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Proinsulin associates with poor β -cell function, glucose-dependent insulintropic peptide, and insulin resistance in persistent type 2 diabetes after Roux-en-Y gastric bypass in humans[†]

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Abstract

Background: The determinants of type 2 diabetes (T2D) remission and/or relapse after gastric bypass (RYGB) remain fully unknown. This study characterized β - and α -cell function, incretin hormone release and insulin sensitivity in individuals with (remitters) or without (non-remitters) diabetes remission after RYGB.

Methods: This is a cross-sectional study of two distinct cohorts of individuals with or without diabetes remission at least 2 years after RYGB. Each individual underwent either an oral glucose (remitters) or a mixed meal (non-remitters) test; glucose, proinsulin, insulin, C-peptide, glucagon, incretins and leptin were measured.

Results: Compared to remitters ($n = 23$), non-remitters ($n = 31$) were older (mean $\pm$ SD] age 56.1 ± 8.2 vs. 46.0 ± 8.9 years, $P < 0.001$), had longer diabetes duration (13.1 ± 10.1 vs. 2.2 ± 2.4 years, $P < 0.001$), were further out from the surgery (5.6 ± 3.3 vs. 3.5 ± 1.7 years, $P < 0.01$), were more insulin resistant (HOMA-IR 4.01 ± 3.65 vs. 2.08 ± 1.22 , $P < 0.001$), but did not differ for body weight. As predicted, remitters had higher β -cell glucose sensitivity (1.95 ± 1.23 vs. 0.86 ± 0.55 pmol/kg/min/mmol, $P < 0.001$) and disposition index (1.55 ± 1.75 vs. 0.33 ± 0.27 , $P = 0.003$), compared to non-remitters, who showed non-suppressibility of glucagon during the oral challenge (time $\times$ group $P = 0.001$). Higher proinsulin (16.55 ± 10.45 vs. 6.62 ± 3.50 PM, $P < 0.0001$), and proinsulin: C-peptide (40.83 ± 29.43 vs. 17.13 ± 7.16 , $P < 0.001$) were strongly associated with non-remission status, while differences in incretins between remitters and non-remitters were minimal.

[†]This study is registered with [Clinicaltrials.gov](https://clinicaltrials.gov) (ID: [NCT01512797](https://clinicaltrials.gov/ct2/show/study/NCT01512797) and [NCT01516320](https://clinicaltrials.gov/ct2/show/study/NCT01516320)).

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DISCLOSURE

The authors have no conflicts of interests to disclose.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

Conclusions: Individual without diabetes remission after gastric bypass have poorer β -cell response and lesser suppression of glucagon to an oral challenge; body weight and incretins differ minimally according to remission status.

Keywords

diabetes remission; gastric bypass; incretins; proinsulin; β -Cell function

1 | INTRODUCTION

Although Roux-en-Y gastric bypass (RYGB) surgery results in the remission of type 2 diabetes (T2D) in approximately 60% of cases,¹ diabetes recurs years after surgery in approximately one-third of individuals who initially experienced remission.² The mechanisms of diabetes remission and/or relapse after RYGB are not completely understood. Prior to surgery, a longer duration of T2D, having poorly controlled T2D, being treated with insulin and/or multiple oral agents, and having poor β -cell function^{3,4} are the main determinants of non-remission and/or relapse after RYGB. Important determinants after surgery include the weight loss amount, the degree of improvement in insulin sensitivity⁵ and clearance,⁶ the enhanced postprandial release of the incretins glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1), and restoration of the incretin effect on insulin secretion, all of which contribute to better glucose control.⁷ Although weight regain plays little role in relapse,² time since surgery is ultimately a key variable because diabetes remission rate decreases over time.^{8–10} There are limited data on long-term pancreatic endocrine function in individuals with persistent and/or relapsing diabetes after RYGB.^{7,11}

The goal of this study was to characterize β - and α -cell function, incretin hormones release, and insulin sensitivity in individuals with (remitters; REM) or without (non-remitters; N-REM) T2D remission after RYGB. We hypothesized that N-REM will have blunted β -cell function, lesser postprandial GLP-1 and GIP release, and exaggerated α -cell response to a nutrient challenge than REM, but that body weight and insulin sensitivity would not differ between the two groups.

2 | METHODS

2.1 | Study design

The present study is a cross-sectional study of two distinct cohorts of individuals with documented T2D status prior to surgery. Diabetes status (remission or no remission) at the time of the study was defined by American Diabetes Association (ADA) criteria.¹² All participants provided written informed consent prior to participation.

2.2 | Subjects

Cohort 1 (REM) comprised individuals with documented T2D before surgery and documented remission at the time of the present study. Subjects were participants in a study of the mechanism of diabetes remission after RYGB,^{13,14} had been recruited from a pool of patients with T2D prior to surgery, and underwent RYGB at St. Luke's Roosevelt Hospital

(New York, NY) between 2005 and 2010. Inclusion criteria were described previously^[13] and included known T2D duration of <3 years, HbA1c <8%, and not being treated with insulin. A subgroup of these patients (n = 23) participated in a follow-up study and underwent yearly oral glucose tolerance tests (OGTTs) from 2 to 8 years after RYGB. Data from their latest OGTTs after RYGB were used for the present study.

