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Limited Recovery of β-Cell Function After Gastric Bypass Despite Clinical Diabetes Remission

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; *2*) 11 severely obese normal glucose-tolerant control subjects; and *3*) 7 lean control subjects. 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 with lean control subjects up to 3 years post-RYGBP. Presurgery, β-cell function, weight loss, and glucagon-like peptide 1 response were all predictors of postsurgery 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.

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Roux-en-Y gastric bypass (RYGBP) remits type 2 diabetes in ~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. Bolus delivery of oral glucose elicits significantly lower plasma glucose excursions compared with 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

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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 glucagon-like peptide 1 (GLP-1) (4), which has been shown to improve glucose tolerance, insulin secretion, and β-cell glucose sensitivity (BCGS) (5–8). In fact, GLP-1 agonists are used for diabetes management.

β-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). β-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 β-cell function after RYGBP has not been directly tested. To investigate this, we examined change in β-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 nonoperated, 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.

RESEARCH DESIGN AND 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, n = 14 for iso-IVGC), and 3 years after RYGBP (OB-DM3Y; n = 13). Diabetes and diabetes remission were defined using American Diabetes Association (ADA) criteria (18). Exclusion criteria included insulin therapy, diabetes duration ≥10 years, current treatment with thiazolidinediones, GLP-1 agonists, or dipeptidyl peptidase-4 inhibitors, and type 1 diabetes. Medications used presurgery, including metformin and/or sulfonylurea (9 of 16 subjects), were withheld 2 to 3 days prior to study visits. Eleven severely OB-NGT and 7 LEAN subjects were used as control subjects (all OB-NGT control subjects: fasting plasma glucose <5.5 mmol/L, 2-h postprandial glucose <7.7 mmol/L, and HbA_{1c} <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

OGTT

After a 12-h overnight fast, subjects underwent a 180-min 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 dipeptidyl peptidase-4 inhibitor (50 μmol/L or 10 μL/mL blood; EMD Millipore, Darmstadt, Germany). Samples were centrifuged at 4°C and stored at −80°C.

Iso-IVGC

Glucose (20% dextrose solution) was infused using a Gemini pump (CareFusion, San Diego, CA) over 180 min 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 min, and glucose infusion rate was adjusted accordingly.

Assays

Plasma glucose was determined at bedside by the glucose oxidase method with an Analox glucose analyzer (Analox Instruments, Lunenburg, MA). Total GLP-1 was measured by radioimmunoassay (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) and 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 radioimmunoassay (Millipore). All hormone and metabolite assays were performed at the Hormonal Core Laboratory at the Obesity Nutrition Research Center. Intra- and interassay 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 (Ortho Clinical Diagnostics, Rochester, NY).

Calculations

Area under the curve (AUC) was calculated using the trapezoidal method for 180 min unless otherwise indicated. Homeostasis model assessment of insulin resistance (HOMA-IR) calculated as: $(\text{fasting-insulin}_{\mu U/mL} \times \text{fasting-glucose}_{mg/dL})/405$. Incretin effect of insulin, C-peptide, and insulin secretion rate (ISR; "X") calculated as difference in β-cell response, or action of the incretin factor, expressed as the percentage of response to oral glucose:

([X_{AUC}OGTT − X_{AUC}iso-IVGC]/[X_{AUC}OGTT]) × 100. Insulin sensitivity index (ISI) calculated as: 10,000/([fasting glucose × fasting insulin × mean $glucose_{0–180\ min}$ × mean $insulin_{0–180\ min}$]$^{0.5}$). ISR calculated by mathematical deconvolution using a two-compartment model for hormone clearance using C-peptide from the OGTT (i.e., O-ISR) and iso-IVGC (i.e., 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), BCGS, and DI. ISX calculated as: ISR AUC/glucose AUC from 0–180 min, 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 × (1/HOMA-IR). DI was alternatively calculated as BCGS × ISI (21,22).

