Long-term effects of bariatric surgery on meal disposal and β-cell function in diabetic and nondiabetic patients.

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Abstract

Gastric bypass surgery leads to marked improvements in glucose tolerance and insulin sensitivity in obese type 2 diabetes; the impact on glucose fluxes in response to a physiological stimulus – such as a mixed meal (MTT) – has not been determined. We administered an MTT to 12 obese type 2 diabetic patients (T2D) and 15 obese nondiabetic subjects (ND) before and one year after surgery (10 T2D and 11 ND) using the double-tracer technique and modeling of β-cell function. In both groups postsurgery, tracer-derived appearance of oral glucose was biphasic, a rapid increase followed by a sharp drop, a pattern that was mirrored by postprandial glucose levels and insulin secretion. In diabetic patients, surgery lowered fasting and postprandial glucose levels; peripheral insulin sensitivity increased in proportion to weight loss (~30%), β-cell glucose sensitivity doubled but did not normalize (viz. 21 nonsurgical obese and lean controls). Endogenous glucose production, however, was less suppressed during the MMT as the combined result of a relative hyperglucagonemia and the rapid fall in plasma glucose and insulin levels.

We conclude that, in type 2 diabetes bypass surgery changes the postprandial response to a dumping-like pattern, improves glucose tolerance, β-cell function, and peripheral insulin sensitivity but worsens endogenous glucose output in response to a physiological stimulus.

Key words: bariatric surgery, mixed meal, hyperglucagonemia, β-cell function, insulin sensitivity
Introduction

Mounting evidence supports bariatric surgery as a powerful intervention to induce remission in patients with type 2 diabetes (T2D) (1,2), and to prevent or delay incident T2D (3). This has engendered enthusiasm for bariatric surgery as a treatment for T2D (4), and has encouraged a broadening of the BMI range as an indication for surgery in diabetic patients (5).

While weight loss and, in the early postoperative period, caloric deficit certainly make a contribution to improve glucose tolerance, surgery itself may trigger weight-independent mechanisms eventually translating into favorable metabolic effects. This postulate is based on early animal studies (6) and, in humans, on evidence that metabolic changes sometimes precede sizeable weight loss or are disproportionate to the amount of weight lost (7). In this regard, there is evidence that different bariatric procedures (e.g., Roux-en-Y gastric bypass (RYGB), biliopancreatic diversion, sleeve gastrectomy) may engage putative weight-independent mechanisms to different extents or involve altogether different mechanisms (8,9).

A number of previous studies have documented the effects of the most popular bariatric operation, RYGB, on glycemic control and incretin hormones (10-24), and mechanistic studies have explored the ability of RYGB to enhance insulin action and β-cell function. The great majority of these studies have utilized methods based on fasting measurements (e.g., HOMAs), OGTT-based surrogate indices of insulin sensitivity and β-cell function, or euglycemic hyperinsulinemic clamp settings (14,16-21). A recent study (24) has taken a more physiological approach by comparing the impact of RYGB and gastric banding on the disposition of a mixed meal – with the use of a double-tracer technique – in nondiabetic subjects studied before and shortly after the operation (~20 weeks). In the present study of morbidly obese patients with type 2 diabetes (T2D), we aimed at measuring the impact of RYGB on chief physiologic determinants of meal disposal long after
surgery – when body weight and metabolic adaptation have stabilized – and assessing their relation to weight loss and the attendant changes in hormonal milieu.

**Materials and methods**

**Subjects** We studied 12 morbidly obese patients with type 2 diabetes (T2D) and 15 gender- and BMI-matched morbidly obese nondiabetic patients (ND). Diabetes was newly diagnosed in 3 patients, while in the other 9 diabetes duration was 3.9±1.2 years (range 1-10). HbA1c was 7.2±0.4% [55±5 mmol/mol], 6 patients were being treated by diet alone and 6 by oral hypoglycemic agents (3 by metformin alone, 3 by metformin plus a sulfonylurea). Antidiabetic medication was discontinued one week before the metabolic studies. These 27 subjects all underwent laparoscopic RYGB; 10 T2D and 11 ND patients were re-studied 1 year after surgery. Two control groups were included, consisting of 7 lean healthy volunteers and 14 obese nondiabetic volunteers whose BMI was matched to that of the RYGB patients at 1 year post-surgery. Thus, a total of 69 complete metabolic studies were performed.

The study was approved by the local Ethics Committee. Nature and purpose of the study were carefully explained to all participants before they provided written consent to participate.