Cohort 2 (N-REM) comprised individuals with documented T2D prior to surgery who had either not experienced T2D remission or had T2D relapse after a period of remission (by self-report), with documented absence of remission at the time of the present study. Individuals from Cohort 2 were recruited for a separate randomized control trial on the effect of a dipeptidyl peptidase (DPP)-4 inhibitor on glucose control in T2D after RYGB.¹⁵ Recruitment for Cohort 2, described previously,¹⁵ was done from 2012 to 2016 as follows: (1) screening of medical records and mass mailing to patients with documented T2D, HbA1c > 7% and/or on insulin treatment prior to RYGB who underwent RYGB at St. Luke's Roosevelt Hospital at least 1 year prior to 2012 (n = 117); or (2) by direct referrals from healthcare providers (n = 7). Interested patients (60 of 124) were then invited for a screening visit and, if the diagnosis of T2D was confirmed, were enrolled after proper consenting (n = 32).

Therefore, REM and N-REM cohorts were recruited separately and underwent different experimental protocols. Post-RYGB cross-sectional data from these two cohorts were used for the present study.

2.3 | Experimental procedures

2.3.1 | Roux-en-Y gastric bypass—The jejunum was divided 30 cm from the ligament of Treitz and anastomosed to a 30-mL proximal pouch. The jejunum was re-anastomosed 150 cm distal to the gastrojejunostomy, as described previously.¹¹

2.3.2 | In vivo testing procedures—Body weight and height were measured with a standardized calibrated scale and stadiometer, and body mass index (BMI; kg/m²) was calculated. Weight loss history (weight at the time of surgery, nadir weight after surgery) was either retrieved from charts or self-reported. Each participant underwent an oral challenge (see below). The morning of the experimental procedure, participants came to the Columbia University clinical and translational science award Clinical Research Center (New York, NY) after an overnight fast, and an intravenous (i.v.) catheter was inserted by a research nurse in the forearm and the i.v. was kept open with infusion of 0.45% saline.

For N-REM participants, oral diabetes medications were held at least 2 days prior to the experiment. Insulin therapy was adjusted so that the last injection was at least 28 hours prior to the meal challenge. The N-REM participants underwent a standardized 200-kcal liquid mixed-meal test (198 mL) with 55% carbohydrates (33 g), 15% protein, 30% fat (Boost; Nestle, Florham Park, NJ), consumed over 10 minutes, in a sitting position. The REM participants underwent a 50-g OGTT (200 mL non-carbonated), consumed over 10 minutes, in a sitting position. Venous blood samples were drawn prior to and 15, 30, 60, 90, 120, and 180 minutes after the start of each oral challenge for hormone and metabolites assays.

2.3.3 | Assays—Blood was collected in two chilled EDTA tubes, one tube containing aprotinin (500 kallikrein inhibitory units [KIU]/mL blood) and DPP-4 inhibitor (10 µL/mL blood; EMD Millipore Corp., Billerica, MA) for measurement of incretins. Glucose was measured at the bedside with an Analox analyzer (Analox Instruments, Atlanta, GA). Insulin, C-peptide, glucagon, proinsulin, leptin, and GLP-1 total were determined by radioimmunoassay (EMD Millipore Corp. Billerica, MA), with inter- and intra-assay coefficients of variation (CV) of 4.7%–8.8% and 3%–6.5% respectively; GIP was determined by ELISA (Millipore), inter- and intra-assay CVs of 1.8%–6.1% and 3.0%–8.8%, respectively, at the Columbia University Diabetes Research Center Hormone and Metabolite Core (New York, NY).

2.4 | Statistical analysis

Calculations were performed as follows:

- insulin secretion rate (ISR; pmol/kg/min) was calculated by mathematical deconvolution of C-peptide using a two-compartment model for hormone clearance (The Chrono-logical Series Analyzer; Van Cauter, Hasak and Leproult, University of Chicago)¹⁶
- β-cell glucose sensitivity (BCGS; pmol/kg/min/mM) was calculated as the slope of ISR_{0-peak} in relation to change of $glucose_{0-peak}$
- fasting and post-prandial insulin clearance rates (ICRs) were calculated as the ratio of ISR to insulin levels
- the insulinogenic index (0–30 minutes [IGI_{0-30}], and 0–180 minutes [IGI_{0-180}]) was calculated as the ratio of the change of insulin for the indicated time to the change of glucose in the same time period (ie, $Insulin_{time} / Glucose_{time}$)
- the Matsuda insulin sensitivity index (ISI) was calculated using the following formula:

$$10000 \times \sqrt{FG \times FINS \times MG_{0-180} \times MINS_{0-180}}$$

where FG is fasting glucose, FINS is fasting insulin, MG is mean glucose and MINS is mean insulin

- homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as $(FINS \times FG)/405$
- the disposition index (DI) was calculated as $BCGS \times 1/HOMA-IR$
- total and incremental areas under the curve (tAUC and iAUC, respectively) were calculated by the trapezoidal rule.¹⁷

Data distribution was assessed and non-parametric tests were used if needed. Log transformations were used for insulin, proinsulin, and C-peptide for analyses. To account for different oral glucose loads, non-transformed hormone data are also presented after adjustment for glucose concentrations (ie, hormone AUCs were divided by the

corresponding glucose AUC). In addition, non-transformed hormone values at each time point during the oral challenge were divided by their corresponding glucose values.