Nomenclature

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

Statistical Analysis

Data are expressed as mean ± SD except in figures, in which mean ± SEM is reported. The study sample consisting of 15 to 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 eight subjects to achieve 80% power ($\alpha = 0.05$) 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 nonparametric tests were used if variables were still not normally distributed. ANOVA with a Bonferroni post hoc test was used to analyze data across all groups presurgery, and Dunnett post hoc test used at all time points postsurgery to compare LEAN and OB-NGT to OB-DM postsurgery. Independent *t* tests were used to compare OB-NGT versus OB-DM, if no LEAN comparison was possible. Paired *t* tests were used to compare data (ISR, ISX, BCGS, and DI) for OGTT versus iso-IVGC. Repeated-measures ANOVA was used to compare plasma glucose matching between the OGTT and iso-IVGC. Mixed-model regression used to compare changes over time in OB-DM and with additional covariates to evaluate predictors of: *1*) β-cell function measured during oral glucose stimulation, and *2*) fasting and postprandial oral glucose after surgery. R^2 values were estimated for predictors in mixed-model regression analyses based on improvements in log likelihoods between baseline and more complex models (e.g., model containing one or more predictors compared with model with slope only) (23). Statistical significance was set at $P < 0.05$ (two-tailed). All analyses were completed using SPSS 19 (IBM, Armonk, NY).

RESULTS

Presurgery Characteristics

Clinical characteristics are provided in Table 1. Fasting and 120-min glucose were significantly higher (Table 1), and β-cell function was significantly lower (Fig. 1), in OB-DM prior to surgery compared with OB-NGT and LEAN. No difference in fasting or postoral incretin concentrations was observed between groups (Supplementary Table 1). Triglycerides were significantly higher in OB-DM versus OB-NGT, and no difference in total, LDL, or 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 with presurgery (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 approximately fivefold at 1 month compared with presurgery, further increased to ~10-fold at 1 year, and remained approximately five- to sevenfold higher at 2 to 3 years. Compared with presurgery 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 control subjects (Supplementary Table 1).

Effects of RYGBP Surgery on the Incretin Effect

Overall, glucose values were well matched between the OGTT and iso-IVGC. However, a significant time × 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 min onwards. Individually, a significant time × test interaction was observed in only the OB-DM1M ($P = 0.04$) and OB-DM2Y ($P = 0.01$) groups; no significant differences were observed in any other

Table 1—Clinical characteristics

	LEAN	OB-NGT	OB-DM0	OB-DM1M	OB-DM1Y	OB-DM2Y	OB-DM3Y
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§
HbA_{1c} (%)		5.4 ± 0.5	7.1 ± 1.0†		5.4 ± 0.4‡	5.5 ± 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-min 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)		193.1 ± 44.3	200.2 ± 41.5		161.5 ± 25.3†‡	146.9 ± 49.8†‡	149.6 ± 32.3†‡
LDL cholesterol (mg/dL)		115.4 ± 41.5	105.5 ± 34.6		83.5 ± 24.7†	83.5 ± 32.0†	73.8 ± 27.1†
HDL cholesterol (mg/dL)		53.7 ± 19.2	48.6 ± 12.3		53.6 ± 16.7	53.5 ± 13.9	54.7 ± 14.6
Triglycerides (mg/dL)		120.7 ± 48.7	234.7 ± 203.3†		129.6 ± 119.5‡	91.7 ± 58.6‡	106.5 ± 93.2‡

Data are mean ± SD. *$P < 0.05$ vs. LEAN; †$P < 0.05$ vs. OB-NGT; ‡$P < 0.05$ vs. OB-DM0; §$P < 0.05$ vs. OB-DM1M.

