Limited recovery of β-cell function after gastric bypass despite clinical diabetes remission

*Short title: β-cell dysfunction after gastric bypass*

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Abstract:

The mechanisms responsible for the remarkable remission of type 2 diabetes after Roux-en Y gastric bypass (RYGBP) are still puzzling. To elucidate the role of the gut, we compared β-cell function assessed during an oral glucose tolerance test (OGTT) and an isoglycemic intravenous glucose clamp (iso-IVGC) in: 1) 16 severely obese patients with type 2 diabetes, up to 3 years post-RYGBP (OB-DM); 2) 11 severely obese normal glucose tolerant controls (OB-NGT); 3) 7 LEAN controls. Diabetes remission was observed after RYGBP. β-cell function during the OGTT, significantly blunted prior to RYGBP, normalized to levels of both control groups after RYGBP. In contrast, during the iso-IVGC, β-cell function improved minimally and remained significantly impaired compared to LEAN controls up to 3 years post-RYGBP. Pre-surgery β-cell function, weight loss and GLP-1 response were all predictors of post-surgery β-cell function, although weight loss appeared to be the strongest predictor. These data show that β-cell dysfunction persists after RYGBP, even in patients in clinical diabetes remission. This impairment can be rescued by oral glucose stimulation, suggesting that RYGBP leads to an important gastrointestinal effect, critical for improved β-cell function after surgery. #NCT00571220 Clinicaltrials.gov.
Introduction

Roux-en-Y gastric bypass (RYGBP) remits type 2 diabetes in approximately 40-80% of cases (1; 2), however mechanisms surrounding this remarkable improvement are still elusive. Although caloric restriction and weight loss are important contributors, evidence suggests that altered gut physiology, including bypass of the proximal small intestine, may also contribute. For example, bolus delivery of oral glucose elicits significantly lower plasma glucose excursions compared to intravenous (IV) bolus delivery, and an isoglycemic IV glucose clamp (iso-IVGC) leads to significantly lower insulin excursions than an oral glucose tolerance test (OGTT) (3). These experiments highlight the importance of gut-mediated factors in the regulation of glucose metabolism and insulin secretion. The difference in postprandial insulin excursion, or incretin effect, is severely blunted in diabetes and normalized shortly after RYGBP, in parallel with a marked increase in the incretin hormone GLP-1 (4), which has been shown to improve glucose tolerance, insulin secretion and ß-cell glucose sensitivity (BCGS) (5-8). In fact, GLP-1 agonists are used for diabetes management.

ß-cell function, often evaluated using BCGS, relating insulin secretion to plasma glucose levels, and the disposition index (DI), which also adds an insulin sensitivity component, has been shown to be an optimal predictor of diabetes risk (9-11). ß-cell function is impaired in diabetes (12; 13) and significantly improved after RYGBP (13-15); evidence suggests this could be GLP-1 mediated (8; 16; 17). However, the contribution of the gastrointestinal tract to improvement in ß-cell function after RYGBP has not been directly tested. To investigate this we examined change in ß-cell function up to 3 years after RYGBP in severely obese individuals with type 2 diabetes who experienced clinical diabetes remission post-RYGBP (OB-DM), and compared
them to both non-operated, obese normal glucose tolerant (OB-NGT) and lean NGT (LEAN) subjects. To assess if improvements in β-cell function after RYGBP were mediated by the gut, we compared measures of β-cell function during an oral and isoglycemic glucose challenge. Lastly, we studied predictors of β-cell function and glucose control after RYGBP.
Methods

Subjects
A total of 185 outpatient testing procedures were performed. Sixteen severely obese subjects with type 2 diabetes of short duration (mean 3.0±2.6 years), were studied before (OB-DM0, n=16) and at 1 month (OB-DM1M, n=16), 1 year (OB-DM1Y, n=15), 2 years (OB-DM2Y, n=16 for OGTT, 14 for iso-IVGC) and 3 years after RYGBP (OB-DM3Y, n=13). Diabetes and diabetes remission were defined using ADA criteria (18). Exclusion criteria included insulin therapy, diabetes duration ≥10 years, current treatment with thiazolidinediones, GLP-1 agonists or dipeptidyl peptidase (DPP)-4 inhibitors, and type 1 diabetes. Medications used pre-surgery, including metformin and/or sulfonylurea (9/16 subjects), were withheld 2-3 days prior to study visits. Eleven severely obese NGT non-operated subjects (OB-NGT) and 7 lean NGT subjects (LEAN) were used as controls (all OB-NGT controls: fasting plasma glucose <5.5 mmol/L, 2-hour postprandial glucose <7.7 mmol/L, HbA1c <6.5%). The study was approved by the Institutional Review Board at St. Luke’s-Roosevelt Hospital Center. OB-NGT and OB-DM were recruited from the bariatric center of our institution, LEAN from the community, and all provided written informed consent.

Surgery
OB-DM subjects underwent laparoscopic RYGBP with a 30-ml gastric pouch, 40-cm afferent limb, 150-cm Roux limb, and 12-mm gastrojejunostomy, as described previously (19).

Experimental procedures
Oral glucose tolerance test
After a 12 hour overnight fast, subjects underwent a 180 minute OGTT (50 g glucose in 200 mL). Blood samples were collected in chilled tubes with EDTA from an antecubital IV catheter from an arterialized arm vein warmed with a heating pad. Blood samples for incretin measurements were treated with aprotinin (500 kallikrein inhibitory U/ml blood; Roche Applied Sciences, Indianapolis, IN) and DPP-4 inhibitor (50 umol/L or 10 ul/ml blood; EMD Millipore, Darmstadt, Germany). Samples were centrifuged at 4ºC and stored at -80ºC.

Isoglycemic IV glucose clamp

Glucose (20% dextrose solution) was infused using a Gemini pump (CareFusion, San Diego, CA, USA) over 180 minutes to match the plasma glucose concentration profiles achieved for each subject during the OGTT. Blood glucose was monitored using contralateral antecubital IV access every 5 minutes, and glucose infusion rate was adjusted accordingly.

Assays

Plasma glucose was determined at bedside by the glucose oxidase method with an Analox glucose analyzer (Lunenburg, MA). Total GLP-1 was measured by RIA (Millipore) after plasma ethanol extraction. The assay reacts 100% with GLP\(_{17-36}\), GLP\(_{19-36}\) and GLP\(_{17-37}\), but not with glucagon (0.2%), GLP-2 (<0.01%) or exendin (<0.01%). Gastric inhibitory peptide (GIP) was determined by ELISA (Millipore), reacts 100% with GIP\(_{1-42}\) and GIP\(_{3-42}\) but not with GLP-1, GLP-2, oxyntomodulin, or glucagon. Plasma insulin and C-peptide were measured by RIA (Millipore). All hormone and metabolite assays were performed at the Hormonal Core Laboratory at the Obesity Nutrition Research Center. Intra- and inter-assay coefficients of variance ranged from 3.4-7.4% and 4.4-7.4%, respectively. Lipids were assayed by Ortho Clinical Diagnostics Vitros Fusion 5.1 (Rochester, NY).
Calculations