**Study design** At baseline and follow-up, subjects received a mixed meal test (MTT) with a double tracer protocol. Briefly, after an overnight (12-h) fast, subjects were admitted to our Clinical Research Unit at 8:00am, and a polyethylene cannula was inserted into an antecubital vein for the infusion of all test substances. A second catheter was inserted retrogradely into an ipsilateral wrist vein on the dorsum of the hand for blood sampling, and the hand was kept in a heated box at 65°C to achieve arterialization of venous blood. Baseline blood samples were drawn to measure plasma glucose, insulin, C-peptide, glucagon, GLP-1, and GIP concentrations, and tracer enrichments. The MTT consisted of 75 g of glucose in 150 ml of water, 40 g of parmesan cheese, and one 50-g egg
(509 kcal, 16% protein, 28% fat, 56% carbohydrate). The glucose solution was drunk after the solid component of the meal was consumed over a period of 10 min. The oral glucose drink was enriched with 1-[3H]glucose in order to trace glucose absorption. A primed (28 µmol kg⁻¹ * [fasting plasma glucose/5])-continuous (0.28 µmol min⁻¹ kg⁻¹) infusion of 6,6-[2H₂]glucose was started (at time –120 min, or at –180 min in the T2D patients) via the antecubital vein catheter, and continued until the end of the study. During the last 20 min of the basal equilibration period (at times –20, –10, and 0 min), blood samples were obtained for the determination of plasma glucose and hormone concentrations, tracer enrichments (when isotopic steady state was achieved). After the basal equilibration period, the meal was consumed over ~10 min. Plasma samples for the determination of plasma glucose and hormone concentrations and glucose tracer enrichments were obtained at 15, 30, 45, 60, 90, 120, 150, 180, 240, and 300 min after meal ingestion.

**Surgical procedure** In subjects undergoing laparoscopic RYGB, a small proximal gastric pouch of 30 ml was created with several firings of a linear stapling endocutter; the jejunum was divided 120 cm distal to the ligament of Treitz and a 2-cm end-to-side gastrojejunostomy was performed by using a hand-sewn technique. A side-to-side jejunoojejunostomy was then created, 150 cm distal to the gastrojejunostomy (17).

**Analytical procedures** Plasma glucose was measured by the glucose-oxidase technique (Analox GM-9), plasma insulin and C-peptide by electrochemiluminescence (on a COBAS e411 instrument, Roche, Indianapolis, USA). Plasma triglycerides and serum high-density lipoprotein (HDL) cholesterol were assayed in duplicate by standard spectrophotometric methods on a Synchron Clinical System CX4 (Beckman Instruments, Fullerton, USA). 6,6-[2H₂]glucose and 1-[3H]glucose enrichments were measured by gas chromatography/mass spectrometry (GCMS) as described previously (25). Plasma glucagon was assayed by radioimmunoassay (Millipore Corporation, Billerica, MA, USA). Plasma GLP-1 and GIP was assayed by radioimmunoassay as described previously (26). No significant cross-reactivity was declared by kits manufacturers. The sensitivity
ranges are: 0.003 nmol/l and 1.39 pmol/l for the C-peptide and insulin assay, respectively, 18.5 pg/ml for glucagon, and 1.5 pM for GLP-1.

Data analysis  Fat-free mass were estimated with the use of electric bioimpedance on a Tanita scale; of note, this method has been validated in very obese individuals against the deuterated water technique (27). Endogenous glucose production (EGP) in the fasting state and throughout the absorptive period and rate of appearance (RaO) of the glucose component of the meal were calculated from the time-course of the plasma tracer/tracee ratio of 6,6-[2H2]glucose and 1-[2H]glucose by 2-compartment modeling, as described (25). Insulin sensitivity was calculated as the mean metabolic clearance rate of glucose (MCRG) during the 5 hours of the MTT (from 6,6-[2H2]glucose kinetics) divided by the mean plasma insulin concentration over the same time interval.

β-cell function was quantitated by mathematical modeling of the plasma C-peptide response, as described (28). Briefly, the model consists of three blocks: a) a model for fitting the glucose concentration profile, the purpose of which is to smooth and interpolate plasma glucose concentrations; b) a model describing the dependence of insulin (or C-peptide) secretion on glucose concentration; and c) a model of C-peptide kinetics, i.e., the two-exponential model proposed by Van Cauter et al. (29), in which the model parameters are individually adjusted to the subject's anthropometric data. The main parameter of the model is β-cell glucose sensitivity, which is calculated as the mean slope of the dose-response function (i.e., relationship between insulin secretion rates and plasma glucose concentrations during corresponding times of the MTT). The model also yields an estimate of glucose rate sensitivity, which is the insulin secretory response to the rate of change in plasma glucose concentrations, and total insulin output (IS), which is the total amount of insulin released over the 5 hours of the meal (28).

Plasma insulin clearance (MCRI) was estimated as the ratio of fasting insulin secretion rate to the fasting plasma insulin concentration.
The pre-hepatic insulin-to-glucagon molar concentration ratio was estimated by the following formula:

\[
\text{ISR}(t)/\text{hPF} + [I(t)]/[Glg(t)] * (1 + \text{MCR}_{\text{Glg}}/\text{hPF})
\]

where ISR(\(t\)) is the insulin secretion rate at time \(t\), hPF is hepatic plasma flow; [I(\(t\))] and [Glg(\(t\))] are the measured peripheral plasma concentrations of insulin and glucagon at time \(t\); and MCR_{\text{Glg}} is the metabolic clearance rate of glucagon. hPF was estimated by multiplying the cardiac index (3.2 L·min^{-1}·m^{-2} (30)) by a plasma-to-blood ratio of 0.6, and by assuming that hepatic blood flow is 30% of cardiac index (= 0.576 L·min^{-1}·m^{-2}) (31). MCR_{\text{Glg}} was taken to be 0.537 L·min^{-1}·m^{-2} (32).

