of the basal and clamp periods using Steele’s equation (28). One patient was excluded from analysis of Ra_{basal} due to preoperative glucosuria. Mean serum C-peptide concentration in the basal period was used to assess basal hepatic insulin sensitivity (HISI_{basal}=10^{6}/[Ra_{basal} \times C\text{-}peptide_{basal}]) while mean serum insulin in the clamp period was used for insulin-adjusted glucose disposal (Rd_{clamp}/Insulin_{clamp}). Suppression of Ra, FA and glucagon was calculated as the difference between basal and clamp levels expressed as a percentage of the basal level. Hepatic insulin clearance at fasting (CI_{fasting}) was calculated as the ratio of fasting serum C-peptide to insulin, while clearance during the clamp was adjusted for endogenous insulin secretion (CI_{clamp}=\text{insulin infusion rate}/(\text{Insulin}_{clamp} – [C\text{-}peptide_{clamp} \times \text{Insulin}_{basal}]/C\text{-}peptide_{basal}) (29).

Incremental AUCs (iAUCs) were calculated using the trapezoidal rule subtracting fasting levels. Early insulin secretion was estimated using insulinogenic index from OGTTs (IGI=ΔC\text{-}peptide_{0-30} /
ΔGlucose_{0-30 \text{min}} and the acute insulin response (AIR) from iv glucose-glucagon tests calculated as the mean serum C-peptide from 6-12 min subtracting fasting levels. Indices of insulin secretion (IGI and AIR, respectively) were related to insulin sensitivity (Rd/I_{clamp}) by calculation of disposition indices (DI_{oral} and DI_{iv}, respectively)(26).

Data are expressed as means ± SEM unless otherwise specified. Postoperative changes were analyzed by ANOVA in a linear mixed effects model using time from surgery and group as fixed effects and individual subjects as random effect. Logarithmic transformation was used if distribution was skewed. Post-hoc comparisons of group differences at a given study-point were performed using unpaired $t$-tests. Pearson’s test was used to evaluate correlations. Prior to the study, we estimated that 8 patients would allow detection of within-group postoperative changes in insulin sensitivity of 20% (power 0.80, level of significance 0.05) based on data from patients with type 2 diabetes (30).

Statistical analyses were performed in $R$ version 2.11.1 (www.R-project.org).

RESULTS

Weight and body composition

Prior to the study, participants in the two groups achieved a similar required diet-induced total weight loss of −9.2±1.2% (figure 1). Weight loss was accelerated after RYGB and initially comparable in the two groups but at 1 year weight loss was larger in the NGT group. Postoperative weight loss was mostly fat mass (% of total weight loss at 3 months T2D 65.5±3.1%, NGT 71.4±1.6%, p=0.10 between groups; at 1 year: T2D 67.1±3.3%, NGT 76.8±2.5%, p=0.03), but fat free mass (FFM) was also reduced by 9-10% at 3 months and 11-13% at 1 year (table 1).

Glycemic control and insulin metabolism

Patients with type 2 diabetes improved glycemic control postoperatively (table 1); fasting P-glucose declined by ~20% at 1 week and was <5.6 mmol/l in 6/10 and in 5/9 patients at 3 months and 1 year,
respectively. HbA1c was also reduced reaching values <6% (<42 mmol/mol) in 7/10 patients at 3 months and 8/9 patients at 1 year, while 2-h P-glucose after OGTT declined by >50% postoperatively. The NGT group experienced postoperative reductions of 5-10% in fasting P-glucose and of ~30% in 2-h P-glucose, while HbA1c was unchanged.

In both groups, fasting serum insulin decreased and to a larger degree than C-peptide pointing to a significant increase in fasting insulin clearance already from 1 week after RYGB. Clearance of exogenous insulin during the clamp increased similarly in the two groups from 1 week post-RYGB and clamp insulin concentration was reduced by 20-30% after surgery (table 2).

**Endogenous glucose production**

Preoperatively, basal glucose production (Ra_{basal}) tended to be higher in patients with type 2 diabetes than in subjects with NGT (p=0.09), while basal hepatic insulin sensitivity (HISI_{basal}) did not differ significantly between groups (table 2). Basal glucose production decreased equally at 1 week after RYGB in the two groups, and basal hepatic insulin sensitivity increased 50% due to concomitant reductions in basal serum C-peptide. While basal glucose production remained reduced in the T2D group, it was no longer significantly changed at 3 months and 1 year in the NGT group. Nevertheless, basal hepatic insulin sensitivity increased 1.5-2 fold in both groups.