**Area under the curve (AUC)** was calculated using the trapezoidal method for 180 minutes unless otherwise indicated. Homeostasis model assessment of insulin resistance (**HOMA-IR**) calculated as: \((\text{fasting-insulin}_{\mu\text{U/mL}} \times \text{fasting-glucose}_{\text{mg/dL}})/405\). **Incretin effect of insulin, C-peptide and ISR (“X”)** calculated as difference in β-cell response, or action of the incretin factor, expressed as the percentage of response to oral glucose: \(\left[(X_{\text{AUCOGTT}}-X_{\text{AUCiso-IVGC}})/(X_{\text{AUCOGTT}})\right] \times 100\). **Insulin sensitivity index (ISI)** calculated as: \(10,000/[(\text{fasting glucose} \times \text{fasting insulin} \times \text{mean glucose}_{0-180\text{min}} \times \text{mean insulin}_{0-180\text{min}})^{0.5}]\). **Insulin secretion rates (ISR)** calculated by mathematical deconvolution using a two-compartment model for hormone clearance using C-peptide from the OGTT (ie, O-ISR) and iso-IVGC (ie, IV-ISR), using the Chronological Series Analyzer (CSA) (Van Cauter, Hasak and Leproult, University of Chicago) (20). ISR was calculated both adjusted and unadjusted for body weight. Measures of β-cell function include **insulin secretion index** (ISX), **β-cell glucose sensitivity** (BCGS), and **disposition index** (DI). ISX calculated as: ISR AUC/Glucose AUC from 0-180 minutes, from either the OGTT (O-ISX) or iso-IVGC (IV-ISX). BCGS calculated as: slope between ISR (pmol/kg/min) and corresponding blood glucose (mmol/L), from baseline to peak glucose level, from OGTT (O-BCGS) and iso-IVGC (IV-BCGS). DI calculated for OGTT (O-DI) and iso-IVGC (IV-DI) as: BCGS x (1/HOMA-IR). DI was alternatively calculated as BCGS x ISI (21; 22).

**Nomenclature**

Variables derived from OGTT and iso-IVGC are preceded by “O-“ and “IV-“, respectively. For example: O-ISX, IV-ISX, O-BCGS, IV-BCGS, O-DI, IV-DI.
Statistical analysis

Data are expressed as mean±SD except in figures where mean±SEM is reported. The study sample consisting of 15-16 subjects was originally powered to compare incretin levels (3; 22). An additional power analysis was completed to justify the use of this sample to look at differences in other outcomes, namely, O-DI and IV-DI (OB-DM0 vs. OB-DM1Y). This indicated that the minimum effect size was 1.15 which required at least 8 subjects to achieve 80 % power (α= 0.05) for a simple paired means comparison of each of these outcomes. We therefore proceeded with these analyses.

Normality was tested, variables were log-transformed if not normally distributed, and non-parametric tests were utilized if variables were still not normally distributed. ANOVA with a Bonferroni post-hoc test was used to analyze data across all groups pre-surgery, and Dunnett’s post-hoc test used at all time points post-surgery to compare LEAN and OB-NGT to OB-DM post-surgery. Independent t-tests were used to compare OB-NGT vs. OB-DM, if no LEAN comparison was possible. Paired t-tests were used to compare data (ISR, ISX, BCGS, DI) for OGTT versus iso-IVGC. Repeated measures ANOVA used to compare plasma glucose matching between the OGTT and iso-IVGC. Mixed model regression (MMR) used to compare changes over time in OB-DM, and with additional covariates to evaluate predictors of: A) β-cell function measured during oral glucose stimulation, B) fasting and postprandial oral glucose, after surgery. R² values were estimated for predictors in MMR analyses based on improvements in log-likelihoods between baseline and more complex models (e.g., model containing one or more predictors compared to model with slope only) (23). Statistical significance was set at p<0.05 (2-tailed). All analyses were completed using SPSS 19 (IBM, Armonk, New York, USA).
Results

Pre-surgery characteristics

Clinical characteristics are provided in Table 1. Fasting and 120’ glucose were significantly higher (Table 1), and β-cell function was significantly lower (Figure 1), in OB-DM prior to surgery, compared to OB-NGT and LEAN. No difference in fasting or post-oral incretin concentrations was observed between groups (Supplementary Table 1). Triglycerides were significantly higher in OB-DM versus OB-NGT and no difference in total, low-density lipoprotein (LDL) or high-density lipoprotein (HDL) cholesterol was observed (Table 1).

Effects of RYGBP surgery on weight loss, glucose metabolism, lipids, and incretin levels

Weight loss was ~11% at 1 month, ~31% at 1 year, and sustained at 2 and 3 years (Table 1). Rate of weight loss was 2.7 kg/week at 1 month and 0.5 kg/week from 1 month through 1 year.

All subjects in OB-DM were in diabetes remission (18) from 1 month onwards except one subject that did not remit until 1 year and relapsed (relapse defined as no longer meeting ADA criteria for remission) at 3 years. Diabetes was significantly improved in this subject, and including or excluding this subject from the data analysis did not alter the overall results (data not shown). Glucose levels, HOMA-IR and ISI all improved as early as 1 month, and glucose and HOMA-IR normalized by 1 year; this was sustained at 2 and 3 years, compared to pre-surgery (Table 1). Similarly, total and LDL cholesterol and triglycerides improved (Table 1).

As expected, plasma concentrations of incretins, in response to oral glucose, were significantly increased after RYGBP. The increase was rapid, with peak GLP-1 elevated ~5 fold at 1 month compared to pre-surgery, further increased to ~10-fold at 1 year, and remained ~5-7-fold higher
at 2-3 years. Compared to pre-surgery values, peak GIP was significantly elevated ~1.4-fold by 1 month and sustained thereafter up to 3 years. At all time points after surgery, GLP-1 and GIP peak responses in OB-DM were significantly higher than both controls (Supplementary Table 1).

Effects of RYGBP surgery on the incretin effect, ISR, BCGS and DI

Overall, glucose values were well matched between the OGTT and iso-IVGC. However, a significant time x test (OGTT vs. iso-IVGC) interaction (p=0.02) was observed, with a trend for slightly higher glucose values during the iso-IVGC from 45 minutes onwards. Individually, a significant time x test interaction was observed in only the OB-DM 1M (p=.04) and OB-DM 2Y (p=0.01) groups; no significant difference was observed in any other groups/conditions. Furthermore, none of the individual group contrasts were significant at any time point overall, or for any group/condition individually, after correcting for the number of comparisons (Supplementary Figure 1).

The severely impaired incretin effect of insulin, C-peptide and ISR in OB-DM rapidly normalized to the level of both controls from 1 month onwards (Figure 2; Supplementary Figures 2-3).

β-Cell response to oral and IV glucose

ISR and β-cell function, assessed during oral glucose stimulation, normalized after RYGBP. O-ISR<sub>AUC0-60min</sub> nearly doubled 1 month after RYGBP and this improvement was maintained up to 3 years post-surgery; in fact, this variable was significantly greater compared to OB-NGT from one year onwards after surgery (Figure 1C). Measures of β-cell function including O-ISX, O-BCGS, and O-DI, severely impaired in OB-DM pre-surgery, normalized to levels of controls
post-surgery (Figure 1B,D,E). O-ISX and O-BCGS both normalized to levels of both controls from 1 month onwards (Figure 1 B,D), and O-DI normalized to OB-NGT levels from 1 month onwards, and to LEAN from 1 year onwards (Figure 1E; Supplementary Figure 4).

Contrary to what was observed during oral glucose stimulation, insulin secretion and β-cell function, measured during the iso-IVGC, improved minimally and never normalized to levels of LEAN, even up to 3 years after RYGBP (Figure 1; Supplementary Figure 4), despite sustained weight loss and clinical diabetes remission. Similar results were obtained when measures of β-cell function were not adjusted for body weight (Supplementary Figure 5).

Figure 3 illustrates the striking difference in the change in DI during oral, versus IV, glucose stimulation. A rapid and significant improvement in O-DI was observed in OB-DM, illustrated by a shift upwards and to the right, equivalent to OB-NGT by 1 month and normalized to the levels of LEAN by 1 year post-RYGBP (Figure 3A). In contrast, during the iso-IVGC, a much smaller, albeit significant, improvement in IV-DI was observed, however this remained significantly impaired compared to LEAN controls up to 3 years after RYGBP (Figure 3B).