The product of mean EGP and mean plasma insulin levels during the meal was taken as an index of hepatic insulin resistance. Areas under time-concentration curves (AUC) were calculated by the trapezium rule.

**Statistical analysis** All data are given as the mean±SEM; parameters that were non-normally distributed are presented as the median [interquartile range]. Mann-Whitney test was used to compare group values, whereas surgery-induced changes were tested by the Wilcoxon signed rank test. Time series were analyzed by ANOVA for repeated measures; for these tests, variables with skewed distribution were log-transformed. Group differences over time series were analyzed by 2-way ANOVA for repeated measures. Simple associations were tested by calculating the Spearman rank correlation coefficient (\(\rho\)). Statistical analyses were performed using JMP® 7.0 and StatView® 5.0. \(p\leq0.05\) was considered statistically significant.

**Results**

**Baseline** The four subject groups were matched for gender and age; T2D patients were older and had moderate fasting hyperglycemia (Table 1). Following meal ingestion, plasma glucose excursions were similar in lean and obese controls and ND surgical patients, while T2D patients...
showed marked hyperglycemia (Fig. 1a). Fasting and postprandial insulin concentrations were higher in all 3 obese groups than in lean controls (Fig. 1b and Table 2). Fasting insulin secretion rate generally increased with obesity as did the total insulin output over the 5 hours of the test (Fig. 1c and Table 2). In T2D patients, total insulin output was not different from that of lean controls despite the hyperglycemia. Consequently, β-GS was markedly impaired in this group (Fig. 1d and Table 2).

On the MTT, the rate of appearance of oral glucose (RaO) was similar in all groups, both in time-course (Fig. 1e) and amount (averaging 51, 52, 50, and 62 g over 5 hours in lean controls, obese controls, ND surgical patients and T2D surgical patients, respectively, Table 2). In contrast, fasting EGP was higher in each obese group (700 [277], 771 [242], and 764 [395] µmol/min) than in lean controls (580 [51] µmol min⁻¹), and remained higher during the MTT despite the postmeal hyperinsulinemia (AUC_{EGP} = 79 [46], 100 [56], and 100 [97] mmol vs 61 [15] of controls). The difference in EGP time-course between each of the obese groups and lean controls (confirmed by repeated-measures ANOVA, p<0.001 for all) was evident after the initial nadir (Fig. 1f). In the whole dataset, both fasting EGP and AUC_{EGP} were positively related to BMI (r = 0.48, p=0.001, and r = 0.30, p=0.05, respectively). Plasma glucose clearance rose during the MTT in all groups; its mean value over 5 hours was reduced in both surgical groups, especially in T2D patients, in comparison with lean controls despite the hyperinsulinemia (Table 2). As a consequence, insulin sensitivity – as the insulin-mediated metabolic clearance rate of glucose – was lower in both obese groups and severely impaired in T2D patients (Table 2).

In all obese groups, fasting plasma glucagon was increased, while the meal-induced glucagon increments were blunted. Fasting GLP-1 and GIP levels were increased only in T2D patients as was AUC_{GIP} (Table 2).

After surgery At 1 year, ND and T2D patients had lost 31% and 35% of their initial body weight, respectively, in an approximate ratio of 1:3 between fat-free mass and fat mass (Table 1).
T2D patients, HbA1c had dropped by an average 1.5%, and all patients were off antidiabetic drug treatment; of them, 2 had IGT and one of these 2 also had impaired fasting glucose (5.9 mmol/L). Four patients experienced dumping-like symptoms (tachycardia, sweating, nausea, diarrhea) following meal ingestion.

On the MTT, the post-surgery plasma glucose and insulin profiles were grossly altered in both ND and T2D subjects, with a large excursion peaking at 60 min followed by a sharp drop to basal levels or below (Fig. 2). In the T2D group, the glucose and insulin AUCs were significantly smaller than preoperatively (Table 2). The time-course of insulin secretion ran parallel to that of plasma glucose (Fig. 3); fasting insulin secretion decreased in both groups, total insulin output decreased in the ND group (Table 2). When viewing insulin secretion in the context of the corresponding plasma glucose levels, β-GS was significantly reduced in the ND group postsurgery – though still within the range of the obese control group – and ~100% increased in T2D patients (though still largely below control values) (Fig. 3). The small decline in β-GS in ND subjects could be due to chronically reduced carbohydrate intake or simply be time-related. Insulin sensitivity improved significantly in both groups (by 2-way ANOVA for repeated measures, F=10.5, p<0.005), to levels similar to those of the obese controls. The estimates of insulin sensitivity fell along the overall relationship describing the association of insulin resistance with BMI, and the surgically-induced changes in insulin sensitivity were correlated with the corresponding changes in body weight (Table 2 and Fig. 4).