Clamp glucose production was incompletely suppressed in patients with type 2 diabetes before surgery (Ra_{clamp} was larger than 0 mg/min, p<0.01) and was not significantly changed at 1 week, but had declined at 3 months (p=0.06) and was completely suppressed by 1 year (Ra_{clamp} not different from 0 mg/min, p=0.30). Subjects with NGT experienced no changes in clamp glucose production which was almost completely suppressed at all time-points (Ra_{clamp} not different from 0 mg/min, p>0.05).
Glucose disposal and fatty acids

Glucose disposal (Rd_{clamp}) was lower in patients with type 2 diabetes compared to subjects with NGT preoperatively and was unchanged at 1 week after surgery but increased by ~50% after 3 months and by ~75% after 1 year (table 2). Glucose disposal in the NGT group decreased by ~30% at 1 week, was unchanged at 3 months and increased by ~60% after 1 year compared with preoperatively. Lower clamp insulin concentration seemed to explain the reduced glucose disposal in the NGT group at 1 week, as glucose disposal adjusted for clamp insulin concentration (Rd/I_{clamp}) was not significantly changed at 1 week in either group. The adjusted glucose disposal tended to be lowest in patients with type 2 diabetes before surgery (Rd/I_{clamp} p=0.07, Rd/I_{ffm} p=0.05) and increased significantly in both groups at 3 months and 1 year. The change in glucose disposal correlated with the weight change at 1 year in the total group of participants (ΔRd_{clamp} r=0.49, p=0.04; ΔRd/I_{clamp} fmf r=0.45, p=0.06), while the correlations were not significant at 3 months.

Fasting plasma FA concentration increased by ~20% at 1 week, returned to preoperative values at 3 months and was slightly decreased after 1 year in both groups (figure 2A, table 2). Suppression of FA in plasma during the clamp did not differ significantly between groups and increased similarly at 3 months and 1 year. At 1 week suppression of FA was reduced in the T2D group and unchanged in the NGT group.

Beta-cell function

Insulin secretion in response to oral glucose was markedly enhanced postoperatively in patients with type 2 diabetes with 2 fold increases in insulinogenic index and iAUC of serum C-peptide and 4 fold increased oral disposition index (figure 3C, table 3). Peak serum C-peptide increased slightly in both groups and time to peak was significantly reduced (p<0.01). Insulinogenic index was unchanged in subjects with NGT after surgery, while iAUC of C-peptide and the oral disposition index increased moderately at 3 months and 1 year, respectively.
Insulin secretion after iv glucose-glucagon was unchanged postoperatively in patients with type 2 diabetes (figure 4, table 3) regardless of disease duration (data not shown), but the iv disposition index increased at 3 months and 1 year due to increased insulin sensitivity. In subjects with NGT, the C-peptide response to iv glucose-glucagon declined after surgery, but the iv disposition index was unchanged.

Disposition index in the NGT group remained higher than in the T2D group after both oral and iv challenges.

**Glucagon, GLP-1 and GIP**

In patients with type 2 diabetes, basal P-glucagon was unchanged at 1 week and decreased at 3 months and 1 year after RYGB, while basal P-glucagon was unchanged postoperatively in subjects with NGT except for a transient increase at 1 week (figure 2B, table 2). The ratio of basal C-peptide to glucagon concentration declined at 1 week after surgery in subjects with NGT and remained low at 3 months and 1 year; whereas it was largely unchanged in patients with type 2 diabetes, except for a decline at 1 year postoperatively. Postoperative changes in the ratio of C-peptide to glucagon were thus not related to changes in basal glucose production. Glucagon suppression during the clamp was highest in the NGT group and did not change postoperatively in either group. Preoperatively, glucagon secretion was suppressed in response to oral glucose in both groups (iAUC was negative), but after surgery postprandial glucagon response increased (figure 3D, table 4).

After RYGB, GLP-1 secretion was exaggerated in both groups with 5-fold increased peak concentration and 12-fold increased iAUC after oral glucose (figure 3E, table 4). Postprandial GIP secretion was largely unchanged, although at 1 year peak GIP increased significantly in patients with type 2 diabetes (figure 3F, table 4) and occurred earlier in subjects with NGT (p<0.01). Fasting concentrations of incretin hormones were unaltered postoperatively.
DISCUSSION

In the present study, changes in insulin sensitivity and beta-cell function were assessed from 1 week after RYGB and throughout the first postoperative year in patients with type 2 diabetes and in obese normal glucose tolerant subjects. Before surgery, patients with type 2 diabetes were more insulin resistant than glucose tolerant subjects, but differences in insulin secretion were more pronounced with >50% lower insulin secretion responses in patients with type 2 diabetes.