Predictors of β-cell function and diabetes control after RYGBP in OB-DM

Results for β-cell function (O-BCGS and O-DI) as outcome were similar. Weight loss, pre-surgery β-cell function and GLP-1 response were all significant predictors of post-surgery β-cell function, although weight loss was consistently the strongest predictor for O-BCGS and both weight loss and pre-surgery O-DI were strong predictors of post-surgery O-DI, based on $R^2$ values (Supplementary Table 2); this remained true when all significant univariate predictors were put into a multivariate model. Age, pre-surgery BMI, and diabetes duration and control were not significant.
Post-surgery β-cell function (O-BCGS) and weight loss were both important, roughly equivalent predictors of fasting glucose after RYGBP in univariate and multivariate models, (Supplementary Table 2). Pre-surgery β-cell function and BMI, age and diabetes duration were not significant.

Weight loss, along with pre- and post-surgery β-cell function, were all predictors of 120’ glucose (Supplementary Table 2) based on univariate modeling. Weight loss and pre-surgery β-cell function (O-DI) both remained significant in a multivariate model, although pre-surgery O-DI was slightly better as a predictor of postprandial 120’ glucose. Age, diabetes duration, pre-surgery BMI, the incretin effect of insulin, GLP-1 and GIP response were not significant.
Discussion

This study demonstrates that impairment in β-cell function persists up to 3 years post-RYGBP, in subjects with “clinical diabetes remission (18).” However, this impairment was only detected when glucose was administered IV, as parameters of β-cell function normalized upon oral glucose stimulation after surgery, highlighting the critical role of gut factors in the improvement in β-cell function after RYGBP. Although engagement of the gut appears important for the stunning improvement in β-cell function and insulin secretion after RYGBP, predictor analyses in our limited, small cohort, suggest that weight loss was the strongest predictor of post-surgical β-cell function, along with pre-surgery β-cell function and GLP-1.

This study is the first to demonstrate the importance of the oral route to improvements in β-cell function after RYGBP, and to show that improvements persist 3 years after surgery. Other studies have observed an improvement in β-cell function after RYGBP, using an oral glucose or meal test (13-15). BCGS, impaired in diabetes, shown here and elsewhere (12; 13), increases acutely (1-6 weeks) after RYGBP, with little further improvement longer-term (3-12 months) (13-15). This is similar to our observations, as O-BCGS markedly increased 1 month after RYGBP, with a more modest increase at 1 year. This increase in BCGS post-RYGBP is not observed in NGT populations (13; 24). Furthermore, although we observed a normalization of O-BCGS in OB-DM after RYGBP, compared to LEAN and OB-NGT controls by 1 year, others (13; 15) observed a lesser improvement at 1 year in subjects with diabetes. However, in these studies (13; 15), diabetes also improved to a lesser extent after RYGBP than in our OB-DM group, thus corroborating the discrepancy in BCGS. Note, after adjustment for insulin sensitivity (ie-DI), similar improvements were observed. DI, impaired in diabetes, shown here and elsewhere, increases after RYGBP regardless of diabetes status (13; 24; 25). We showed that O-
DI normalized to the levels of both control groups, LEAN and OB-NGT, by 1 year. This is similar to Jorgensen et al, which showed that by one year post-surgery, DI in subjects with diabetes approached levels of unoperated obese NGT populations (13).

Despite the rapid and marked improvement in β-cell function and increase in ISR during oral glucose stimulation after RYGBP, exposure to equivalent plasma glucose levels via an IV glucose clamp (iso-IVGC), to calculate the incretin effect (8), elicited a much smaller response, suggesting that gastrointestinal factors are important for the remarkable improvement in β-cell function after RYGBP. We observed that BCGS and DI were not significantly different between the OGTT and iso-IVGC in OB-DM subjects prior to surgery, however this differential was restored after surgery. This is in agreement with Muscelli et al., that observed greater BCGS during an OGTT versus an iso-IVGC in NGT individuals, but not individuals with diabetes (12). Furthermore, the rapid normalization of the incretin effect one month after surgery, in agreement with previous studies (4), is sustained up to 3 years.

GLP-1, significantly increased after RYGBP and a significant predictor of post-surgery β-cell function in our study, may be one of the factors that explain the difference in β-cell function after oral and iso-IVGC glucose stimulation. Indeed, infusion of GLP-1 with a hyperglycemic clamp in healthy subjects significantly increased the slope of ISR versus plasma glucose (6). Furthermore, the blunted stimulation in insulin, C-peptide and ISR during an iso-IVGC can be rescued, and even further amplified compared to an OGTT, with an IV GLP-1 infusion (17). Additionally, exendin (9-39), a GLP-1 receptor antagonist, has been used to illustrate GLP-1’s role in postprandial insulin secretion in post-RYGBP subjects (5; 8; 16; 26). These studies highlight the crucial role of GLP-1 in glucose-stimulated insulin secretion, and implicate the robust GLP-1 response after RYGBP in the improvement in insulin secretion and β-cell function.
observed in our study after oral, but not IV glucose, stimulation. However, the effect of GLP-1 on glucose control, albeit significant, may be small. Exendin (9-39) administration does not severely worsen glucose tolerance in individuals after RYGBP (8; 16). Other factors such as accelerated gastric emptying (GE) and GIP, as well as yet unknown intestinal factors, may be important. Although the GIP response after RYGBP was not a significant predictor of β-cell function in our study, others have shown that GIP infusion mildly enhances insulin secretion (17). The development of GIP antagonists for human studies will clarify GIP’s role in insulin secretion and glucose metabolism.

Despite the important influence of intestinal factors, we cannot discount the contribution of weight loss to improvement in β-cell function after RYGBP. Although GLP-1 and pre-surgical β-cell function predicted post-surgical β-cell function, weight loss appears to be a superior predictor. Weight loss, along with post-surgical β-cell function (O-BCGS) predicted fasting glucose, and weight loss, along with pre-surgical β-cell function (O-DI), predicted 120’ glucose. Yet, these predictor analyses should be interpreted with caution due to the small sample size.

The importance of weight loss versus an independent effect of the gut after RYGBP is a topic of fervent investigation. Bradley et al. showed that in a non-diabetic population, equivalent 20% weight loss after RYGBP and gastric banding similarly improved DI during a meal test coupled with a clamp (24). However, diabetes remission has been reported after duodenal bypass, sans weight loss (27). Future studies comparing β-cell function in a diabetic population, at matched weight loss after RYGBP, compared to caloric restriction and/or restrictive bariatric surgery will help elucidate the impact of weight loss versus gut-mediated factors.
Although this study has merits, with a unique comparison of β-cell function comparison after oral and IV glucose, in a cohort of patients with diabetes followed for three years post-surgery, there are limitations to its interpretation. There are some technical issues that should be discussed. First, during the latter part of the experiment, plasma glucose levels during the iso-IVGC were slightly greater than during the OGTT; however, this would actually favor increased insulin secretion during IV versus oral glucose. Secondly, the amount of glucose administered during an iso-IVGC is ~50% of that administered during a 50g OGTT (54% reported (28) and 46% in OB-NGT) in a NGT individual; however, the amount delivered IV is similar (88% in our OB-DM group) in individuals with diabetes (28). Thirdly, a lower amount of glucose is delivered IV after RYGBP, and it could be hypothesized that this may elicit a lesser insulin response. Yet, the amount of glucose delivered IV post-surgery was higher than both the LEAN and OB-NGT groups, suggesting that the lackluster improvement in IV β-cell function post-surgery is not an artifact of the lower amount of glucose infused. To correct for this we presented ISR versus glucose concentrations (ISX; Figure 1), and still did find an increase in ISR during IV glucose stimulation after RYGBP. Fourth, we did not report glucagon levels in this study, although effects of RYGBP on glucagon have been reported by our group (29) and others (16). Interestingly, with type 2 diabetes, glucagon suppression is impaired during oral, but not IV, glucose stimulation (30) and this may be secondary to a change in the balance in incretin levels (17).