In both ND and T2D subjects postsurgery, the rate of appearance of oral glucose was similar in total amount (Table 2) but distorted in time-course, in a manner resembling the plasma glucose curves closely (Fig. 5). Thus, most oral glucose was absorbed during the first hour after meal ingestion, as the area under the curve calculated in the first 60 minutes showed (AUC pre vs postsurgery: 122 [63] vs 186 [37] mmol in ND, 129 [81] vs 190 [94] mmol in T2D, p<0.04 vs postsurgery for both). EGP was unchanged in ND as well as T2D patients in the fasting state, but
rebounded significantly above presurgery values during meal absorption, particularly over the second half of the postcibal period when plasma glucose concentrations were back to basal or below. The EGP time-course was increased in both groups (with no significant difference between T2D and ND patients, \( p=0.74 \)), whether expressed in absolute terms (Fig. 5) or normalized by fat-free mass (\( F=133.9, \ p<0.0001 \)) (Table 2).

Fasting glucagon levels dropped in both ND and T2D, but the response to the meal (as the incremental AUC) was greatly enhanced in both groups (Table 2 and Fig. 6). After surgery, the estimated prehepatic insulin-to-glucagon molar concentration ratio was higher than pre-surgery (especially in T2D patients) for the first 80-100 min, but then fell below preoperative levels for the remainder of the MTT in both groups (Supplemental Fig. 1).

The GLP-1, but not the GIP, response was increased in ND as well as T2D; the time profile of the GLP-1 and GIP responses showed a sharper early rise and a rapid drop thereafter (Fig. 6).

The metabolic clearance rate of insulin – \( \text{MCR}_I \), calculated as the ratio of fasting insulin output to fasting peripheral plasma insulin concentrations – was slightly reduced in the obese groups as compared to the lean controls, and was significantly increased postsurgery in both ND and T2D (2-way ANOVA)

**Discussion**

Before the operation, the metabolic response to the mixed meal in the nondiabetic obese subjects was characterized by insulin resistance of glucose disposal and insulin hypersecretion, with preserved \( \beta \)-cell glucose sensitivity and rate sensitivity, i.e., the metabolic picture of obesity. In the T2D patients, insulin resistance was worse, and both \( \beta \)-cell glucose sensitivity and rate sensitivity were markedly impaired, thereby accounting for the hyperglycemia. While the rate of appearance of oral glucose was similar in time-course and total amount in all groups, in obese and T2D patients
EGP was higher at baseline and was incompletely suppressed during the meal – in some proportion to the degree of obesity – despite the hyperinsulinemia, thereby manifesting liver insulin resistance. In the T2D patients, the blunted rise in the prehepatic insulin-to-glucagon ratio during the first 2 hours postmeal (Supplemental Fig. 1) likely contributed to the elevated EGP.

One year following RYGB, at a time when drastic weight loss had occurred and body weight had stabilized, fasting plasma glucose was similar in nondiabetic and diabetic patients, but in these latter 2-hour glucose concentrations were significantly higher (5.93±0.53 vs 4.23±0.31 mmol/l, p=0.02) – and mean glucose levels during the 5-hour meal tended to be higher (Table 2) – than in nondiabetic surgical patients. Insulin sensitivity improved in both surgical groups to levels close to those of the BMI-matched control group, i.e., in rough proportion to the weight loss and without reaching the values of the lean control subjects (Fig. 4). This result, obtained by tracer analysis of a dynamic test such as the MTT, confirms the findings of steady-state (clamp) measurements of insulin sensitivity (17), namely, that RYGB does not potentiate insulin action beyond, or independently of, the effect on weight loss. ß-cell glucose sensitivity worsened slightly in the nondiabetic surgical patients whereas it doubled in the diabetic patients, in whom, however, it remained markedly inferior to that of BMI-matched nondiabetic subjects. This outcome prevailed despite the large increase in GLP-1 response, a consistent change after bariatric surgery (10-17) – even as long as 10 years after surgery (33) – which is quantitatively related to enhanced insulin secretion (19). In both groups, rate sensitivity improved postsurgery, likely reflecting the rapid plasma glucose excursions as well as, at least in part, the heightened GLP-1 surge (Supplemental Fig. 2A). The latter finding is compatible with evidence that a rapid rather than delayed delivery of insulin improves glucose tolerance irrespective of the degree of insulin resistance (34), and may explain why meal tolerance in our patients was preserved a year after surgery despite the abnormal ß-cell glucose sensitivity.

Thus, in T2D patients recovery of both insulin sensitivity and glucose sensitivity was sizeable but incomplete, leaving behind a trace of glucose intolerance. These results confirm previous
findings in both nondiabetic and T2D subjects – using a liquid formula meal and HOMA-IR as a surrogate index of insulin resistance (35) – or an OGTT (18). Further increments in both functions may occur if more weight is lost or as removal of the toxic effects of hyperglycemia continues; on the other hand, diabetes may relapse if weight is regained or insulin resistance otherwise worsens or else if the disease itself should progress. In addition, as previously shown, in T2D patients the outcome of glucose tolerance at 1 year post-RYGB may be better or worse than in the present series of patients depending on the initial degree of β-cell dysfunction (18).