After RYGB, our main finding was an early increase in hepatic insulin sensitivity at 1 week in both study groups as indicated by reduced basal glucose production and increased basal hepatic insulin sensitivity. Hepatic insulin clearance and clearance of exogenous insulin were furthermore increased at 1 week and because insulin clearance has been suggested to be initiated by receptor mediated endocytosis, this could indicate a common mechanism responsible for the early improvement in hepatic insulin action and clearance (31). In contrast, peripheral insulin sensitivity was not improved after 1 week, but glucose disposal and suppression of FA increased in both groups after 3 months and 1 year pointing towards improvements in muscle and fat tissue insulin sensitivity.

Insulin secretion in response to oral glucose increased in patients with type 2 diabetes, while insulin secretion was unchanged after iv stimulation. In subjects with NGT, the time course of insulin secretion after oral glucose changed with higher and earlier peak C-peptide, but insulinogenic index was unchanged. In response to iv glucose-glucagon, insulin secretion declined in subjects with NGT.

Immediate improvement in hepatic insulin sensitivity without changes in peripheral sensitivity is the typical response to calorie restriction in obese subjects regardless of glucose tolerance and is associated with an early decrease in liver fat (30,32–34). Thus, we suggest that the observed increase in hepatic insulin sensitivity and insulin clearance 1 week after RYGB in our study could be the result of postoperative calorie restriction, perhaps due to a rapid decrease in hepatic fat content. In
fact, changes in basal glucose production and basal concentrations of glucose and insulin have been reported to be even larger after 1 week of strict dieting (600 kcal/day) (30), hence calorie restriction per se is sufficient to cause changes of this magnitude. Furthermore, several studies have reported comparable changes in HOMA-IR after RYGB and calorie restriction (7,13,15,22,35,36) although a few studies found larger improvements in HOMA-IR after RYGB than after restrictive surgery (37,38) or diet alone (38,39). Better compliance to the diet in RYGB-operated patients could possibly explain some, if not all, of these differences.

Glucose disposal was largely unchanged 1 week after RYGB, except for a decline in $R_d$ in the NGT group that could be attributed to the lower clamp insulin concentration brought about by increased insulin clearance. Also at 1 week after surgery, glucose disposal could still be influenced by postoperative stress (40,41) possibly counteracting a beneficial effect of surgery. However, lack of improvement in glucose disposal is in line with hyperinsulinemic clamp studies performed 4 weeks after RYGB when surgical stress has abated (17,42). Elevated FA also act to reduce glucose disposal (43,44) and were seen in both groups 1 week after RYGB in accordance with previous studies early after RYGB (12,22,42) and calorie restriction (22). Notably, improved hepatic insulin sensitivity is not seen in response to increased FA (43) or surgical stress (41). Peripheral insulin sensitivity increased at 3 months and 1 year in both groups probably due to weight loss (45,46) as indicated by the positive correlation between changes in glucose disposal and weight loss at 1 year post-surgery.

Increased insulin secretion after ingestion of oral glucose in patients with type 2 diabetes is in accordance with previous findings after RYGB (9,14,37) and is likely caused by the large postoperative increase in GLP-1 as demonstrated in studies using pharmacological blockade of the GLP-1 receptor (19,20). Additionally, relief of gluco- and lipotoxicity have been proposed as potential contributors to improved beta-cell function based on the gradual improvement in insulin
secretion in response to an iv glucose bolus reported previously (12,14,22–24). However, in our study, patients with type 2 diabetes displayed unchanged insulin secretion in response to the iv challenge despite reductions in fasting glucose concentrations and FA levels (at 1 year). This discrepancy may be explained by the use of the combined glucose-glucagon stimulus (47), which may be less influenced by differences in fasting glucose concentrations (48,49). Of note, the concomitantly improved insulin sensitivity makes the insulin secretion less inadequate, as seen by increased DIiv in the T2D group. Insulin secretion after the iv test declined postoperatively in glucose tolerant subjects, likely as an adaptation to improved insulin sensitivity (i.e. DIiv was unchanged). Postoperative improvements in beta-cell secretory capacity per se are thus not supported by this study (37,50).