The significance of differences between groups in the hormone response during the oral challenge was tested using a general linear model (GLM) with repeated measures (time $\times$ group interaction); unpaired *t* tests and Mann-Whitney *U* tests for non-parametric values were used to calculate group differences between fasting values and AUCs. Mixed-model regression was used to assess predictors of β -cell function and HOMA-IR. Statistical analyses were performed using SPSS 24 (IBM, Armonk, New York) and two-sided $P < 0.05$ was considered significant. Data are expressed as the mean $\pm$ SD, except in figures, where the data are presented as the mean $\pm$ SEM. Based on the difference of increment of BCGS between 0 and 3 months after RYGB between individuals with (1.551 ± 1.031 PMol/kg/min/mM) and without (0.491 ± 0.531 PMol/kg/min/mM) diabetes remission, we estimated at least 15 individuals in each group were needed for 80% power to detect significant differences at the $P < 0.05$ level. Every attempt was made to limit bias: the study had a strict definition of diabetes status, documentation of date and type of surgery; validated experimental procedures were performed in the Clinical Research Center and a certified laboratory was used to measure glucose and hormone outcomes; and rigorous statistical analysis was performed.

3 | RESULTS

3.1 | Subject characteristics

Fifty-four individuals were included in this study. Individuals in the N-REM group ($n = 31$) were older, had a longer known duration of T2D prior to RYGB, and were further out from RYGB than those in the REM group ($n = 23$). However, body weight at the time of surgery, nadir weight, maximum total and percentage weight loss after RYGB, weight at the time of study, and leptin concentrations, a marker of fat mass, were not different between the two groups (Table 1). Individuals in the N-REM group were more insulin resistant and, as expected, had higher HbA1c and glucose concentrations than those in the REM group (Table 1; Fig. 1a).

3.2 | β -Cell biomarkers: proinsulin, insulin, C-peptide and ISR

Fasting log[proinsulin], log(proinsulin tAUC), log(proinsulin iAUC), and log[proinsulin] during the oral challenge (time $\times$ group interaction, $P < 0.001$) and the proinsulin/insulin (PI:I) ratio (fasting, iAUC) were significantly higher in the N-REM than REM group (Fig. 1c; Table 2; Table S1). After correcting for glucose, fasting proinsulin and the time $\times$ group interaction remained significantly higher in the N-REM than REM group (Fig. 2b; Table S1).

The REM group had a more robust insulin secretory reserve with significantly higher fasting log[insulin], log(insulin iAUC), peak log[insulin], and insulin concentrations during the oral challenge (time $\times$ group interaction, $P < 0.001$; Table 2). After correcting for glucose, the REM group had a higher tAUC, peak insulin and insulin concentrations during the oral challenge (time $\times$ group interaction, $P = 0.010$) than the N-REM group (Figure S1A,B).

Fasting C-peptide and fasting ISR did not differ between groups (Table 2). Indices of C-peptide secretion were also higher in the REM than N-REM cohort (Table 2; Fig. 1d). With the exception of C-peptide iAUC, all group differences remained significant after correcting for glucose levels (Fig. 2c; Table S1). The ISR after oral stimulus (ISR tAUC, ISR iAUC, peak ISR) and ISR (time $\times$ group interaction, $P < 0.001$) was 2.2- to 3.5-fold greater in the REM than N-REM cohort (Table 2; Fig. 1b). All group differences for ISR remained significant after correcting for glucose levels, except ISR iAUC (Fig. 2c; Table S1). Interestingly, the insulinogenic index (IGI_{0-30}), a measure of the early phase insulin secretion, did not differ between groups (Table 2).

3.3 | Comparison of HOMA-IR, ISI, and ICR between the REM and N-REM groups

Although the body weights at the time of the study were not significantly different between the two groups, the N-REM cohort tended to be heavier (Table 1) and were significantly more insulin resistant than the REM cohort. In the entire cohort, HOMA-IR was positively correlated with weight ($n = 54$; $R^2 = 0.439$, $P = 0.001$). This association, not present in the REM group ($P = 0.469$, $R^2 = 0.159$), was driven by the N-REM cohort ($P = 0.004$, $R^2 = 0.504$). The difference in HOMA-IR between the REM and NREM groups disappeared when the analysis was done in a sub-group of REM and N-REM well matched for body weight (data not shown). The ISI did not differ between groups (Table 2).

The REM cohort had higher fasting and postprandial ICR levels, by a factor of 2, than the N-REM cohort (Table 2).

There was a strong positive correlation between HOMA-IR and fasting proinsulin ($r = 0.428$, $P = 0.002$), but a negative correlation between HOMA-IR and iAUC proinsulin: iAUC insulin ($r = -0.464$, $P = 0.001$), fasting ICR ($r = -0.396$, $P = 0.003$), and tAUC ICR ($r = -0.453$, $P = 0.001$).