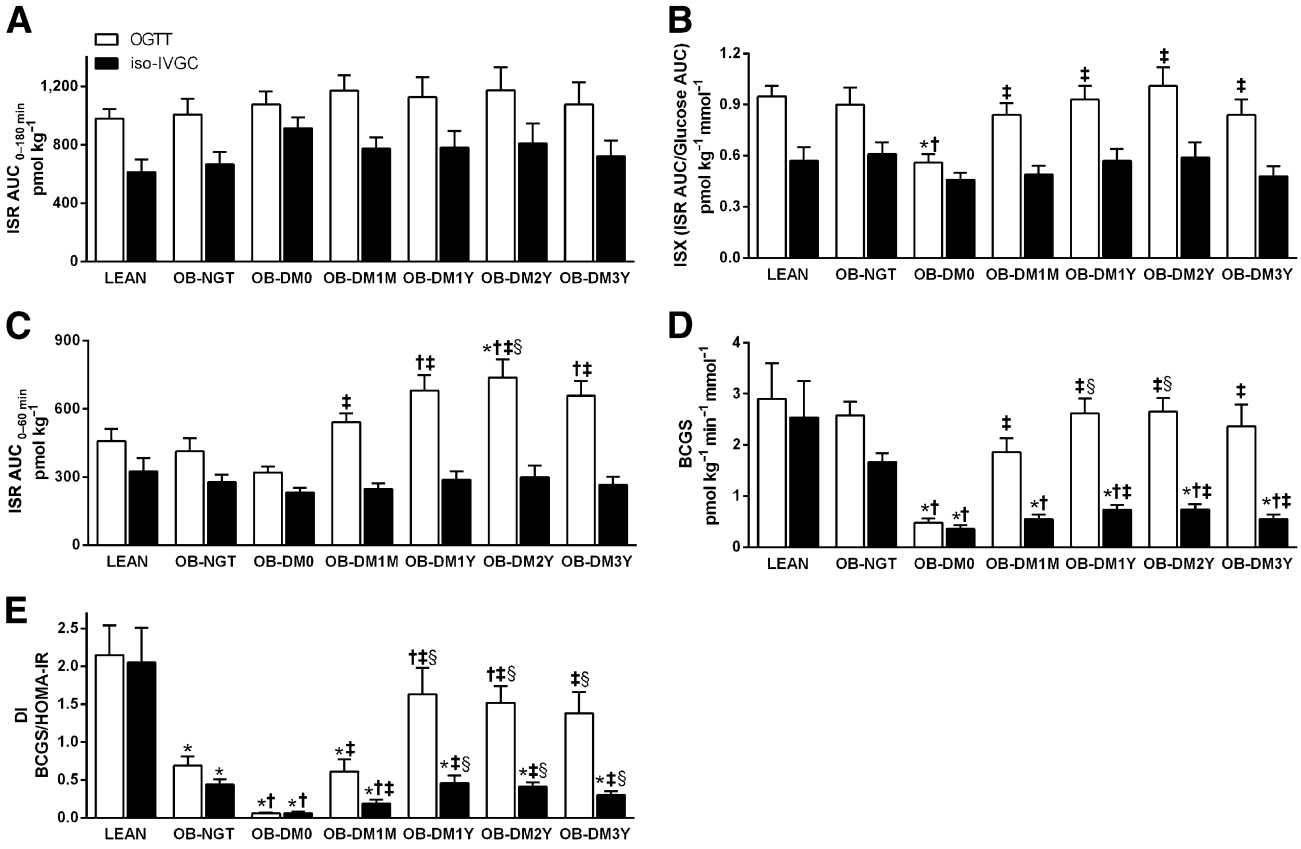

Figure 1—Effect of RYGBP on ISR and β-cell function. *A*: ISR during the OGTT or iso-IVGC over the entire experiment (180 min) was not different between groups. In all groups and conditions, ISR $AUC_{0–180\ min}$ was significantly greater during the OGTT vs. iso-IVGC ($P < 0.05$). *B*: ISX was significantly impaired during the OGTT in the OB-DM group presurgery and significantly increased immediately after surgery; this increase was maintained up to 3 years postsurgery. However, no increase in ISX was observed during the iso-IVGC. In all groups and conditions, ISX was significantly greater during the OGTT vs. iso-IVGC ($P < 0.05$). *C*: ISR during the first 60 min of the OGTT was significantly increased immediately after surgery and this increase was maintained up to 3 years postsurgery. 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. iso-IVGC ($P < 0.05$). *D*: In the OB-DM group presurgery, 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 to 2 years. In contrast, IV-BCGS remained significantly lower compared with both control groups up to 3 years. BCGS was significantly greater during the OGTT vs. iso-IVGC in the OB-NGT group and OB-DM group at all conditions postsurgery ($P < 0.05$), but not in the LEAN or OB-DM group prior to surgery. *E*: In the OB-DM group presurgery, DI during the OGTT and iso-IVGC was significantly impaired compared with 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 with the LEAN control subjects up to 3 years postsurgery. DI was significantly greater during the OGTT vs. iso-IVGC in the OB-NGT group and in the OB-DM group at all conditions postsurgery ($P < 0.05$), but not in the LEAN or OB-DM group prior to surgery. Data are mean ± SEM. *$P < 0.05$ vs. LEAN; †$P < 0.05$ vs. OB-NGT; ‡$P < 0.05$ vs. OB-DM0; §$P < 0.05$ vs. OB-DM1M.

groups or 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 Fig. 1).