In both groups, relative increases in insulin secretion after oral glucose exceeded changes after iv stimulation whether expressed independently or as DI, highlighting the importance of gut-related potentiating factors. Increased postprandial GLP-1 has consistently been reported after RYGB (6) and is likely related to the changed gut anatomy as calorie restriction and weight loss do not change GLP-1 secretion substantially (7,13,35). GIP secretion has been reported unchanged in some (8,9,35,37) but not all previous studies (14,18), while increased postprandial glucagon secretion is a common finding after RYGB (8,9,18,19,35), although somewhat paradoxical considering the high concomitant levels of GLP-1 and glucose. The increase may represent glucagon of gut origin resulting from excess postoperative stimulation of L-cells (18). At any rate, glucagon suppression during the clamp was unchanged postoperatively confirming intact alpha cell responsiveness to iv glucose and/or insulin (19).

The study has limitations; firstly participants were included after a preoperative weight loss of 9%, likely to improve several metabolic parameters, especially hepatic insulin sensitivity (32), thus preoperative characteristics of study participants may not be comparable to RYGB candidates or
patients with type 2 diabetes not subjected to a preoperative diet. Nevertheless, postoperative metabolic improvements were still observed, although the magnitude probably would have been greater, if participants had not been subjected to the preoperative diet. Secondly, the study was powered to detect postoperative changes in insulin sensitivity within the groups and minor changes in other parameters or minor differences between groups may thus not have reached significance. Finally, we did not include a non-operated group subjected to the same postoperative diet, which would be of major interest provided that diet-adherence can be controlled.

In conclusion, RYGB increases basal hepatic insulin sensitivity already 1 week after surgery in patients with type 2 diabetes and in obese glucose tolerant subjects. Concomitant increases in insulin clearance further highlight the liver as an important organ responsible for the early effects on glucose metabolism after surgery. Later improvements in peripheral insulin sensitivity 3 months and 1 year postoperatively are likely related to the reduction in body weight. Insulin secretion increases after RYGB in patients with type 2 diabetes but only in response to oral glucose, underscoring the importance of the changed gut anatomy and exaggerated GLP-1 secretion.

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Author contributions: K.N.BM and C.D. wrote the study protocol, identified eligible participants, conducted the study, researched data, performed data analysis, contributed to the discussion and wrote the manuscript. N.B.J., S.H.J, A.K.S., P.H.A., V.B.K., L.N., D.L.H, and D.W. contributed to data analysis and discussion and reviewed/edited the manuscript. S.M., J.J.H., J.F.P.W., B.K. and E.A.R. contributed to the design of the study protocol, data generation and analysis, discussion and reviewed/edited the manuscript. All authors have approved the final version of the manuscript. K.N.BM 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.
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FIGURE LEGENDS

Figure 1: Preoperative and postoperative total body weight loss (%) in patients with type 2 diabetes (black squares) and NGT (black triangles) undergoing RYGB at t=0. Values are mean ± sem.

Changes in BMI was analyzed with mixed-effects ANOVA (Time: p<0.01, Group: p=0.52, Time×Group: p=0.01). # p<0.05 for difference in response between groups, ** p < 0.01 for the change from preoperative level within the group.

Figure 2: Plasma FA (A) and glucagon (B) at fasting and during hyperinsulinemic euglycemic clamp (initiated at t=0 min) in patients with type 2 diabetes (upper panels) and NGT (lower panels) before (solid line, black triangles), 1 week (solid line, black squares), 3 months (dotted line, white squares) and 1 year (dotted line, white triangles) after RYGB. Values are mean ± sem.

Figure 3: Plasma glucose (A), serum insulin (B), serum C-peptide (C), plasma glucagon (D), plasma total GLP-1 (E) and plasma total GIP (F) in response to oral glucose tolerance test in patients with type 2 diabetes (left) and NGT (right) before (solid line, black triangles), 3 months (dotted line, white squares) and 1 year (dotted line, white triangles) after RYGB. Values are mean ± sem.