As found by Bradley et al. In nondiabetic subjects (24), RYGB drastically changed the shape of the glucose and hormonal responses to the meal, with a markedly biphasic pattern reminiscent of a dumping syndrome. Glucose fluxes confirmed that this was the consequence of the altered delivery of gastric contents to the peripheral circulation. A somewhat unexpected consequence of the altered pattern of transit of alimentary glucose was the reduced suppression of endogenous glucose release during the meal. Under euglycemic clamp conditions, hepatic and peripheral insulin resistance typically are somewhat interrelated and change consensually (36). A mixed meal, however, creates a hormonal makeover by stimulating both insulin and glucagon secretion, especially in diabetic patients in whom glucagon responses are exaggerated (37). In our surgical patients, both fasting insulin and fasting glucagon concentrations fell significantly postsurgery; correspondingly, fasting EGP did not change after the operation. Following the meal, the prehepatic insulin-to-glucagon molar ratio normally rises in a time-course roughly parallel to that of plasma glucose concentrations and insulin secretion rates; in T2D patients, the ratio shows the blunted initial rise followed by a sustained increase typical of the insulin secretory response (Supplemental Fig. 1). After surgery, however, plasma glucagon rose sharply and remained above basal levels throughout the absorption period both in ND and T2D patients (Fig. 6), such that the prehepatic insulin-to-glucagon ratio, after peaking at ~1 hour postmeal, dropped rapidly to levels below presurgery (Supplemental Fig. 1), in phase with the lower plasma glucose levels. Thus, the raised postmeal EGP was the integrated liver
response to lower insulin, higher glucagon, and falling glucose levels. The correlation between the calculated index of hepatic insulin resistance and the glucagon response supports the role of the relative hyperglucagonemia (Supplemental Fig. 2B). It should be noted that, in the cited study in nondiabetic subjects (24), the time-course of EGP was similar to ours but the glucagon response was rather flat and unchanged from presurgery. This difference may be due to the use of a smaller meal (~300 kcal with only 9 g of protein viz 500 kcal in the present study) and/or the shorter time interval from surgery (22 weeks), a time at which typically patients are still losing weight. The stimulus for the meal-induced hyperglucagonemia 1 year after surgery – which has been noted before (15) – remains undetermined. Diminished paracrine control of α-cell activity by insulin (38) seems unlikely as the glucagon excursions in our patients were essentially synchronous with those of plasma insulin. Potential mechanisms are: enhanced postprandial neural stimulation (39), overstimulation of glucagon release by GIP (40) or GLP-2 (41), and co-secretion of glucagon and GLP-1/GIP by intestinal cells (42). In our data, the postsurgery time-courses of glucagon and GIP were in-phase, lending some support to an effect of GIP on α-cells. It should also be considered that the standard glucagon assay has limitations in terms of its ability to discriminate between pancreatic glucagon and other sources (43).

While gastric emptying was not measured in the present studies, our previous work in post-RYGB patients (using a scintigraphic technique) has confirmed that the operation causes accelerated gastric emptying even 14-26 months later (44); this result is fully compatible with the accelerated appearance of oral glucose in the present series.

In summary, the long-term outcome of RYGB is a comparable weight reduction and a proportional improvement in insulin sensitivity in nondiabetic and well controlled, recent-onset diabetic patients. In both, delivery of oral glucose to the peripheral circulation is maintained in quantity but strikingly changed in time-course, with a ‘dumping’ pattern resulting from the modified anatomy. Glucose tolerance is preserved in nondiabetic and much improved in diabetic patients; in
the latter, a detectable degree of glucose intolerance persists. In diabetic patients, \( \beta \)-cell function improves presumably as a combined result of reversal of glucose toxicity, lower insulin secretory burden, and incretin-mediated potentiation, but \( \beta \)-cell glucose sensitivity remains subnormal. This may predispose patients to recurrent diabetes. The surgery-induced change in glucose delivery triggers not only a heightened GLP-1 response but also an exaggerated glucagon response synchronous with the glucose excursions, which likely contributes to maintain euglycemia through elevated rates of EGP.
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intestinal transit, and exaggerated gut hormone responses after Roux-en-Y gastric bypass.

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Author Contributions: S.C. designed the study, carried out the *in vivo* studies, analyzed the data and wrote the manuscript, E.M. carried out the *in vivo* studies and contributed to the discussion, A.G. and D.C. analyzed the tracer data, B.A. and M.N. carried out the *in vivo* studies, S.B was responsible for laboratory measurements, M.A. operated on the patients, A.M. analyzed the β-cell function data, J.H. was responsible for hormone measurements and contributed to the discussion, E.F. contributed to the design of the study, analyzed the data and wrote the manuscript.