The 50 g OGTT also introduces some limitations. This lower load was used to circumvent dumping post-RYGBP and has been used previously to derive indices of insulin secretion (28; 31). However, this may have underestimated diabetes status in OB-NGT and post-surgery OB-DM subjects. Diabetes remission was defined according to ADA criteria (18), however the
interpretation of remission after RYGBP is controversial (32; 33), as faster GE and episodes of hypoglycemia complicate the interpretation of postprandial glucose and HbA1c levels. Using more stringent criteria (32; 33), we observed some deterioration of glucose control at 3 years, which is consistent with recent data showing diabetes remission wanes from 1 to 3 years (34). It is possible that the persistent defect in β-cell function, only revealed with IV glucose stimulation, may contribute to the relapse in diabetes observed years after surgery.

Another point worthy of discussion is the calculation of the DI. OGGTT-derived DI (from a 75-g OGTT) has been shown to correlate with DI derived from frequently-sampled intravenous glucose tolerance tests (FSIVGTT) (35), as well as predict future diabetes status (11). To calculate DI, we measured the slope of the relationship between ISR versus plasma glucose levels (ie-BCGS) from a 50-g OGTT or matched iso-IVGC, and adjusted this for insulin sensitivity—using HOMA-IR or ISI. Using HOMA-IR circumvents obvious changes in postprandial glucose dynamics after RYGBP and has been reported previously post-RYGBP (13). However, ISI provides a measure of whole-body insulin sensitivity that correlates well with clamp-derived measures (21), and has been suggested for use in DI calculations (22). In this study, HOMA-IR in OB-DM normalized to the level of LEAN from 1-3 years post-surgery, despite obesity (BMI=30). ISI improved post-RYGBP but still remained significantly lower versus LEAN, and levels slightly deteriorated from 1-3 years. Using either HOMA-IR or ISI in the DI measurement showed a similar trend, with a far greater improvement in DI after oral, than after IV, glucose stimulation. However, using ISI (Supplementary Figure 4) revealed a more pronounced deterioration in DI at 2-3 years post-surgery. Furthermore, it should be acknowledged that the rapid GE and resulting leftward shift in glucose and insulin curves (Supplementary Table 1 and 2) post-RYGBP may complicate the comparison to OB-NGT and
LEAN, and potentially overestimate BCGS and DI after surgery; however, the remarkable paradox in β-cell function after surgery between the oral and IV glucose measurements remains unaffected by this.

This study shows that in the setting of clinical diabetes remission and large, sustained weight loss, RYGBP does not rescue impairment in insulin secretion and β-cell function when the gastrointestinal tract is not engaged. However, oral glucose stimulation rescues impairment rapidly, at 1 month, and this is sustained up to 3 years after RYGBP, demonstrating the essential role of the gut in this effect. Predictor analyses showed that weight loss, GLP-1 and pre-surgery β-cell function are all important contributors to post-surgical β-cell function. Evidence from the literature suggests GLP-1 may mediate some of this remarkable effect, however it is possible that there are other gut-mediated factors, aside from incretins, that are important in this phenomenon.
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BL designed the study. JM referred subjects. RD, KB, PB, FP, PH, BL collected and/or analyzed the data. FP, PH provided statistical consultation. RD and BL wrote the manuscript. AC, JM, PH reviewed and/or edited manuscript. BL 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. Authors do not have any conflict of interest.

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Figure 1. Effect of RYGBP on Insulin Secretion Rate and β-Cell Function.

(A) ISR during the OGTT or iso-IVGC over the entire experiment (180 minutes) was not different between groups. In all groups and conditions, ISR AUC 0-180 min was significantly greater during the OGTT vs. the iso-IVGC (p<.05). (B) ISX was significantly impaired during the OGTT in the OB-DM group pre-surgery, significantly increased immediately after surgery, and this increase was maintained up to 3 years post-surgery. However, no increase in ISX was observed during the iso-IVGC. In all groups and conditions, ISX was significantly greater during the OGTT vs. the iso-IVGC (p<.05). (C) ISR during the first 60 minutes of the OGTT was significantly increased immediately after surgery and this increase was maintained up to 3 years post-surgery. No increase in ISR during the iso-IVGC was observed. In all groups and conditions, ISR AUC 0-60 min was significantly greater during the OGTT vs. the iso-IVGC (p<.05). (D) In the OB-DM group pre-surgery, BCGS during the OGTT and iso-IVGC was significantly lower than both control groups. After surgery, O-BCGS normalized to the levels of both control groups by 1 month, and was further increased at 1-2 years. In contrast, IV-BCGS remained significantly lower compared to both control groups up to 3 years. BCGS was significantly greater during the OGTT vs. the iso-IVGC in the OB-NGT group and in the OB-DM group at all conditions post-surgery (p<.05), but not in the LEAN or OB-DM group prior to surgery. (E) In the OB-DM group pre-surgery, DI during the OGTT and iso-IVGC was significantly impaired compared to both control groups. After surgery, O-DI normalized to the levels of the OB-NGT group by 1 month and LEAN by 1 year. In contrast, IV-DI remained significantly lower compared to the LEAN controls up to 3 years post-surgery. DI was significantly greater during the OGTT vs. the iso-IVGC in the OB-NGT group and in the OB-DM group
at all conditions post-surgery (p<.05), but not in the LEAN or OB-DM group prior to surgery.

OGTT=White bar; Iso-IVGC=Black bar. ISR, Insulin Secretion Rate; ISX, Insulin Secretion Index; BCGS, β-cell Glucose Sensitivity; DI, Disposition Index. LEAN=Lean; OBGNGT=Obese, normal glucose tolerant controls; OB-DM0=Obese group with diabetes prior to RYGBP surgery; OB-DM1M, OB-DM1Y, OB-DM2Y, OB-DM3Y=Obese, group with diabetes at 1 month, 1 year, 2 years and 3 years after RYGBP surgery.

Values are Mean±SEM; *p<.05 vs. LEAN, †p<.05 vs. OBGNGT, ‡p<.05 vs. OB-DM0, §p<.05 vs. OB-DM1M.

Figure 2. ISR during the OGTT and iso-IVGC and the Incretin Effect of ISR. (A–G) ISR AUC (pmol/kg/min) was significantly greater during the OGTT compared to the iso-IVGC under all groups and conditions (p<.05). (H) The incretin effect of ISR was blunted in subjects with diabetes prior to surgery (D0), but normalized from 1 month onwards after surgery.

Closed circle=OGTT; Open circle=iso-IVGC. ISR, Insulin secretion rate. LEAN/L=Lean; OBGNGT/OB=Obese normal glucose tolerant controls; OB-DM0/D0=Obese group with diabetes prior to RYGBP surgery; OB-DM1M/D1M, OB-DM1Y/D1Y, OB-DM2Y/D2Y, OB-DM3Y/D3Y=Obese group with diabetes at 1 month, 1 year, 2 years and 3 years after RYGBP surgery.

Values are Mean±SEM; *p<.05 vs. LEAN, †p<.05 vs. OB-NGT, ‡p<.05 vs. OB-DM0.

Figure 3. Effect of RYGBP on the Disposition Index during the OGTT and Iso-IVGC.
(A) OGGT: O-DI, significantly impaired in OB-DM prior to surgery (p<.05 vs. both LEAN and OB-NGT groups), improved rapidly and significantly, as illustrated by a shift upwards and to the right. O-DI in the OB-DM group normalized to levels of OB-NGT controls by 1 month and was not significantly different from the LEAN from 1 year onwards (p<.05). (B) Iso-IVGC: IV-DI was significantly impaired in OB-DM prior to surgery (p<.05 vs. both LEAN and OB-NGT groups). Contrary to the O-DI, IV-DI improved minimally, albeit significantly (p<.05), with a shift to the right and minimal shift upward, after RYGBP. However, IV-DI remained significantly lower than the OB-NGT controls at 1 month and lower than the LEAN controls at all time points post-surgery (p<.05).