Figure 4: Serum C-peptide in response to iv glucose-glucagon test in patients with type 2 diabetes (left) and NGT (right) before (solid line, black triangles), 1 week (solid line, black squares), 3 months (dotted line, white squares) and 1 year (dotted line, white triangles) after RYGB. Values are mean ± sem.
Table 1: Weight, fat free mass, glycemic control and fasting measurements of insulin and C-peptide in patients with type 2 diabetes and NGT before, 1 week, 3 months and 1 year after Roux-en-Y gastric bypass

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<td>8 (4/4)</td>
<td>10 (4/6)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>121.5±8.9</td>
<td>118.1±9.5*</td>
<td>103.3±7.8**</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>73.3±6.9</td>
<td>-</td>
<td>66.8±6.0**</td>
</tr>
<tr>
<td>Fasting P-glucose (mmol/L)</td>
<td>8.3±0.6</td>
<td>6.6±0.4**</td>
<td>5.7±0.2**</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.0±0.3</td>
<td>-</td>
<td>5.9±0.2**</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>53±3.3</td>
<td>-</td>
<td>41±2.2**</td>
</tr>
<tr>
<td>2 h P-glucose after OGTT (mmol/L)</td>
<td>15.2±1.0</td>
<td>-</td>
<td>7.7±0.7**</td>
</tr>
<tr>
<td>Fasting S-insulin (pmol/L)</td>
<td>97±13</td>
<td>89±18*</td>
<td>51±8**</td>
</tr>
<tr>
<td>Fasting S-C-peptide (pmol/L)</td>
<td>1256±113</td>
<td>1287±217</td>
<td>925±103**</td>
</tr>
<tr>
<td>CI_{fasting}</td>
<td>13.1</td>
<td>(11.8;16.0)</td>
<td>14.7*</td>
</tr>
</tbody>
</table>

Values are mean±sem. CI_{fasting} is reported as median (IQR) due to skewed distribution.
* p < 0.05, ** p < 0.01 for the change from preoperative level within the group (post-hoc estimates from mixed effect model)
†p < 0.05, ††p < 0.01 for differences between the groups at a given study session (post-hoc unpaired t-test)
FFM=fat free mass, OGTT=oral glucose tolerance test, CI_{fasting}=Clearance of insulin at fasting (fasting serum C-peptide to serum insulin ratio)
Table 2: Endogenous glucose production, glucose disposal, fatty acids and glucagon in patients with type 2 diabetes and NGT before, 1 week, 3 months and 1 year after Roux-en-Y gastric bypass. Results from the basal state and from the hyperinsulinemic euglycemic clamp.

<table>
<thead>
<tr>
<th></th>
<th>Type 2 diabetes</th>
<th>NGT</th>
<th>Mixed effect model, ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>1 week</td>
<td>3 months</td>
</tr>
<tr>
<td>Basal period</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ra&lt;sub&gt;b&lt;/sub&gt; (mg/min)</td>
<td>207±15</td>
<td>174±11**</td>
<td>176±14**</td>
</tr>
<tr>
<td>H&lt;sub&gt;I&lt;/sub&gt;S&lt;sub&gt;b&lt;/sub&gt;</td>
<td>4.8±1.0</td>
<td>7.7±2.8*</td>
<td>9.0±1.9**</td>
</tr>
<tr>
<td>P-FA&lt;sub&gt;b&lt;/sub&gt; (µmol/l)</td>
<td>705±56</td>
<td>841±36*</td>
<td>741±52</td>
</tr>
<tr>
<td>P-Glucagon &lt;sub&gt;b&lt;/sub&gt; (pmol/l)</td>
<td>10.7±3.4</td>
<td>10.3±2.9</td>
<td>7.4±2.0**</td>
</tr>
<tr>
<td>C-peptide to glucagon ratio &lt;sub&gt;b&lt;/sub&gt;</td>
<td>284±65</td>
<td>189±64</td>
<td>221±49</td>
</tr>
<tr>
<td>Clamp period</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Ra&lt;sub&gt;c&lt;/sub&gt; (mg/min)</td>
<td>55±13</td>
<td>44±8</td>
<td>30±7</td>
</tr>
<tr>
<td>Suppression of Ra (%)</td>
<td>70±5</td>
<td>71±5</td>
<td>83±4</td>
</tr>
<tr>
<td>Rd&lt;sub&gt;d&lt;/sub&gt; (mg/kg/min)</td>
<td>3.5±0.5</td>
<td>3.5±0.5</td>
<td>5.4±0.5**</td>
</tr>
<tr>
<td>Rd&lt;sub&gt;d&lt;/sub&gt;/I&lt;sub&gt;e&lt;/sub&gt; (µg/kg/min/µM)</td>
<td>7.7±1.1</td>
<td>9.2±1.6</td>
<td>14.9±1.8**</td>
</tr>
<tr>
<td>Rd&lt;sub&gt;d&lt;/sub&gt;/I&lt;sub&gt;e&lt;/sub&gt; (µg/kg&lt;sub&gt;min&lt;/sub&gt;/min/µM)</td>
<td>14.0±2.2</td>
<td>-</td>
<td>24.7±3.1**</td>
</tr>
<tr>
<td>P-Glucose&lt;sub&gt;c&lt;/sub&gt; (mmol/L)</td>
<td>5.4±0.09</td>
<td>5.4±0.04</td>
<td>5.5±0.03</td>
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<tr>
<td>S-Insulin&lt;sub&gt;c&lt;/sub&gt; (pmol/L)</td>
<td>469±30</td>
<td>395±25**</td>
<td>380±25**</td>
</tr>
<tr>
<td>S-C-peptide&lt;sub&gt;c&lt;/sub&gt; (pmol/L)</td>
<td>622±101</td>
<td>528±103</td>
<td>509±84</td>
</tr>
<tr>
<td>CI&lt;sub&gt;c&lt;/sub&gt; (mL/min/ kg)</td>
<td>13.9±0.9</td>
<td>16.4±1.2*</td>
<td>18.0±1.2**</td>
</tr>
<tr>
<td>Suppression of P-FA (%)</td>
<td>80±3</td>
<td>69±4**</td>
<td>89±2**</td>
</tr>
<tr>
<td>Suppression of P-Glucagon (%)</td>
<td>51±6</td>
<td>49±10</td>
<td>55±10</td>
</tr>
</tbody>
</table>