S.C. and E.F are the guarantors of this work and, as such, had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Legend to the figures

**Figure 1** – Plasma glucose (a) and insulin concentrations (b), insulin secretion rates (ISR) (c), insulin secretion dose-response (d) rates of appearance of oral (RaO) (e), and endogenous glucose (EGP) (f) in the 4 groups of study subjects at baseline. The grey shaded areas are the mean±SEM for the lean control group, the green shaded area is the mean±SEM for the obese control group.

**Figure 2** – Plasma glucose and insulin concentrations in the patients before and after RYGB. The corresponding data for the obese control group is shown by the grey line.

**Figure 3** – Insulin secretion rate and dose-response function in the patients before and after RYGB. The corresponding data for the obese control group is shown by the shaded areas.

**Figure 4** – Insulin sensitivity (as the ratio of mean glucose clearance to mean insulin concentration during a 5-hour mixed meal test) plotted against the corresponding BMI value in the 4 study groups. The points to the far right are those for the surgical groups before the operation. Plots are median [interquartile range]. The dotted line is a power function fit of the plots. The inset shows the relationship between the change in insulin sensitivity and the percent change in body weight in the surgical patients; the linear fit and the 95% confidence interval are shown.

**Figure 5** – Rate of appearance of oral (RaO) and endogenous glucose (EGP) glucose metabolic clearance rate in the patients before and after RYGB. The corresponding data for the obese control group is shown by the shaded areas. By 2-way ANOVA, the time-course of EGP is significantly higher postsurgery than presurgery (F=27.6, p<0.0001) in both ND and T2D.

**Figure 6** – Plasma glucagon, GLP-1, and GIP response to the meal in the patients before and after RYGB. The corresponding data for the obese control group is shown by the grey lines.
<table>
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<th>ND</th>
<th>Pre-surgery</th>
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<th>Pre-surgery</th>
<th>1 year</th>
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<td>0.003</td>
<td>40.9 ± 2.0§</td>
<td>45.9 ± 2.9§*</td>
<td>0.005</td>
<td>31.8 ± 3.1##</td>
<td></td>
</tr>
<tr>
<td><strong>Fasting [G] (mmol/L)</strong></td>
<td>5.2 ± 0.2</td>
<td>5.3 ± 0.1</td>
<td>5.47 ± 0.13</td>
<td>0.02</td>
<td>5.05 ± 0.13</td>
<td>8.79 ± 0.87§##</td>
<td>0.005</td>
<td>4.97 ± 0.16</td>
<td></td>
</tr>
<tr>
<td><strong>Fasting [I] (pmol/L)</strong></td>
<td>54 [28]</td>
<td>81 [38]§</td>
<td>143 [40]§*</td>
<td>0.003</td>
<td>44 [18]*</td>
<td>163 [145]§</td>
<td>0.005</td>
<td>60 [25]*</td>
<td></td>
</tr>
<tr>
<td><strong>Fasting C-peptide (nmol/L)</strong></td>
<td>0.46 [0.40]</td>
<td>0.52 [0.24]</td>
<td>1.41 [0.61]§*</td>
<td>0.003</td>
<td>0.48 [0.18]</td>
<td>0.88 [0.54]§*</td>
<td>0.01</td>
<td>0.54 [0.24]</td>
<td></td>
</tr>
</tbody>
</table>