BCGS, β-cell Glucose Sensitivity; DI, Disposition Index. X=Individual points of LEAN group; Closed square=Obese normal glucose tolerant group; Closed circle=OB-DM 0 (Obese group with diabetes, pre-surgery); Open circle=OB-DM 1M (Obese group with diabetes, 1 month post-surgery); Closed diamond=OB-DM 1Y (Obese group with diabetes, 1 year post-surgery); Closed triangle=OB-DM 2Y (Obese group with diabetes, 2 years post-surgery); Open triangle=OB-DM 3Y (Obese group with diabetes, 3 years post-surgery).

Mean±SEM for all groups except LEAN. LEAN presented as each individual subject.
|                         | LEAN       | OB-NGT     | OB-DM 0    | OB-DM 1M   | OB-DM 1Y   | OB-DM 2Y   | OB-DM 3Y   |
|-------------------------|------------|------------|------------|------------|------------|------------|------------|
| Age (years)             | 35.4±8.3   | 36.3±7.6   | 47.1±8.5*† |            |            |            |            |
| Weight (kg)             | 73.8±11.2  | 122.6±18.8*| 113.7±16.2*| 101.2±14.6*†‡| 78.0±12.0†‡§| 79.0±12.5†‡§| 81.0±11.8†‡§|
| BMI (kg/m^2)            | 23.5±2.1   | 44.7±6.9*  | 43.9±4.9*  | 39.2±5.1*†‡| 30.3±3.7*†‡§| 30.5±3.6*†‡§| 31.1±3.0*†‡§|
| Weight loss (kg)        |            |            |            |            | 12.5±4.9   | 35.4±10.5§  | 34.7±10.9§  | 35.0±10.4§  |
| HbA1c (%)               |            |            |            |            | 5.4±0.5    | 7.1±1.0†    | 5.4±0.4‡    | 5.7±0.4‡    |
| Fasting glucose (mmol/L)| 4.96±0.44  | 5.17±0.26  | 8.05±2.62*†| 5.86±1.00*†‡| 4.79±0.66‡§| 4.98±0.83‡§| 5.17±0.68‡§|
| Fasting insulin (pmol/L)| 47.3±27.8  | 144.2±66.0*| 185.1±64.5*| 117.4±50.6*‡| 73.2±36.8†‡§| 70.2±28.9†‡§| 66.7±33.6†‡§|
| 120' glucose (mmol/L)   | 4.79±0.66  | 6.04±0.88* | 11.33±3.08*†| 6.47±1.74*‡| 5.00±1.38†‡§| 4.37±1.37†‡§| 5.08±1.73†‡§|
| HOMA-IR                 | 1.51±0.97  | 4.61±2.08  | 9.48±5.46*†| 4.44±2.54*‡| 2.22±1.24†‡§| 2.23±1.14†‡§| 2.19±1.25†‡§|
| ISI composite           | 10.55±6.85 | 3.47±1.95* | 1.97±0.89*†| 3.27±1.57*‡| 5.25±2.79*‡§| 4.88±1.83*‡§| 4.52±1.58*‡§|
| Total Cholesterol (mg/dL)|           |            |            |            |            |            |            |
| LDL Cholesterol (mg/dL) |           |            |            |            |            |            |            |
| HDL Cholesterol (mg/dL) |           |            |            |            |            |            |            |
| Triglycerides (mg/dL)   |           |            |            |            |            |            |            |
Table 1: Clinical Characteristics
Mean ±SD. *p<.05 vs. LEAN, †p<.05 vs. OB-NGT, ‡p<.05 vs. OB-DM 0, §p<.05 vs. OB-DM 1M.

HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; ISI, Insulin Sensitivity Index; LDL, Low-density Lipoprotein; HDL, High-density Lipoprotein.
Figure 1. Effect of RYGBP on Insulin Secretion Rate and β-Cell Function.
(A) ISR during the OGTT or iso-IVGC over the entire experiment (180 minutes) was not different between groups. In all groups and conditions, ISR AUC 0-180 min was significantly greater during the OGTT vs. the iso-IVGC (p<.05). (B) ISX was significantly impaired during the OGTT in the OB-DM group pre-surgery, significantly increased immediately after surgery, and this increase was maintained up to 3 years post-surgery. However, no increase in ISX was observed during the iso-IVGC. In all groups and conditions, ISX was significantly greater during the OGTT vs. the iso-IVGC (p<.05). (C) ISR during the first 60 minutes of the OGTT was significantly increased immediately after surgery and this increase was maintained up to 3 years post-surgery. No increase in ISR during the iso-IVGC was observed. In all groups and conditions, ISR AUC 0-60 min was significantly greater during the OGTT vs the iso-IVGC (p<.05). (D) In the OB-DM group pre-surgery, BCGS during the OGTT and iso-IVGC was significantly lower than both control groups. After surgery, O-BCGS normalized to the levels of both control groups by 1 month, and was further increased at 1 and 2 years. In contrast, IV-BCGS remained significantly lower compared to both control groups up to 3 years. BCGS was significantly greater during the OGTT vs. the iso-IVGC in the OB-NGT group and in the OB-DM group at all conditions post-surgery (p<.05), but not in the LEAN or OB-DM group prior to surgery. After surgery, O-DI normalized to the levels of the OB-NGT group by 1 month and LEAN by 1 year. In contrast, IV-DI remained significantly lower compared to the LEAN controls up to 3 years post-surgery. DI was significantly greater during the OGTT vs. the iso-IVGC in the OB-NGT group and in the OB-DM group at all conditions post-surgery (p<.05), but not in the LEAN or OB-DM group prior to surgery. Mean±SEM.

OGTT=White bar; Iso-IVGC=Black bar
ISR, Insulin Secretion Rate; ISX, Insulin Secretion Index; BCGS, β-cell Glucose Sensitivity; DI, Disposition Index
ISX=ISR AUC/Glucose AUC (0-180’); BCGS=Slope of ISR vs. plasma glucose, calculated from Time 0 to the time of the peak glucose value.
LEAN=Lean; OB-NGT=Obese, normal glucose tolerant controls; OB-DM0=Obese group with diabetes prior to RYGBP surgery; OB-DM1M, OB-DM1Y, OB-DM2Y, OB-DM3Y=Obese, group with diabetes at 1 month, 1 year, 2 years and 3 years after RYGBP surgery
Values are Mean±SEM; *p<.05 vs. LEAN, †p<.05 vs. OB-NGT, ‡p<.05 vs. OB-DM0, §p<.05 vs. OB-DM1M
Figure 2. ISR during the OGTT and iso-IVGC and the incretin effect of ISR. (A-G) ISR AUC (pmol/kg/min) was significantly greater during the OGTT compared to the iso-IVGC under all groups and conditions (p<.05). The incretin effect of ISR was blunted in subjects with diabetes prior to surgery (D0), but normalized from 1 month onwards after surgery. Closed circle=OGTT; Open circle=iso-IVGC
LEAN/L=Lean; OB-NGT/OB=Obese normal glucose tolerant controls; OB-DM0/D0=Obese group with diabetes prior to RYGBP surgery; OB-DM1M/D1M, OB-DM1Y/D1Y, OB-DM2Y/D2Y, OB-DM3Y/D3Y=Obese group with diabetes at 1 month, 1 year, 2 years and 3 years after RYGBP surgery; ISR, Insulin secretion rate

Values are Mean±SEM; *p<.05 vs. LEAN, †p<.05 vs. OB-NGT, ‡p<.05 vs. OB-DM0
Figure 3. Effect of RYGBP on the Disposition Index during the OGTT and the Iso-IVGC.