Values are mean±sem. Days from surgery is expressed as median (IQR). * p < 0.05, ** p < 0.01 for the change from preoperative level within the group (post-hoc estimates from mixed effect model), † p < 0.05, †† p < 0.01 for differences between the groups at a given study session (post-hoc unpaired t-test). basal=mean value from the last 30 min of basal period, clamp=mean value from the last 30 min of clamp period, Ra = Rate of appearance of glucose, H<sub>I</sub>S = Hepatic insulin sensitivity index, Rd = Rate of disappearance of glucose, Rd/I = Rate of disappearance of glucose adjusted for clamp insulin concentration, Rd/I<sub>min</sub> = Rate of disappearance of glucose adjusted for clamp insulin concentration and fat free mass (ffm), CI<sub>c</sub> = Clearance rate of insulin during clamp, FA=fatty acids.
Table 3: Insulin secretion in response to oral glucose tolerance test (OGTT) and intravenous glucose-glucagon test in patients with type 2 diabetes and NGT before, 1 week, 3 months and 1 year after Roux-en-Y gastric bypass

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>1 week</th>
<th>3 months</th>
<th>1 year</th>
<th>Before</th>
<th>1 week</th>
<th>3 months</th>
<th>1 year</th>
<th>Time</th>
<th>Group</th>
<th>T x G</th>
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<tr>
<td><strong>OGTT</strong></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days from surgery</td>
<td>−17</td>
<td>−17</td>
<td>−21</td>
<td>−21</td>
<td>−21</td>
<td>−21</td>
<td>−21</td>
<td>−21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iAUC C-peptide (mmol/L × min)</td>
<td>125±25</td>
<td>257±40**</td>
<td>236±38**</td>
<td>237±35</td>
<td>321±54**</td>
<td>286±57</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
<td>0.18</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>(99;112)</td>
<td>(99;112)</td>
<td>(119;164)</td>
<td>(115;169)</td>
<td>(119;164)</td>
<td>(115;169)</td>
<td>(119;164)</td>
<td>(115;169)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak C-peptide (pmol/L)</td>
<td>306±409</td>
<td>4044±584*</td>
<td>3710±599</td>
<td>3720±390</td>
<td>4783±625**</td>
<td>4255±637</td>
<td></td>
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<td>&lt;0.01</td>
<td>0.38</td>
<td>0.90</td>
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<tr>
<td></td>
<td>(81;262)</td>
<td>(81;262)</td>
<td>(230;496)</td>
<td>(230;496)</td>
<td>(230;496)</td>
<td>(230;496)</td>
<td>(230;496)</td>
<td>(230;496)</td>
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</tr>
<tr>
<td>IGI (pmol/L/mM)</td>
<td>121</td>
<td>237**</td>
<td>260**</td>
<td>705††</td>
<td>741††</td>
<td>747††</td>
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<td>&lt;0.01</td>
<td>&lt;0.01</td>
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<tr>
<td></td>
<td>(81;262)</td>
<td>(81;262)</td>
<td>(149;404)</td>
<td>(149;404)</td>
<td>(149;404)</td>
<td>(149;404)</td>
<td>(149;404)</td>