*p vs Pre-surgery by Wilcoxon test; * p≤0.05 vs obese controls and § p≤0.05 vs lean controls by Mann Whitney U-test # p≤0.05 ND vs T2D pre or 1-year.
<table>
<thead>
<tr>
<th></th>
<th>Lean cts</th>
<th>Obese cts</th>
<th></th>
<th>NS</th>
<th>ND</th>
<th>Obese cts</th>
<th>T2D</th>
<th>1 year</th>
<th>1 year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean glucose [G] (mmol/L)</td>
<td>6.1 ± 0.2</td>
<td>6.1 ± 0.1</td>
<td>6.0 ± 0.2</td>
<td>ns</td>
<td>5.9 ± 0.1</td>
<td>9.3 ± 0.9*#</td>
<td>0.005</td>
<td>6.4 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>AUCG (mol/L)</td>
<td>1.80 [0.17]</td>
<td>1.84 [0.21]</td>
<td>1.76 [0.26]</td>
<td>ns</td>
<td>1.79 [0.17]</td>
<td>2.58 [0.99]*#</td>
<td>0.01</td>
<td>1.87 [0.19]</td>
<td></td>
</tr>
<tr>
<td>Mean insulin [I] (pmol/L)</td>
<td>181 [81]</td>
<td>353 [208]§</td>
<td>493 [269]§</td>
<td>ns</td>
<td>416 [274]§</td>
<td>353 [581]§</td>
<td>0.02</td>
<td>320 [247]</td>
<td></td>
</tr>
<tr>
<td>AUCI (nmol)</td>
<td>56 [15]</td>
<td>92 [65]§</td>
<td>148 [81]§</td>
<td>ns</td>
<td>125 [82]§</td>
<td>106 [113]§</td>
<td>0.03</td>
<td>96 [60]</td>
<td></td>
</tr>
<tr>
<td>Mean C-peptide (nmol/L)</td>
<td>1.59 [0.71]</td>
<td>1.41 [1.10]</td>
<td>2.90 [1.03]§*</td>
<td>0.02</td>
<td>1.80 [0.71]</td>
<td>1.85 [1.23]</td>
<td>ns</td>
<td>1.74 [0.91]</td>
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<tr>
<td>AUCC_pep (μmol)</td>
<td>0.48 [0.21]</td>
<td>0.42 [0.33]</td>
<td>0.87 [0.31]*</td>
<td>0.01</td>
<td>0.54 [0.21]</td>
<td>0.56 [0.37]*</td>
<td>ns</td>
<td>0.52 [0.27]</td>
<td></td>
</tr>
<tr>
<td>Fasting ISR (pmol min⁻¹ m⁻²)</td>
<td>54 [28]</td>
<td>74 [25]</td>
<td>147 [69]*</td>
<td>0.004</td>
<td>57 [28]</td>
<td>104 [62]</td>
<td>0.02</td>
<td>73 [35]</td>
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<tr>
<td>Total IS (nmol m⁻²)</td>
<td>64 [23]</td>
<td>59 [37]</td>
<td>100 [29]*</td>
<td>0.03</td>
<td>68 [30]</td>
<td>66 [28]*</td>
<td>ns</td>
<td>65 [44]</td>
<td></td>
</tr>
<tr>
<td>MCRI (L min⁻¹ m⁻²)</td>
<td>1.66 [1.25]</td>
<td>0.95 [0.32]§</td>
<td>1.17 [0.73]</td>
<td>ns</td>
<td>1.50 [0.67]*</td>
<td>1.00 [0.73]</td>
<td>0.02</td>
<td>1.37 [0.26]*</td>
<td></td>
</tr>
<tr>
<td>β-GS (pmol min⁻¹ m⁻² mM⁻¹)</td>
<td>96 [60]</td>
<td>110 [92]</td>
<td>122 [41]</td>
<td>0.02</td>
<td>91 [19]</td>
<td>33 [25]*</td>
<td>0.007</td>
<td>61 [38]*</td>
<td></td>
</tr>
<tr>
<td>Rate sensitivity (nmol m⁻²)</td>
<td>4.3 [3.0]</td>
<td>4.1 [5.0]</td>
<td>5.2 [4.9]</td>
<td>0.04</td>
<td>15.0 [13.3]</td>
<td>2.6 [3.2]*</td>
<td>0.01</td>
<td>10.9 [8.4]*</td>
<td></td>
</tr>
<tr>
<td>Fasting EGP (μmol kg⁻¹ min⁻¹)</td>
<td>13.3 [1.7]</td>
<td>13.1 [4.0]</td>
<td>12.1 [0.4]</td>
<td>ns</td>
<td>11.9 [0.6]</td>
<td>11.9 [0.9]</td>
<td>ns</td>
<td>13.3 [0.8]</td>
<td></td>
</tr>
<tr>
<td>AUC_EGP (mmol kg⁻¹ FFm⁻¹)</td>
<td>1.41 [0.22]</td>
<td>1.39 [0.87]</td>
<td>1.57 [0.80]</td>
<td>0.008</td>
<td>2.18 [0.89]*</td>
<td>1.36 [1.39]</td>
<td>(0.09)</td>
<td>2.04 [1.15]*</td>
<td></td>
</tr>
<tr>
<td>MCRG (mL min⁻¹ kg⁻¹ FFm⁻¹)</td>
<td>4.4 [1.1]</td>
<td>4.2 [0.4]</td>
<td>3.6 [1.1]*</td>
<td>ns</td>
<td>3.6 [0.9]</td>
<td>2.3 [0.8]*</td>
<td>&lt;0.01</td>
<td>3.4 [1.2]</td>
<td></td>
</tr>
<tr>
<td>MCR_G/I (mL min⁻¹ kg⁻¹ FFm⁻¹ nM⁻¹)</td>
<td>24.4 [8.4]</td>
<td>10.5 [12.0]§</td>
<td>7.6 [5.2]§</td>
<td>ns</td>
<td>9.1 [9.2]§</td>
<td>6.7 [3.6]*</td>
<td>0.008</td>
<td>11.3 [9.2]</td>
<td></td>
</tr>
<tr>
<td>Fasting glucagon (pg mL⁻¹)</td>
<td>38 [17]</td>
<td>69 [33]§</td>
<td>69 [40]§</td>
<td>0.006</td>
<td>50 [23]*</td>
<td>69 [49]#</td>
<td>0.03</td>
<td>32 [16]*</td>
<td></td>
</tr>
<tr>
<td>∂AUC_Glucagon (ng mL⁻¹)</td>
<td>2.8 [3.7]</td>
<td>0.6 [3.9]§</td>
<td>0.6 [7.4]§</td>
<td>0.006</td>
<td>10.5 [8.4]*</td>
<td>0.3 [3.0]§</td>
<td>0.007</td>
<td>5.7 [4.5]*</td>
<td></td>
</tr>
<tr>
<td>Fasting GIP (pmol/L)</td>
<td>4.5 [5.5]</td>
<td>10.0 [7.0]</td>
<td>9.0 [12.5]</td>
<td>ns</td>
<td>7.0 [4.0]</td>
<td>11.5 [10.0]§</td>
<td>ns</td>
<td>9.5 [11.0]</td>
<td></td>
</tr>
<tr>
<td>∂AUC_GIP (nmol L⁻¹)</td>
<td>11.9 [12.3]</td>
<td>3.6 [4.4]§</td>
<td>4.1 [3.1]</td>
<td>ns</td>
<td>4.5 [3.2]</td>
<td>8.3 [7.2]</td>
<td>ns</td>
<td>5.7 [6.0]</td>
<td></td>
</tr>
<tr>
<td>Fasting GLP-1 (pmol/l)</td>
<td>11.0 [12.0]</td>
<td>7.0 [4.5]</td>
<td>9.0 [7.0]</td>
<td>ns</td>
<td>11.0 [7.0]*</td>
<td>12.5 [12.0]*</td>
<td>ns</td>
<td>11.5 [7.0]*</td>
<td></td>
</tr>
<tr>
<td>AUCGLP-1 (nmol L⁻¹)</td>
<td>6.3 [3.0]</td>
<td>4.5 [2.2]</td>
<td>6.2 [3.6]</td>
<td>0.008</td>
<td>16.8 [9.3]*</td>
<td>4.7 [3.7]</td>
<td>0.02</td>
<td>12.1 [18.5]*</td>
<td></td>
</tr>
</tbody>
</table>
° ISR = insulin secretion rate; IS = total insulin output; MCR\textsubscript{i} = insulin clearance; β-GS = β-cell glucose sensitivity; EGP = endogenous glucose production; AUC = area-under-curve; δAUC = incremental AUC; RaO = rate of appearance of oral glucose; MCR\textsubscript{G} = metabolic clearance rate of glucose; p vs Pre-surgery by Wilcoxon test; * p ≤ 0.05 vs obese controls and § p ≤ 0.05 vs lean controls by Mann Whitney test; # p ≤ 0.05 ND vs T2D pre or 1-year.
Plasma glucose (a) and insulin concentrations (b), insulin secretion rates (ISR) (c), insulin secretion dose-response (d) rates of appearance of oral (RaO) (e), and endogenous glucose (EGP) (f) in the 4 groups of study subjects at baseline. The grey shaded areas are the mean±SEM for the lean control group, the green shaded area is the mean±SEM for the obese control group.
Plasma glucose and insulin concentrations in the patients before and after RYGB. The corresponding data for the obese control group is shown by the grey line.