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

Effects of RYGBP Surgery on the β-Cell Response to Oral and IV Glucose

ISR and β-cell function, assessed during oral glucose stimulation, normalized after RYGBP. O-ISR during the first 60 minutes nearly doubled 1 month after RYGBP, and this improvement was maintained up to 3 years postsurgery; in fact, this variable was significantly greater compared with OB-NGT from 1 year onwards after surgery (Fig. 1*C*). Measures of β-cell function including O-ISX, O-BCGS, and O-DI, severely impaired in OB-DM presurgery, normalized to levels of control subjects postsurgery (Fig. 1*B*, *D*, and *E*). O-ISX and O-BCGS both normalized to levels of both control groups from 1 month onwards (Fig. 1*B* and *D*), and O-DI normalized to OB-NGT levels from 1 month onwards and to LEAN from 1 year onwards (Fig. 1*E*).

Contrary to what was observed during oral glucose stimulation, insulin secretion and β-cell function, measured during the iso-IVGC, improved minimally and never

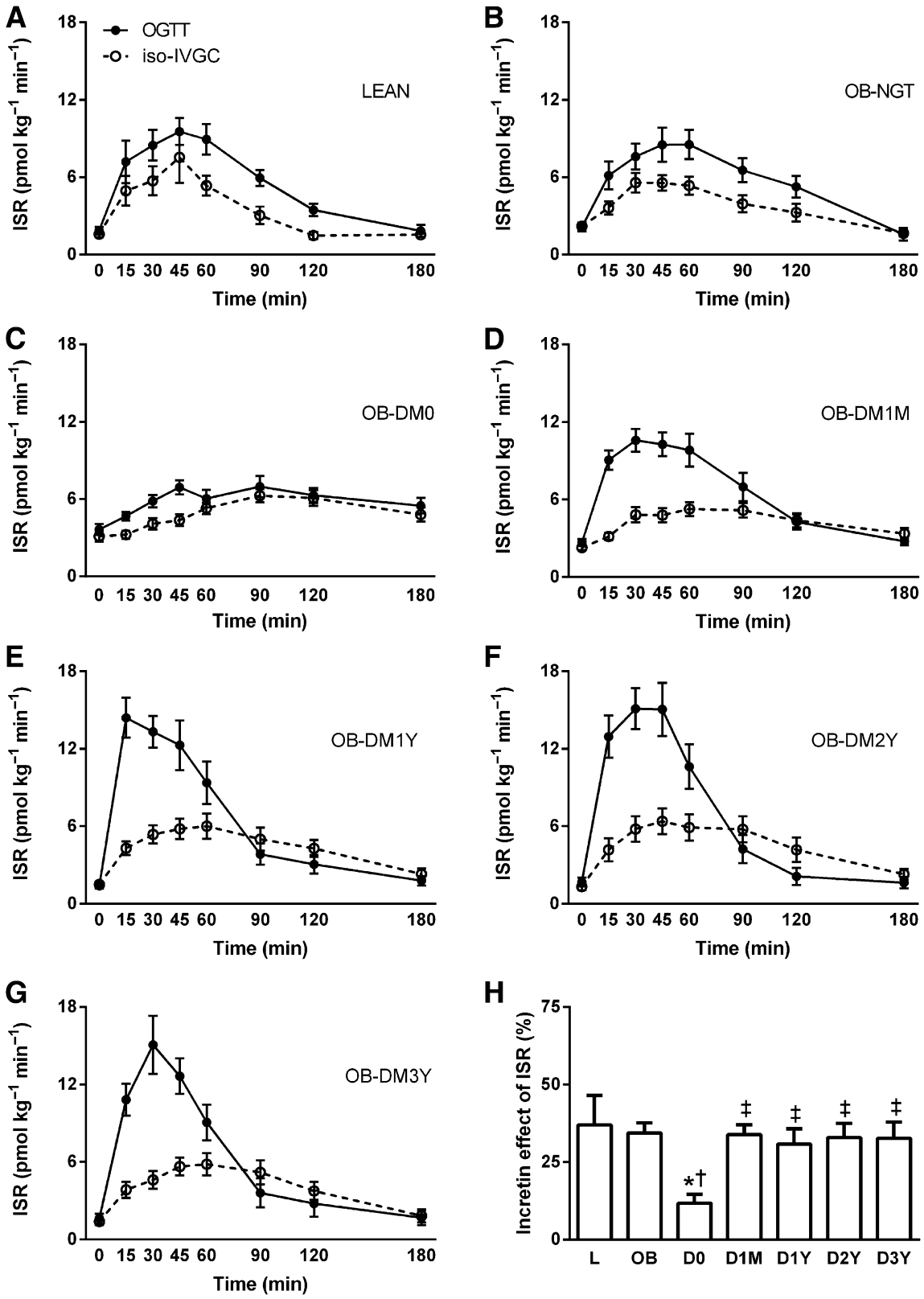

Figure 2—ISR during the OGTT and iso-IVGC and the incretin effect of ISR. *A–G*: ISR AUC (pmol/kg/min) was significantly greater during the OGTT compared with iso-IVGC under all groups and conditions ($P < 0.05$). *H*: The incretin effect of ISR was blunted in subjects with diabetes prior to surgery (D0), but normalized from 1 month onwards after surgery. Data are mean ± SEM. *$P < 0.05$ vs. LEAN; †$P < 0.05$ vs. OB-NGT; ‡$P < 0.05$ vs. OB-DM0. D0, obese group with diabetes prior to RYGBP surgery; D1M, D1Y, D2Y, D3Y, obese group with diabetes at 1 month, 1 year, 2 years, and 3 years after RYGBP surgery, respectively; L, lean; OB, obese NGT control subjects.

normalized to levels of LEAN, even up to 3 years after RYGBP (Fig. 1), 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 Fig. 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 (Fig. 3*A*). In contrast, during the iso-IVGC, a much smaller, albeit significant, improvement in IV-DI was observed; however, this remained significantly impaired compared with LEAN control subjects up to 3 years after RYGBP (Fig. 3*B*).