(A) OGTT: O-DI, significantly impaired in OB-DM prior to surgery (p<.05 vs. both LEAN and OB-NGT groups), improved rapidly and significantly, as illustrated by a shift upwards and to the right. O-DI in the OB-DM group normalizes to levels of OB-NGT controls by 1 month and is not significantly different from the LEAN from 1 year onwards. (B) Iso-IVGC: IV-DI was significantly impaired in OB-DM prior to surgery (p<.05 vs. both LEAN and OB-NGT groups). Contrary to the O-DI, IV-DI improved minimally, albeit significantly (p<.05), with a shift to the right and minimal shift upward, after RYGBP. However, IV-DI remained significantly lower than the OB-NGT controls at 1 month and lower than the LEAN controls at all time points post-surgery (p<.05). Mean±SEM for all groups except LEAN. LEAN presented as each individual subject.

BCGS, β-cell Glucose Sensitivity; DI, Disposition Index
X=Individual points of LEAN group; Closed square=Obese normal glucose tolerant group; Closed circle=OB-DM 0 (Obese group with diabetes, pre-surgery); Open circle=OB-DM 1M (Obese group with diabetes, 1 month post-surgery); Closed diamond=OB-DM 1Y (Obese group with diabetes, 1 year post-surgery); Closed triangle=OB-DM 2Y (Obese group with diabetes, 2 years post-surgery); Open triangle=OB-DM 3Y (Obese group with diabetes, 3 years post-surgery).
**Supplementary Figure 1. Matching of Plasma Glucose Levels during the OGTT and Iso-IVGC.** (A-G) A significant time x test interaction was observed in the OB-DM 1M (p=.04) and OB-DM 2Y (p=0.01) groups, with a trend for higher glucose values during the iso-IVGC. No significant difference was observed in the LEAN, OB-NGT, OB-DM0, OB-DM 1Y, or OB-DM 3Y groups. None of the individual group contrasts were significant for any group/condition individually after correcting for the number of comparisons. (H) Overall, glucose values were well matched between the OGTT and iso-IVGC. However, a significant time x test (OGTT vs. Iso-IVGC) interaction (p=0.02) was observed, with a trend for slightly higher glucose values during the iso-IVGC from 45 minutes onwards. None of the individual group contrasts were significant at any time point overall, after correcting for the number of comparisons.

Closed circle=OGTT; Open circle=Iso-IVGC. LEAN=Lean; OB-NGT=Obese, normal glucose tolerant controls; OB-DM0=Obese group with diabetes prior to RYGBP surgery; OB-DM1M, OB-DM1Y, OB-DM2Y, OB-DM3Y=Obese group with diabetes at 1 month, 1 year, 2 years and 3 years after RYGBP surgery

Values are Mean±SEM.

**Supplementary Figure 2. Insulin Levels during the OGTT and Iso-IVGC and the Incretin Effect of Insulin.** (A-G) Insulin AUC (pmol/L/min) was significantly greater during the OGTT compared to the iso-IVGC under all groups and conditions (p<.05). (H) The incretin effect of insulin was blunted in subjects with diabetes prior to surgery (D0), but normalized from 1 month onwards after surgery.
Supplementary Figure 3. C-peptide levels during the OGTT and iso-IVGC and the Incretin Effect of C-peptide. (A-G) C-peptide AUC (pmol/L/min) was significantly greater during the OGTT compared to the iso-IVGC under all groups and conditions (p<.05). (H) The incretin effect of C-peptide was blunted in subjects with diabetes prior to surgery (D0), but normalized from 1 month onwards after surgery.

Values are Mean±SEM; *p<.05 vs. LEAN, †p<.05 vs. OB-NGT, ‡p<.05 vs. OB-DM0
Supplementary Figure 4. Effect of RYGBP on the Disposition Index using ISI for Insulin Sensitivity.

In the OB-DM group pre-surgery, DI (ISI) during the OGTT and iso-IVGC was significantly impaired compared to both control groups. After surgery, O-DI normalized to the levels of the OB-NGT group by 1 month and LEAN by 1 year, although only normalization to the OB-NGT persisted up to 3 years post-surgery. In contrast, IV-DI remained significantly lower compared to the OB-NGT controls at 1 month and 3 years post-surgery, and LEAN controls up to 3 years post-surgery. DI was significantly greater during the OGTT vs. the iso-IVGC in the OB-NGT group and OB-DM group at all conditions post-surgery (p<.05), but not in the LEAN or OB-DM group prior to surgery.

OGTT=White bar; Iso-IVGC=Black bar. DI, Disposition Index. LEAN=Lean; OB-NGT=Obese, normal glucose tolerant controls; OB-DM0=Obese group with diabetes prior to RYGBP surgery; OB-DM1M, OB-DM1Y, OB-DM2Y, OB-DM3Y=Obese, group with diabetes at 1 month, 1 year, 2 years and 3 years after RYGBP surgery

Values are Mean±SEM; *p<.05 vs. LEAN, †p<.05 vs. OB-NGT, ‡p<.05 vs. OB-DM0, §p<.05 vs. OB-DM1M, || vs. OB-DM2Y
Supplementary Figure 5. Effect of RYGBP on ISR and β-cell Function (Without Adjustment for Body Weight).

(A) ISR during the OGTT and iso-IVGC over the entire experiment (180 minutes) was significantly greater in the OB-DM0 group compared to lean controls. ISR during the OGTT and iso-IVGC were significantly reduced by 1 year and 1 month post-surgery, respectively, so that this was no longer greater the lean controls. In all groups and conditions, ISR AUC 0-180 min was significantly greater during the OGTT vs. the iso-IVGC (p<.05). (B) ISX was significantly impaired during the OGTT in the OB-DM group pre-surgery, and was unchanged after surgery. No increase in ISX was observed during the iso-IVGC. In all groups and conditions, ISX was significantly greater during the OGTT vs. the iso-IVGC (p<.05). (C) ISR during the first 60 minutes of the OGTT was significantly increased immediately after surgery and this increase was maintained up to 3 years post-surgery. No increase in ISR during the iso-IVGC was observed. In all groups and conditions, ISR AUC 0-60 min was significantly greater during the OGTT vs. the iso-IVGC (p<.05). (D) In the OB-DM group pre-surgery, BCGS during the OGTT and iso-IVGC was significantly lower than both control groups. After surgery, O-BCGS normalized to the levels of both control groups by 1 month, and this was maintained up to 3 years post-surgery. In contrast, IV-BCGS did not significantly change over time and remained significantly lower compared to both control groups up to 3 years post-surgery. BCGS was significantly greater during the OGTT vs. the iso-IVGC in the OB-NGT group and in the OB-DM group at all conditions post-surgery (p<.05), but not in the LEAN or OB-DM group prior to surgery. (E) In the OB-DM group pre-surgery, DI (HOMA-IR) during the OGTT and iso-IVGC was significantly impaired compared to both control groups. After surgery, O-DI normalized to the levels of the OB-NGT group by 1 month and LEAN by 1 year. In contrast, IV-DI remained significantly lower compared to the LEAN controls up to 3 years post-
surgery. **DI** was significantly greater during the OGTT vs. the iso-IVGC in the OB-NGT group and in the OB-DM group at all conditions post-surgery (p<.05), but not in the LEAN or OB-DM group prior to surgery. (F) In the OB-DM group pre-surgery, **DI (ISI)** during the OGTT and iso-IVGC was significantly impaired compared to both control groups. After surgery **O-DI** normalized to the levels of the OB-NGT group by 1 month and LEAN by 1 year. In contrast, **IV-DI** remained significantly lower compared to the LEAN controls up to 3 years post-surgery. **DI** was significantly greater during the OGTT vs. the iso-IVGC in the OB-DM group at all conditions post-surgery (p<.05), but not in the LEAN, OB-NGT or OB-DM group prior to surgery.