3.4 | β -Cell glucose sensitivity and DI

As predicted, BCGS and DI were two- and 5fold greater, respectively, in the REM than N-REM group (Table 2). The difference in DI was driven by the greater insulin secretion capacity in the REM cohort. Interestingly, there was significant overlap in the DI of the REM and N-REM cohorts (Fig. 1; Table S2). We performed a subanalysis of these “overlappers” (O; REM-O and N-REM-O). Although the total weight loss and current weight did not differ between the N-REM-O and REM-O cohorts, those in the N-REM-O group had significantly larger weight regain from self-reported nadir to current weight (Table S2). Consistent with the analysis in the entire cohorts, age, years since surgery, HbA1c, and known T2D duration prior to surgery were all higher in the N-REM-O than REM-O group (Table S2). Furthermore, the N-REM-O cohort had worse β -cell function, higher glucagon levels that did not suppress during the oral challenge, worse ICR, higher proinsulin and PI: I ratios, and a higher GIP response but similar GLP-1 (Table S2).

3.5 | Glucagon

The N-REM group had hyperglucagonemia, both under the fasting condition and during the oral challenge (time $\times$ group interaction, $P < 0.001$; Table 2; Fig. 1g). Adjustment of

glucagon for glucose concentrations showed that N-REM did not suppress glucagon after the oral load, whereas those in the REM group did (time $\times$ group interaction, $P < 0.001$; Table 2; Fig. 2d). The insulin: glucagon ratio (peak, average, and AUC) was significantly higher in the REM than N-REM cohort, even after correcting for glucose (Table S1).

3.6 | Glucagon-like peptide-1 and GIP

Fasting and postprandial GLP-1 concentrations were not significantly different between the N-REM and REM cohorts (Table 2; Fig. 1f). However, after correcting for glucose levels, the N-REM group had overall lower GLP-1 concentrations: lower 120-minute GLP-1 concentrations (1.70 ± 0.82 vs 3.55 ± 3.44 pM; $P = 0.019$) and a trend for lower GLP-1 tAUC (2.78 ± 1.46 vs 4.44 ± 3.62 pM/min; $P = 0.063$; Fig. 2d; Table S1).

Although fasting GIP and GIP tAUC and iAUC were significantly higher in the N-REM than REM group, all differences disappeared after correcting for glucose levels (Table 2; Figs 1E, 2E; Table S1).

The GLP-1 AUC and iAUC, but not GIP, were weakly correlated with BCGS ($r = 0.265$ [$P = 0.055$] and $r = 0.286$ [$P = 0.038$], respectively). Glucagon-like peptide-1 secretion was not associated with proinsulin, or the PI: I ratio (data not shown); however, there was a strong correlation between GIP tAUC ($r = 0.490$; $P < 0.0001$), GIP iAUC ($r = 0.456$, $P = 0.001$) and peak GIP ($r = 0.392$, $P = 0.006$) and fasting proinsulin. Similarly, strong correlations were observed between the same GIP variables and proinsulin tAUC (data not shown).

3.7 | Predictors of β -cell function and HOMA-IR

Neither age nor weight loss (total or percentage) predicted HOMA-IR or β -cell function (BCGS). However, self-reported known diabetes duration was a strong predictor of β -cell function ($P = 0.002$) and HOMA-IR ($P = 0.007$).

4 | DISCUSSION

The availability of two well-characterized cohorts allowed for the unique comparison of metabolic profiles and gut and pancreatic endocrine function in individuals with and without diabetes remission after RYGB. As predicted, individuals with diabetes remission (REM) had better β -cell function, were less insulin resistant, and had better ICRs and more favorable insulin and glucagon response to an oral challenge. There were no significant differences in body weight, GLP-1, and GIP concentrations between the REM and N-REM groups; GIP was strongly associated with proinsulin.

Consistent with past literature,^{18,19} our data confirm that N-REM individuals had a longer known duration of T2D,²⁰ were more likely to use insulin prior to RYGB, were older, and further out from their surgery.

Interestingly, weight loss, nadir weight after surgery, weight regain, and weight and BMI at the time of the study were not significantly different between the two cohorts, which also had similar leptin levels, an indirect biomarker of fat mass.²¹ Weight regain was previously shown not to be the main determinant of diabetes relapse 5 to 8 years after RYGB.²

However, N-REM tended to be heavier, which could explain, in part, their greater insulin resistance, as assessed by HOMA-IR. Indeed, when matched for weight loss, the difference in insulin resistance between groups disappeared.

β -Cell function, as expected, was significantly greater in the REM group. This is in agreement with many studies showing that β -cell function is the key predictor of diabetes remission after RYGB.⁴ An elevated PI: I ratio, a hallmark of T2D β -cell defect,^{22–25} was higher in the N-REM group, highlighting a persistent defect on the prohormone processing of insulin, a hallmark of T2D.

We have shown that the hyperproinsulinemia observed in individuals with T2D prior to RYGB normalizes 6 months after RYGB and remains normal in individuals experiencing diabetes remission.²⁶ In the present study, the PI: I ratio was associated poor β -cell function (BCGS) and with insulin resistance; therefore, the PI: I ratio could be used as a biomarker of diabetes relapse after RYGB. The β -cell of the N-REM group seems to have all the characteristics of individuals with T2D prior to bypass.