<td>(149;404)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DI&lt;sub&gt;oral&lt;/sub&gt; × 10&lt;sup&gt;−3&lt;/sup&gt;</td>
<td>0.9</td>
<td>4.5**</td>
<td>3.9**</td>
<td>7.3††</td>
<td>12.8††</td>
<td>13.8***††</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>(0.5;1.1)</td>
<td>(0.5;1.1)</td>
<td>(2.9;4.8)</td>
<td>(2.9;4.8)</td>
<td>(2.9;4.8)</td>
<td>(2.9;4.8)</td>
<td>(2.9;4.8)</td>
<td>(2.9;4.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>IV glucose-glucagon</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days from surgery</td>
<td>−5</td>
<td>9</td>
<td>98</td>
<td>387</td>
<td>−5</td>
<td>9</td>
<td>99</td>
<td>376</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>(−8;−4)</td>
<td>(7;9)</td>
<td>(93;102)</td>
<td>(383;402)</td>
<td>(−6;−4)</td>
<td>(9;10)</td>
<td>(93;108)</td>
<td>(369;385)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP-Glucose&lt;sub&gt;0−2 min&lt;/sub&gt; (mmol/L)</td>
<td>13.2±1.2</td>
<td>13.5±1.1</td>
<td>12.4±1.1</td>
<td>10.7±2.3</td>
<td>14.5±1.2</td>
<td>15.8±1.4</td>
<td>14.4±1.3</td>
<td>13.3±1.8</td>
<td>0.12</td>
<td>0.21</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>(969;1885)</td>
<td>(879;1754)</td>
<td>(868;2498)</td>
<td>(868;2498)</td>
<td>(973;1778)</td>
<td>(1581;4479)</td>
<td>(1581;4479)</td>
<td>(1581;4479)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIR (pmol/L)</td>
<td>1049</td>
<td>1351</td>
<td>1029</td>
<td>3614††</td>
<td>2912*</td>
<td>2308**</td>
<td>1866**</td>
<td>1866**</td>
<td>0.04</td>
<td>0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>(969;1885)</td>
<td>(879;1754)</td>
<td>(868;2498)</td>
<td>(868;2498)</td>
<td>(973;1778)</td>
<td>(1581;4479)</td>
<td>(1581;4479)</td>
<td>(1581;4479)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DI&lt;sub&gt;iv&lt;/sub&gt; × 10&lt;sup&gt;−3&lt;/sup&gt;</td>
<td>10.4</td>
<td>26.1**</td>
<td>21.9**</td>
<td>37.4††</td>
<td>29.6†</td>
<td>40.8††</td>
<td>37.6†</td>
<td>37.6†</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>(6.8;12.7)</td>
<td>(9.1;13.6)</td>
<td>(17.4;28.5)</td>
<td>(17.4;28.5)</td>
<td>(17.4;28.5)</td>
<td>(17.4;28.5)</td>
<td>(17.4;28.5)</td>
<td>(17.4;28.5)</td>
<td></td>
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</tr>
</tbody>
</table>

Values are mean±sem. Days from surgery, IGI, AIR and DI are expressed as medians (IQR) due to skewed distribution.

* p < 0.05, ** p < 0.01 for the change from preoperative level within the group (post-hoc estimates from mixed effect model).
†p < 0.05, ††p < 0.01 for differences between the groups at a given study session (post-hoc unpaired t-test).
OGTT=oral glucose tolerance test, iAUC=incremental area-under-the-curve, IGI=insulinogenic index, DI=disposition index, AIR=acute insulin response.
Table 4: Glucagon, GLP-1 and GIP in response to oral glucose tolerance test (OGTT) in patients with type 2 diabetes and normal glucose tolerance (NGT) before, 3 months and 1 year after Roux-en-Y gastric bypass