OGTT=White bar; Iso-IVGC=Black bar. **ISR**, Insulin Secretion Rate; **ISX**, Insulin Secretion Index; **BCGS**, β-cell Glucose Sensitivity; **DI**, Disposition Index. **LEAN**=Lean; **OB-NGT**=Obese, normal glucose tolerant controls; **OB-DM0**=Obese group with diabetes prior to RYGBP surgery; **OB-DM1M**, **OB-DM1Y**, **OB-DM2Y**, **OB-DM3Y**=Obese, group with diabetes at 1 month, 1 year, 2 years and 3 years after RYGBP surgery

Values are Mean±SEM; *p<.05 vs. LEAN, †p<.05 vs. OB-NGT, ‡p<.05 vs. OB-DM0, §p<.05 vs. OB-DM1M
## Supplementary Table 1: Additional clinical characteristics, amount of glucose infused, and incretin levels.

|                          | LEAN          | OB-NGT        | OB-DM 0       | OB-DM 1M      | OB-DM 1Y      | OB-DM 2Y      | OB-DM 3Y      |
|--------------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| Glucose AUC (mmol/L/min) | 5.75±0.88     | 6.21±0.49     | 11.26±3.04†   | 7.83±1.53†‡   | 6.61±1.39‡§   | 6.46±1.33‡§   | 7.04±1.49‡    |
| Glucose peak (mmol/L)    | 8.08±2.00     | 7.81±1.18     | 13.84±3.33*†  | 11.21±2.01*‡   | 10.51±2.24*‡   | 11.08±2.14*‡   | 11.89±2.18*†  |
| Insulin AUC (pmol/L/min) | 186.0±101.0   | 460.3±232.5*  | 394.2±172.7   | 345.5±145.0   | 309.2±173.0   | 289.9±110.0†  | 348.2±173.8*  |
| Glucose infused-IVGC (g) | 18.6±9.4      | 23.1±9.1      | 43.5±13.3*†   | 35.2±18.9*†   | 30.1±13.2‡    | 31.4±10.3‡*   | 37.7±15.8*†   |
| Incretin effect-insulin (pmol/L/min) | 93.4±51.4 | 163.8±123.0   | 79.3±86.0     | 174.8±106.5†  | 172.3±138.3†  | 180.3±115.8†  | 189.5±138.3†  |
| GLP-1AUC (pmol/L/min)    | 6.65±2.59     | 5.24±1.48     | 8.34±7.83     | 26.45±9.45*‡   | 47.41±38.69*‡   | 33.89±36.16*‡   | 27.96±13.23*†   |
| Fasting GLP-1 (pmol/L)   | 5.65±2.61     | 5.86±3.53     | 8.33±8.63     | 6.94±4.31     | 16.21±19.12   | 13.32±23.91   | 10.62±8.33    |
| Peak GLP-1(pmol/L)       | 14.89±6.18    | 8.96±3.29     | 14.67±9.22    | 77.89±48.34*‡  | 149.00±95.10*†‡   | 103.58±96.18*†‡  | 75.99±33.70*†‖   |
| GIP AUC (pmol/L/min)     | 18.57±4.03    | 23.05±10.35   | 20.24±7.27    | 23.39±7.95    | 21.93±7.71    | 24.01±10.57   | 24.91±8.49    |
| Fasting GIP (pmol/L)     | 10.48±13.01   | 7.17±4.20     | 7.25±4.83     | 7.24±3.07     | 7.42±2.88     | 8.84±2.52     | 9.87±4.92     |
| Peak GIP (pmol/L)        | 29.94±8.94    | 34.75±14.95   | 36.93±13.76   | 50.76±16.02*‡   | 51.52±23.54*‡   | 55.38±22.63*‡   | 57.43±23.94*‖   |

Mean ±SD. *p<.05 vs. LEAN, †p<.05 vs. OB-NGT, ‡p<.05 vs. OB-DM 0, §p<.05 vs. OB-DM 1M, ‖p<.05 vs. OB-DM 1Y; All glucose and incretin measurements are from the OGTT. **AUC**, Area under the curve; **GLP-1**, glucagon-like peptide-1; **GIP**, Gastric inhibitory peptide

LEAN=Lean; OB-NGT=Obese, normal glucose tolerant controls; OB-DM0=Obese group with diabetes prior to RYGBP surgery; OB-DM1M, OB-DM1Y, OB-DM2Y, OB-DM3Y=Obese group with diabetes at 1 month, 1 year, 2 years and 3 years after RYGBP surgery.
Supplementary Table 2: Predictors of post-surgery β-cell function and glucose control

All mixed model regression (MMR) analyses included time relative to surgery (TRS), to control for it; predictor analyses are presented after controlling for TRS. Predictors found to be significant based on univariate results were entered into a multivariate model. In the multivariate analysis for post-surgery postprandial glucose, only weight loss and pre-surgery O-DI, along with TRS, were tested due to multicollinearity. R² values could not be calculated for non-significant predictors.

In the multivariate model, R² values for post-surgery O-BCGS, O-DI, fasting glucose and postprandial glucose were 0.159, 0.350, 0.282, 0.246, respectively.

O-BCGS, β-cell glucose sensitivity from the oral glucose tolerance test; O-DI, Disposition index from the oral glucose tolerance test; AUC, Area under the curve

| Outcome variable                        | Predictors                      | Univariate |                           | Multivariate |                           |
|----------------------------------------|---------------------------------|------------|---------------------------|--------------|---------------------------|
|                                        |                                 | R²         | p-value                   | R²           | p-value                   |
|                                        |                                 | a          | a                         | a            | a                         |
| Post-surgery O-BCGS                    | TRS                             | 0.041      | 0.100                     | N/A b        | 0.512                     |
|                                        | Pre-surgery O-BCGS              | 0.041      | 0.030                     | 0.024        | 0.056                     |
|                                        | GLP-1 response (AUC)            | 0.021      | 0.030                     | 0.016        | 0.030                     |
|                                        | Weight loss                     | 0.092      | 0.002                     | 0.062        | 0.008                     |
|                                        | Pre-surgery HbA1c               | 0.008      | 0.030                     | N/A b        | 0.200                     |
| Post-surgery O-DI                      | TRS                             | 0.173      | 0.004                     | N/A b        | 0.862                     |
|                                        | Pre-surgery O-DI                | 0.090      | 0.020                     | 0.066        | 0.001                     |
|                                        | GLP-1 response (AUC)            | 0.017      | 0.030                     | 0.011        | 0.042                     |
|                                        | Weight loss                     | 0.088      | 0.002                     | 0.051        | 0.010                     |
| Post-surgery fasting glucose          | TRS                             | 0.174      | 0.010                     | N/A b        | 0.711                     |
|                                        | Post-surgery O-BCGS             | 0.060      | 0.004                     | 0.055        | 0.019                     |
|                                        | Weight loss                     | 0.089      | 0.002                     | 0.020        | 0.048                     |
| Post-surgery postprandial 120' glucose| TRS                             | 0.061      | 0.004                     | N/A b        | 0.191                     |
|                                        | Weight loss                     | 0.093      | 0.004                     | 0.078        | 0.001                     |
|                                        | Pre-surgery O-BCGS              | 0.023      | 0.032                     | N/T c        | N/T c                     |
|                                        | Post-surgery O-BCGS             | 0.134      | <0.001                    | N/T c        | N/T c                     |
|                                        | Pre-surgery O-DI                | 0.106      | <0.001                    | 0.091        | <0.001                    |
|                                        | Post-surgery O-DI               | 0.114      | <0.001                    | N/T c        | N/T c                     |
\textsuperscript{a} $R^2$ and p-values for all predictors aside from time relative to surgery (TRS) were calculated after controlling for TRS.
\textsuperscript{b} N/A; Not appropriate.
\textsuperscript{c} N/T; Variables not put into model.
Supplementary Figure 1. Matching of plasma glucose levels during the OGTT and Iso-IVGC. (A-G) A significant time x test interaction was observed in the OB-DM 1M (p=.04) and OB-DM 2Y (p=0.01) groups, with a trend for higher glucose values during the iso-IVGC. No significant difference was observed in the LEAN, OB-NGT, OB-DM0, OB-DM 1Y, or OB-DM 3Y groups. None of the individual group contrasts were significant for any group/condition individually after correcting for the number of comparisons. (H) Overall, glucose values were well matched between the OGTT and iso-IVGC. However, a significant time x test (OGTT vs. Iso-IVGC) interaction (p=0.02) was observed, with a trend for slightly higher glucose values during the iso-IVGC from 45 minutes onwards. None of the individual group contrasts were significant at any time point overall, after correcting for the number of comparisons. Closed circle=OGTT; Open circle=iso-IVGC