Although both groups showed similarly enhanced post-prandial GLP-1 and GIP, in concentrations similar to those reported in previous studies,^{13,14} the difference in incretin concentrations between REM and N-REM were small. Concentrations of GLP-1 tended to be higher in the REM group. Because of the potentiating effect of GLP-1 on glucose-stimulated insulin secretion in vivo,²⁷ the enhanced post-prandial GLP-1 secretion, and incretin effect after RYGB,¹³ we tested the association between GLP-1 release and β -cell function. Our data confirm an association, albeit weak, of GLP-1, but not GIP, with BCGS. Others have shown that GLP-1 response, together with β -cell function, is associated with diabetes remission after RYGB.^{3,4} However, GLP-1 may be only one of many mediators of glucose control after RYGB, because the infusion of the GLP-1 receptor inhibitor exendin (9–39) worsens postprandial glucose only minimally after RYGB.^{28–30}

Concentrations of GIP were higher in the N-REM than REM group. This is contrary to another study that showed no difference in GIP during a mixed-meal test between REM and N-REM groups 12 months after RYGB.³¹ The contribution of endogenous GIP after RYGB has been less studied than GLP-1, due, in part, to a less robust effect of RYGB on GIP between studies^{13,32} and, foremost, because of the lack of a specific GIP receptor inhibitor. Our data show a strong correlation between GIP and proinsulin. Previous studies have shown that GIP potently stimulates proinsulin gene transcription³³ and disproportionately stimulates proinsulin secretion in glucose-intolerant individuals.³⁴

The significant overlap between REM and N-REM DI, not explained by age or weight, suggests that variables other than β -cell function and insulin resistance may be associated with T2D remission. Although specific metabolomic^{35–38} and microbiome³⁹ signatures, and increased metabolism of the remodeled small intestine after RYGB,⁴⁰ have been shown to be associated with glucose control and/or improved insulin sensitivity after RYGB, there is still a need to identify biomarkers of diabetes relapse after RYGB.

Overall, individuals who were in diabetes remission had more robust insulin secretion, greater insulin clearance and glucagon suppression, had lower proinsulin, and were less

insulin resistant than N-REM individuals. The PI: I ratio, rather than incretin response, was an important discriminatory factor of diabetes remission after RYGB.

This study has many strengths. It presents novel data on two relatively large, well-characterized unique cohorts of individuals with and without diabetes remission, studied 3 to 5 years after RYGB. The few studies that present data on similar parameters are short term, often limited to 12 months after RYGB.³ In addition, the data in the present study include β - and α -cell biomarkers and incretin concentrations under fasting and after an oral stimulus, allowing calculation of the ISR and ICR.

However, this study has several limitations. First two different carbohydrate loads were used: N-REM participants received a standardized 200-kcal liquid meal (33 g carbohydrates), whereas REM participants received a 50-g glucose drink. This could affect not only glucose levels during the glucose tolerance tests, but also some gut hormones that respond to macronutrients other than glucose. Second, the N-REM cohort was 2 years further out from surgery than the REM cohort. Insulin resistance was estimated by HOMA-IR rather than measured by clamp, which would have allowed assessment of both components of insulin resistance, hepatic and peripheral. The lack of data on dietary intake, physical activity, and body composition limits assessment of determinants of insulin resistance. The cross-sectional design for the N-REM group did not allow us to distinguish between individuals who may have gone into remission then relapsed from those who had never experience diabetes remission, because the mechanisms of relapse may differ from those of non-remission. However, the phenotype of the N-REM group was similar to that of people with T2D.

In brief, we show a distinct defect of pancreatic endocrine function in non-remitters compared with remitters, but minimal difference in body weight and gut endocrine response.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Buchwald H, Estok R, Fahrbach K, et al. Weight and type 2 diabetes after bariatric surgery: Systematic review and meta-analysis. *Am J Med.* 2009;122:248–256.e245. [PubMed: 19272486]