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, presurgery β-cell function, and GLP-1 response were all significant predictors of postsurgery β-cell function, although weight loss was consistently the strongest predictor for O-BCGS, and both weight loss and presurgery O-DI were strong predictors of postsurgery 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, presurgery BMI, and diabetes duration and control were not significant.

Postsurgery, 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). Presurgery β-cell function and BMI, age, and diabetes duration were not significant.

Weight loss along with pre- and postsurgery β-cell function were all predictors of 120-min glucose (Supplementary Table 2) based on univariate modeling. Weight loss and presurgery β-cell function (O-DI) both remained significant in a multivariate model, although presurgery O-DI was slightly better as a predictor of postprandial 120-min glucose. Age, diabetes duration, presurgery BMI, the incretin effect of insulin, and 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 postsurgical β-cell function, along with presurgery 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 in this study and in others (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 with LEAN and OB-NGT control subjects 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 (i.e., DI), similar improvements were observed. DI, impaired in diabetes, shown in this study and in others (13,24,25), increases after RYGBP regardless of diabetes status. We showed that O-DI normalized to the levels of both control groups, LEAN and OB-NGT, by 1 year. This is similar to Jørgensen et al. (13), who showed that by 1 year postsurgery, DI in subjects with diabetes approached levels of unoperated obese NGT populations.

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 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. (12), who observed greater BCGS during an OGTT versus an iso-IVGC in NGT individuals, but not individuals with diabetes. Furthermore, the rapid normalization of the incretin effect 1 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 postsurgery β-cell function in our study, may be one of the factors that explains 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