<table>
<thead>
<tr>
<th></th>
<th>Type 2 diabetes</th>
<th></th>
<th>NGT</th>
<th></th>
<th></th>
<th>Mixed effect model, ANOVA</th>
</tr>
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<tr>
<td></td>
<td>Before</td>
<td>3 months</td>
<td>1 year</td>
<td>Before</td>
<td>3 months</td>
<td>1 year</td>
</tr>
<tr>
<td><strong>Fasting glucagon</strong></td>
<td>18.2±3.8</td>
<td>7.6±1.5**</td>
<td>10.1±1.7**</td>
<td>12.8±2.8</td>
<td>9.9±0.8</td>
<td>8.9±1.3</td>
</tr>
<tr>
<td>(pmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.01 0.59 0.05</td>
</tr>
<tr>
<td><strong>iAUC glucagon</strong></td>
<td>−342±147</td>
<td>370±136**</td>
<td>280±142**</td>
<td>−403±218</td>
<td>352±124**</td>
<td>398±228**</td>
</tr>
<tr>
<td>(pmol/L×min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.01 0.64 0.77</td>
</tr>
<tr>
<td><strong>Peak glucagon</strong></td>
<td>21.7±4.4</td>
<td>16.2±1.4</td>
<td>21.0±3.1</td>
<td>14.7±2.7</td>
<td>17.3±1.5</td>
<td>17.6±1.8</td>
</tr>
<tr>
<td>(pmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.41 0.34 0.12</td>
</tr>
<tr>
<td><strong>Fasting GLP-1</strong></td>
<td>9.4±0.9</td>
<td>7.2±1.2</td>
<td>10.2±0.7</td>
<td>9.7±0.8</td>
<td>8.6±0.7</td>
<td>11.4±0.8</td>
</tr>
<tr>
<td>(pmol/L)</td>
<td></td>
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<td></td>
<td></td>
<td>0.01 0.23 0.79</td>
</tr>
<tr>
<td><strong>iAUC GLP-1</strong></td>
<td>333±141</td>
<td>4022±377**</td>
<td>4822±538**</td>
<td>425±108</td>
<td>5232±571**</td>
<td>5199±670**</td>
</tr>
<tr>
<td>(pmol/L×min)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>&lt;0.01 0.27 0.26</td>
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<tr>
<td><strong>Peak GLP-1</strong></td>
<td>15.0±1.3</td>
<td>76.1±8.5**</td>
<td>104.7±9.3**</td>
<td>18.6±2.0</td>
<td>91.1±17.9**</td>
<td>146.5±34.6**</td>
</tr>
<tr>
<td>(pmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.01 0.28 0.33</td>
</tr>
<tr>
<td><strong>Fasting GIP</strong></td>
<td>7.3±1.1</td>
<td>6.4±1.0</td>
<td>8.4±0.6</td>
<td>7.2±1.0</td>
<td>8.1±1.1</td>
<td>6.8±1.0</td>
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<td>(pmol/L)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>0.81 0.96 0.16</td>
</tr>
<tr>
<td><strong>iAUC GIP</strong></td>
<td>3497±312</td>
<td>3800±386</td>
<td>3600±449</td>
<td>3895±533</td>
<td>3515±469</td>
<td>3169±523</td>
</tr>
<tr>
<td>(pmol/L×min)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>0.64 0.86 0.41</td>
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<tr>
<td><strong>Peak GIP</strong></td>
<td>48.7±3.8</td>
<td>59.7±4.6</td>
<td>72.7±8.2**</td>
<td>52.0±5.4</td>
<td>58.2±6.3</td>
<td>62.8±9.2</td>
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<tr>
<td>(pmol/L)</td>
<td></td>
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<td></td>
<td></td>
<td>&lt;0.01 0.72 0.41</td>
</tr>
</tbody>
</table>

Values are mean±sem.  
* p < 0.05, ** p < 0.01 for the change from preoperative level within the group (post-hoc estimates from mixed effect model)  
†p < 0.05, ††p < 0.01 for differences between the groups at a given study session (post-hoc unpaired t-test)  
iAUC=incremental area-under-curve, GLP-1=Glucagon-like-peptide 1, GIP = Glucose-dependent insulinotropic polypeptide
Figure 1
76x5mm (300 x 300 DPI)

<table>
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<tr>
<th></th>
<th>pre-diet</th>
<th>-1</th>
<th>+1</th>
<th>+15</th>
<th>+54</th>
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<tbody>
<tr>
<td>BMI T2D</td>
<td>42.4±1.7</td>
<td>38.9±1.6**</td>
<td>37.3±1.8**</td>
<td>33.1±1.5**</td>
<td>30.8±1.7**</td>
</tr>
<tr>
<td>BMI NGT</td>
<td>44.9±1.0</td>
<td>40.2±0.8**</td>
<td>37.9±0.9**</td>
<td>33.2±1.1**</td>
<td>28.5±1.5**</td>
</tr>
</tbody>
</table>
Figure 2
88x43mm (300 x 300 DPI)
Figure 3
228x587mm (300 x 300 DPI)
Figure 4
58x38mm (300 x 300 DPI)