LEAN=Lean; OB-NGT=Obese, normal glucose tolerant controls; OB-DM0=Obese group with diabetes prior to RYGBP surgery; OB-DM1M, OB-DM1Y, OB-DM2Y, OB-DM3Y=Obese group with diabetes at 1 month, 1 year, 2 years and 3 years after RYGBP surgery
Values are Mean±SEM

247x338mm (300 x 300 DPI)
Supplementary Figure 2. Insulin levels during the OGTT and Iso-IVGC and the incretin effect of insulin. Insulin AUC (pmol/L/min) was significantly greater during the OGTT compared to the iso-IVGC under all groups and conditions (p<.05). The incretin effect of insulin was blunted in subjects with diabetes prior to surgery (D0), but normalized from 1 month onwards after surgery. Closed circle=OGTT; Open circle=Iso-IVGC.

LEAN/L=Lean; OB-NGT/OB=Obese normal glucose tolerant controls; OB-DM0/D0=Obese group with diabetes prior to RYGBP surgery; OB-DM1M/D1M, OB-DM1Y/D1Y, OB-DM2Y/D2Y, OB-DM3Y/D3Y=Obese group with diabetes at 1 month, 1 year, 2 years and 3 years after RYGBP surgery.

Values are Mean±SEM; *p<.05 vs. LEAN, †p<.05 vs. OB-NGT, ‡p<.05 vs. OB-DM0.

234x304mm (300 x 300 DPI)
Supplementary Figure 3. C-peptide levels during the OGTT and iso-IVGC and the incretin effect of C-peptide. (A-G) C-peptide AUC (pmol/L/min) was significantly greater during the OGTT compared to the iso-IVGC under all groups and conditions (p<.05). (H) The incretin effect of C-peptide was blunted in subjects with diabetes prior to surgery (D0), but normalized from 1 month onwards after surgery. Closed circle=OGTT; Open circle=iso-IVGC
LEAN/L=Lean; OB-NGT/OB=Obese normal glucose tolerant controls; OB-DM0/D0=Obese group with diabetes prior to RYGBP surgery; OB-DM1M/D1M, OB-DM1Y/D1Y, OB-DM2Y/D2Y, OB-DM3Y/D3Y=Obese group with diabetes at 1 month, 1 year, 2 years and 3 years after RYGBP surgery
Values are Mean±SEM; *p<.05 vs. LEAN, †p<.05 vs. OB-NGT, ‡p<.05 vs. OB-DM0

234x306mm (300 x 300 DPI)
Supplementary Figure 4. Effect of RYGBP on the Disposition Index using ISI for insulin sensitivity.

In the OB-DM group pre-surgery, DI (ISI) during the OGTT and iso-IVGC was significantly impaired compared to both control groups. After surgery, O-DI normalized to the levels of the OB-NGT group by 1 month and LEAN by 1 year, although only normalization to the OB-NGT persisted up to 3 years post-surgery. In contrast, IV-DI remained significantly lower compared to the OB-NGT controls at 1 month and 3 years post-surgery, and LEAN controls up to 3 years post-surgery. DI was significantly greater during the OGTT vs. the iso-IVGC in the OB-NGT group and OB-DM group at all conditions post-surgery (p<.05), but not in the LEAN or OB-DM group prior to surgery.

OGTT=White bar; Iso-IVGC=Black bar
DI, Disposition Index
LEAN=Lean; OB-NGT=Obese, normal glucose tolerant controls; OB-DM0=Obese group with diabetes prior to RYGBP surgery; OB-DM1M, OB-DM1Y, OB-DM2Y, OB-DM3Y=Obese, group with diabetes at 1 month, 1 year, 2 years and 3 years after RYGBP surgery
Values are Mean±SEM; *p<.05 vs. LEAN, †p<.05 vs. OB-NGT, ‡p<.05 vs. OB-DM0, §p<.05 vs. OB-DM1M, || vs. OB-DM2Y

78x34mm (600 x 600 DPI)
Supplementary Figure 5. Effect of RYGBP on ISR and β-cell function (not adjusted for body weight).

(A) ISR during the OGTT and iso-IVGC over the entire experiment (180 minutes) was significantly greater in the OB1DM0 group compared to lean controls. ISR during the OGTT and iso-IVGC were significantly reduced by 1 year and 1 month, respectively, so that it was no longer greater the lean controls. In all groups and conditions, ISR AUC 0-180 min was significantly greater during the OGTT vs the iso-IVGC (p<.05). (B) ISX was significantly impaired during the OGTT in the OB-DM group pre-surgery, and was unchanged after surgery. No increase in ISX was observed during the iso-IVGC. In all groups and conditions, ISX was significantly greater during the OGTT vs. the iso-IVGC (p<.05). (C) ISR during the first 60 minutes of the OGTT was significantly increased immediately after surgery and this increase was maintained up to 3 years post-surgery. No increase in ISR during the iso-IVGC was observed. In all groups and conditions, ISR AUC 0-60 min was significantly greater during the OGTT vs. the iso-IVGC (p<.05). (D) In the OB-DM group pre-surgery, BCGS during the OGTT and iso-IVGC was significantly lower than both control groups. After surgery, O-BCGS normalized to the levels of both control groups by 1 month, and maintained up to 3 years post-surgery. In contrast, IV-BCGS did not significantly change over time and remained significantly lower compared to both control groups up to 3 years. BCGS was significantly greater during the OGTT vs. the iso-IVGC in the OB-NGT group and in the OB-DM group at all conditions post-surgery (p<.05), but not in the LEAN or OB-DM group prior to surgery. (E) In the OB-DM group pre-surgery, DI (HOMA-IR) during the OGTT and iso-IVGC was significantly impaired compared to both control groups. After surgery, O-DI normalized to the levels of the OB-NGT group by 1 month and LEAN by 1 year. In contrast, IV-DI remained significantly lower compared to the LEAN controls up to 3 years post-surgery. DI was significantly greater during the OGTT vs. the iso-IVGC in the OB-NGT group and in the OB-DM group at all conditions post-surgery (p<.05), but not in the LEAN or OB-DM group prior to surgery. (F) In the OB-DM group pre-surgery, DI (ISI) during the OGTT and iso-IVGC was significantly impaired compared to both control groups. After surgery, O-DI normalized to the levels of the OB-NGT group by 1 month and LEAN by 1 year. In contrast, IV-DI remained significantly lower compared to the LEAN controls up to 3 years post-surgery. DI was significantly greater during the OGTT vs. the iso-IVGC in the OB-DM group at all conditions post-surgery (p<.05), but not in the LEAN, OB-NGT or OB-DM group prior to surgery.

OGTT=White bar; Iso-IVGC=Black bar

ISR, Insulin Secretion Rate; ISX, Insulin Secretion Index; BCGS, β-cell Glucose Sensitivity; DI, Disposition Index

ISX=ISR AUC/Glucose AUC (0-180'); BCGS=Slope of ISR vs. plasma glucose, calculated from Time 0 to the time of the peak glucose value

80x51mm (600 x 600 DPI)