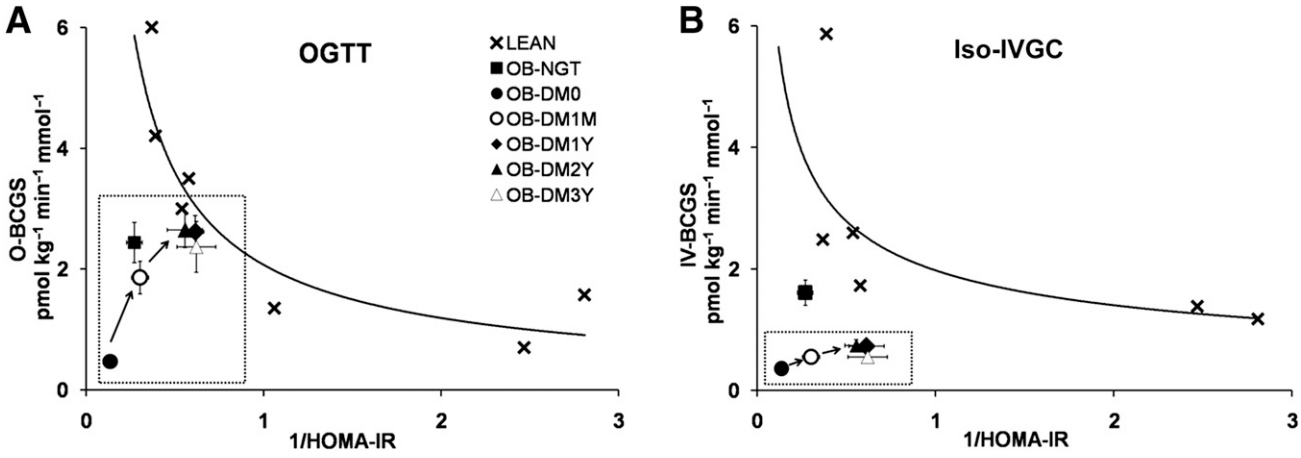

Figure 3—Effect of RYGBP on the DI during the OGTT and iso-IVGC. *A*: OGTT. O-DI, significantly impaired in OB-DM prior to surgery ($P <$ 0.05 vs. both LEAN and OB-NGT groups), improved rapidly and significantly, as illustrated by a shift upwards and to the right. O-DI in the OB-DM group normalized to levels of OB-NGT control subjects by 1 month and was not significantly different from the LEAN from 1 year onwards ($P < 0.05$). *B*: Iso-IVGC. IV-DI was significantly impaired in OB-DM prior to surgery ($P < 0.05$ vs. both LEAN and OB-NGT groups). Contrary to the O-DI, IV-DI improved minimally, albeit significantly ($P < 0.05$), with a shift to the right and minimal shift upward, after RYGBP. However, IV-DI remained significantly lower than the OB-NGT control subjects at 1 month and lower than the LEAN control subjects at all time points postsurgery ($P < 0.05$). Mean ± SEM for all groups except LEAN. LEAN presented as each individual subject.

amplified compared with 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 presurgical β-cell function predicted postsurgical β-cell function, weight loss appears to be a superior predictor. Weight loss, along with postsurgical β-cell function (O-BCGS) predicted fasting glucose, and weight loss, along with presurgical β-cell function (O-DI), predicted 120-min 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. (24) showed that in a nondiabetic population, equivalent 20% weight loss after RYGBP and gastric banding similarly improved DI during a meal test coupled with a clamp. 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 with 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 after oral and IV glucose, in a cohort of patients with diabetes followed for 3 years postsurgery, 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. Second, the amount of glucose administered during an iso-IVGC is ~50% of that administered during a 50-g OGTT (54% reported [28] and 46% in OB-NGT) in an NGT individual; however, the amount delivered IV is similar (88% in our OB-DM group) in individuals with diabetes (28). Third, 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 postsurgery was higher than both the LEAN and OB-NGT groups, suggesting that the lackluster improvement in IV

β-cell function postsurgery is not an artifact of the lower amount of glucose infused. To correct for this we presented ISR versus glucose concentrations (ISX; Fig. 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 postsurgery 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 HbA_{1c} 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. OGTT-derived DI (from a 75-g OGTT) has been shown to correlate with DI derived from frequently sampled IV glucose tolerance tests (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 (i.e., 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 postsurgery, 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 Fig. 4) revealed a more pronounced deterioration in DI at 2 to 3 years postsurgery. Furthermore, it should be acknowledged that the rapid GE and resulting leftward shift in glucose and insulin curves (Supplementary Figures 1 and 2) post-RYGBP may complicate the comparison with 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 presurgery β-cell function are all important contributors to postsurgical 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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