2. Arterburn DE, Bogart A, Sherwood NE, et al. A multisite study of long-term remission and relapse of type 2 diabetes mellitus following gastric bypass. *Obes Surg*. 2013;23:93–102. [PubMed: 23161525]
3. Nannipieri M, Mari A, Anselmino M, et al. The role of β -cell function and insulin sensitivity in the remission of type 2 diabetes after gastric bypass surgery. *J Clin Endocrinol Metab*. 2011;96:E1372–E1379.
4. Blanco J, Jiménez A, Casamitjana R, et al. Relevance of β -cell function for improved glycemic control after gastric bypass surgery. *Surg Obes Relat Dis*. 2014;10:9–13. [PubMed: 24342036]
5. Bradley D, Conte C, Mittendorfer B, et al. Gastric bypass and banding equally improve insulin sensitivity and β cell function. *J Clin Invest*. 2012;122:4667–4674. [PubMed: 23187122]
6. Shah A, Holter MM, Rimawi F, et al. Insulin clearance after oral and intravenous glucose following gastric bypass and gastric banding weight loss. *Diabetes Care*. 2019;42:311–317. [PubMed: 30523032]
7. Holst JJ, Madsbad S. Mechanisms of surgical control of type 2 diabetes: GLP-1 is key factor. *Surg Obes Relat Dis*. 2016;12:1236–1242. [PubMed: 27313194]
8. Sjöström L, Lindroos A-K, Peltonen M, et al. Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery. *N Engl J Med*. 2004;351:2683–2693. [PubMed: 15616203]
9. Dicker D, Golan R, Aron-Wisniewsky J, et al. Prediction of long-term diabetes remission after RYGB, sleeve gastrectomy, and adjustable gastric banding using DiaRem and advanced-DiaRem scores. *Obes Surg*. 2019;29:796–804. [PubMed: 30467708]
10. Madsen LR, Baggesen LM, Richelsen B, Thomsen RW. Effect of roux-en-Y gastric bypass surgery on diabetes remission and complications in individuals with type 2 diabetes: A Danish population-based matched cohort study. *Diabetologia*. 2019;62:611–620. [PubMed: 30734055]
11. Holter MM, Dutia R, Stano SM, et al. Glucose metabolism after gastric banding and gastric bypass in individuals with type 2 diabetes: weight loss effect. *Diabetes Care*. 2017;40:7–15. [PubMed: 27999001]
12. American Diabetes Association. Classification and diagnosis of diabetes: Standards of medical care in diabetes – 2018. *Diabetes Care*. 2018;41(Suppl. 1):S13–S27. [PubMed: 29222373]
13. Laferrère B, Heshka S, Wang K, et al. Incretin levels and effect are markedly enhanced 1 month after roux-en-Y gastric bypass surgery in obese patients with type 2 diabetes. *Diabetes Care*. 2007;30:1709–1716. [PubMed: 17416796]
14. Laferrère B, Teixeira J, McGinty J, et al. Effect of weight loss by gastric bypass surgery versus hypocaloric diet on glucose and incretin levels in patients with type 2 diabetes. *J Clin Endocrinol Metab*. 2008;93:2479–2485. [PubMed: 18430778]
15. Shah A, Levesque K, Pierini E, et al. Effect of sitagliptin on glucose control in type 2 diabetes mellitus after roux-en-Y gastric bypass surgery. *Diabetes Obes Metab*. 2018;20:1018–1023. [PubMed: 29072800]
16. Van Cauter E, Mestrez F, Sturis J, Polonsky KS. Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes*. 1992;41:368–377. [PubMed: 1551497]
17. Allison D, Paultre F, Maggio C, Mezzitis N, Pi-Sunyer F. The use of areas under curves in diabetes research. *Diabetes Care*. 1995; 18:245–250. [PubMed: 7729306]
18. Still CD, Wood GC, Benotti P, et al. A probability score for preoperative prediction of type 2 diabetes remission following RYGB surgery. *Lancet Diabetes Endocrinol*. 2014;2:38–45. [PubMed: 24579062]
19. Kassem MAM, Durda MA, Stoicea N, Cavus O, Sahin L, Rogers B. The impact of bariatric surgery on type 2 diabetes mellitus and the management of hypoglycemic events. *Front Endocrinol*. 2017;8:37.
20. Panunzi S, Carlsson L, De Gaetano A, et al. Determinants of diabetes remission and glycemic control after bariatric surgery. *Diabetes Care*. 2016;39:166–174. [PubMed: 26628418]
21. Liuzzi A, Savia G, Tagliaferri M, et al. Serum leptin concentration in moderate and severe obesity: relationship with clinical, anthro-pometric and metabolic factors. *Int J Obes Relat Disord*. 1999;23: 1066–1073.