254x366mm (72 x 72 DPI)
Insulin secretion rate and dose-response function in the patients before and after RYGB. The corresponding data for the obese control group is shown by the shaded areas.

254x366mm (72 x 72 DPI)
Insulin sensitivity (as the ratio of mean glucose clearance to mean insulin concentration during a 5-hour mixed meal test) plotted against the corresponding BMI value in the 4 study groups. The points to the far right are those for the surgical groups before the operation. Plots are median [interquartile range]. The dotted line is a power function fit of the plots. The inset shows the relationship between the change in insulin sensitivity and the percent change in body weight in the surgical patients; the linear fit and the 95% confidence interval are shown.
Rate of appearance of oral (RaO) and endogenous glucose (EGP) glucose metabolic clearance rate in the patients before and after RYGB. The corresponding data for the obese control group is shown by the shaded areas. By 2-way ANOVA, the time-course of EGP is significantly higher postsurgery than presurgery (F=27.6, p<0.0001) in both ND and T2D.
Plasma glucagon, GLP-1, and GIP response to the meal in the patients before and after RYGB. The corresponding data for the obese control group is shown by the grey lines.
Supplemental Figure 1 – Time-course of the estimated insulin-to-glucagon molar concentration ratio (mean±SEM) in ND and T2D patients before and after surgery. The corresponding data for the lean control groups are shown by the green area, those for the obese control group are shown by the grey area.

Supplemental Figure 2 – Direct relationship between rate sensitivity of insulin secretory response to the meal and the incremental area under the GLP-1 response (A); relationship between the hepatic sensitivity index (= product of mean EGP and mean plasma insulin) and the area under the glucagon response (B).
Time-course of the estimated insulin-to-glucagon molar concentration ratio (mean±SEM) in ND and T2D patients before and after surgery. The corresponding data for the lean control groups are shown by the green area, those for the obese control group are shown by the grey area.
Direct relationship between rate sensitivity of insulin secretory response to the meal and the incremental area under the GLP-1 response (A); relationship between the hepatic sensitivity index (= product of mean EGP and mean plasma insulin) and the area under the glucagon response (B).