22. Kahn SE, Leonetti DL, Prigeon RL, Boyko EJ, Bergstrom RW, Fujimoto WY. Proinsulin as a marker for the development of NIDDM in Japanese-American men. *Diabetes*. 1995;44:173–179. [PubMed: 7859937]
23. Åhrén B, Larsson H. Relative hyperproinsulinemia as a sign of islet dysfunction in women with impaired glucose tolerance. *J Clin Endocrinol Metab*. 1999;84:2068–2074. [PubMed: 10372712]
24. Mykkanen L, Haffner S, Kuusisto J, Pyorala K, Hales C, Laakso M. Serum proinsulin levels are disproportionately increased in elderly prediabetic subjects. *Diabetologia*. 1995;38: 1176–1182. [PubMed: 8690169]
25. Kahn SE, Halban PA. Release of incompletely processed proinsulin is the cause of the disproportionate proinsulinemia of NIDDM. *Diabetes*. 1997;46:1725–1732. [PubMed: 9356018]
26. Bose M, Teixeira J, Olivan B, et al. Weight loss and incretin responsiveness improve glucose control independently after gastric bypass surgery. *J Diabetes*. 2010;2:47–55. [PubMed: 20676394]
27. Kjems LL, Holst JJ, Vølund A, Madsbad S. The influence of GLP-1 on glucose-stimulated insulin secretion. *Diabetes*. 2003;52: 380–386. [PubMed: 12540611]
28. Vidal J, de Hollanda A, Jiménez A. GLP-1 is not the key mediator of the health benefits of metabolic surgery. *Surg Obes Relat Dis*. 2016;12:1225–1229. [PubMed: 27313195]
29. Jimenez A, Casamitjana R, Viaplana-Masclans J, Lacy A, Vidal J. GLP-1 action and glucose tolerance in subjects with remission of type 2 diabetes after gastric bypass surgery. *Diabetes Care*. 2013; 36:2062–2069. [PubMed: 23359363]
30. Morinigo R, Lacy AM, Casamitjana R, Delgado S, Gomis R, Vidal J. GLP-1 and changes in glucose tolerance following gastric bypass surgery in morbidly obese subjects. *Obes Surg*. 2006;16: 1594–1601. [PubMed: 17217635]
31. Kyoung KM, Chan LH, Hwan LS, et al. The difference of glucostatic parameters according to the remission of diabetes after roux-en-Y gastric bypass. *Diabetes Metab Res Rev*. 2012;28:439–446. [PubMed: 22407971]
32. Lee CJ, Brown TT, Cheskin LJ, et al. Effects of meal composition on postprandial incretin, glucose and insulin responses after surgical and medical weight loss. *Obes Sci Pract*. 2015;1:104–109. [PubMed: 27774253]
33. Fehmann H-C, Göke B. Characterization of GIP(1–30) and GIP (1–42) as stimulators of proinsulin gene transcription. *Peptides*. 1995;16:1149–1152. [PubMed: 8532600]
34. Eiel JN, Nyirjesy S, Peleckis AJ, et al. Effects of GLP-1 and GIP on insulin secretion in glucose intolerant, pancreatic insufficient cystic fibrosis. *Diabetes*. 2018;67:P1820.
35. Li Q-R, Wang Z-M, Wewer Albrechtsen NJ, et al. Systems signatures reveal unique remission-path of type 2 diabetes following roux-en-Y gastric bypass surgery. *EBioMedicine*. 2018;28: 234–240. [PubMed: 29422288]
36. Narath SH, Mautner SI, Svehlikova E, et al. An untargeted metabolomics approach to characterize short-term and long-term metabolic changes after bariatric surgery. *PLoS One*. 2016;11: e0161425.
37. Zhao L, Ni Y, Yu H, et al. Serum stearic acid/palmitic acid ratio as a potential predictor of diabetes remission after roux-en-Y gastric bypass in obesity. *FASEB J*. 2017;31:1449–1460. [PubMed: 28007782]
38. Laferrère B, Reilly D, Arias S, et al. Differential metabolic impact of gastric bypass surgery versus dietary intervention in obese diabetic subjects despite identical weight loss. *Sci Transl Med*. 2011; 3:80re82.
39. Murphy R, Tsai P, Jüllig M, Liu A, Plank L, Booth M. Differential changes in gut microbiota after gastric bypass and sleeve gastrectomy bariatric surgery vary according to diabetes remission. *Obes Surg*. 2017;27:917–925. [PubMed: 27738970]
40. Saeidi N, Meoli L, Nestoridi E, et al. Reprogramming of intestinal glucose metabolism and glycemic control in rats after gastric bypass. *Science*. 2013;341:406–410. [PubMed: 23888041]

Highlights

- Both pancreatic endocrine function (β - and α -cells) and insulin clearance rate are key differentiators of diabetes remission status after gastric bypass.
- Neither weight loss nor glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP) concentrations differ between individuals with and without diabetes remission after gastric bypass.
- Proinsulin is significantly elevated in non-remitters and could be a biomarker for diabetes relapse after gastric bypass surgery.

FIGURE 1.

Metabolic and hormone responses during the oral challenge in individuals with (remitters) and without (non-remitters) type 2 diabetes remission after Roux-en-Y gastric bypass. Data are the mean $\pm$ SEM. * $P < 0.05$ tested by GLM with repeated measures, if time $\times$ group interaction was significant. A, glucose; B, insulin secretion rate (ISR); C, proinsulin; D, C-peptide; E, glucose-dependent insulintropic polypeptide (GIP); F, glucagon-like peptide-1 (GLP-1); G, glucagon; H, disposition index, represented by the relationship between β -cell

glucose sensitivity (BCGS) and the homeostatic model assessment of insulin resistance (HOMA-IR)

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FIGURE 2.

Metabolic and hormone responses during the oral challenge, adjusted for glucose concentrations, in remitters and non-remitters. Data are the mean $\pm$ SEM. * $P < 0.05$ GLM with repeated measures, with significant time $\times$ group interaction. A, insulin secretion rate (ISR); B, proinsulin; C, C-peptide; D, glucagon; E, glucose-dependent insulinotropic polypeptide (GIP); F, glucagon-like peptide-1 (GLP-1)

TABLE 1

Characteristics of participants with (remitters) and without (non-remitters) diabetes remission after Roux-en-Y gastric bypass surgery

	Remitters	Non-remitters	P-value
No. subjects	23	31	
Age (y)	46.0 ± 8.9	56.1 ± 8.2	<0.001
% Female	87.0	90.6	NS
Time since surgery (y) [range]	3.5 ± 1.7 [2–8]	5.6 ± 3.3 [1.1–11.2]	0.005
T2D duration prior to RYGB (y)	2.2 ± 2.4	13.1 ± 10.1	<0.001
Weight at surgery (kg)	114.5 ± 15.6	123.9 ± 23.2	0.118
Current weight (kg) *	81.7 ± 16.8	90.9 ± 18.0	0.127
Current BMI (kg/m ²)	31.4 ± 4.6	34.4 ± 6.7	0.174
Current weight loss (kg)	33.8 ± 12.9	33.0 ± 12.8	0.937
Current percentage weight loss	29.8 ± 11.0	26.4 ± 8.4	0.357
Nadir weight after RYGB (kg)	75.9 ± 12.9	78.2 ± 16.1	0.587
Maximum weight loss after RYGB (kg)	38.6 ± 9.3	45.7 ± 17.6	0.079
Maximal percentage weight loss after RYGB	33.7 ± 6.6	36.4 ± 10.2	0.275
Fasting leptin (nM)	14.44 ± 6.7	17.53 ± 8.33	0.179
HOMA-IR	2.08 ± 1.22	4.01 ± 3.65	<0.001
HbA1c (%)	5.6 ± 0.6	7.2 ± 0.8	<0.001
Fasting glucose (mM)	5.16 ± 0.43	6.65 ± 1.40	<0.001
120 minutes glucose (mM)	4.87 ± 1.43	7.13 ± 1.63	<0.001
Glucose AUC (mM/min)	6.83 ± 1.32	7.96 ± 1.56	0.007

Unless indicated otherwise, data are given as the mean ± SD. Group differences were determined by independent *t* test or the Mann-Whitney *U* test.

* Current weight, BMI, weight and BMI at time of present study. Current weight loss and current percentage weight loss= weight loss at time of present study, calculated from pre-surgery body weight, as described in Methods.

AUC, area under the curve; BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; RYGB, Roux-en-Y gastric bypass; T2D, type 2 diabetes.

Metabolic and hormone responses during the oral challenge in participants with (remitters) and without (non-remitters) diabetes remission after Roux-en-Y gastric bypass surgery

TABLE 2

	Remitters	Non-remitters	P-value
Fasting insulin (pM)	64.40 ± 36.37	97.16 ± 87.11	0.050 [‡]
Insulin 120 minutes (pM)	79.45 ± 41.05	110.61 ± 74.98	0.027 [‡]
Insulin AUC (pM/min)	250.45 ± 123.92	213.60 ± 103.06	0.317 [‡]
Insulin iAUC (pM/min)	186.06 ± 115.24	115.74 ± 71.49	0.013
Fasting Proinsulin (pM)	6.62 ± 3.50	16.55 ± 10.45	<0.001
Proinsulin AUC (pM/min)	22.34 ± 13.71	29.25 ± 15.63	0.022 [‡]
Proinsulin iAUC (pM/min)	15.72 ± 11.17	12.83 ± 6.88	<0.001 [‡]
Fasting C-peptide (nM)	0.46 ± 0.29	0.43 ± 0.19	0.901 [‡]
C-Peptide AUC (nM/min)	1.53 ± 0.63	0.77 ± 0.33	<0.001
C-Peptide iAUC (nM/min)	1.07 ± 0.48	0.34 ± 0.17	<0.001
Fasting ISR (pmol/kg)	1.43 ± 0.89	1.22 ± 0.50	0.729
ISR 120 minutes (pmol/kg)	1.88 ± 2.19	1.49 ± 0.77	0.426
Mean ISR (pmol/kg)	4.54 ± 2.04	2.11 ± 0.81	<0.001
Peak ISR (pmol/kg)	13.14 ± 4.95	4.81 ± 2.46	<0.001
ISR AUC (pmol/kg/min)	4.81 ± 2.15	2.17 ± 0.84	<0.001
ISR iAUC (pmol/kg/min)	3.38 ± 1.65	0.96 ± 0.45	<0.001
Fasting ICR (mL/kg/min)	28.06 ± 24.05	17.39 ± 9.05	0.025
ICR (mL/kg/min)	21.75 ± 10.25	11.57 ± 4.65	<0.001
BCGS (pmol/kg/min/mmol)	1.95 ± 1.23	0.86 ± 0.55	<0.001
Disposition index	1.55 ± 1.75	0.33 ± 0.27	<0.001
ISI	6.30 ± 3.66	5.07 ± 2.78	0.249
Insulinogenic index ₀₋₃₀	0.90 ± 0.79	0.86 ± 0.59	0.795
Fasting glucagon (pM)	11.18 ± 4.87	13.02 ± 3.38	0.026
Glucagon 120 minutes (pM)	11.66 ± 4.02	13.51 ± 3.63	0.111
Glucagon AUC (pM/min)	12.03 ± 3.77	15.17 ± 2.94	0.002
Glucagon iAUC (pM/min)	0.85 ± 2.60	2.18 ± 2.16	0.076

	Remitters	Non-remitters	P-value
Fasting GIP (pM)	10.60 ± 6.27	12.98 ± 4.64	0.045
GIP 120 minutes (pM)	13.26 ± 5.46	22.25 ± 6.84	<0.001
Mean GIP (pM)	33.48 ± 11.37	40.57 ± 10.12	0.026
GIP AUC (pM/min)	29.30 ± 10.09	36.83 ± 9.35	0.010
GIP iAUC (pM/min)	18.70 ± 7.92	23.91 ± 7.48	0.023
Fasting GLP-1 (pM)	10.08 ± 6.89	9.17 ± 3.45	0.808
GLP-1 120 minutes (pM)	16.27 ± 15.91	11.54 ± 4.72	0.859
Mean GLP-1 (pM)	30.86 ± 20.35	23.82 ± 12.42	0.197
GLP-1 tAUC (pM/min)	28.26 ± 19.95	21.50 ± 11.08	0.249
GLP-1 iAUC (pM/min)	18.18 ± 16.20	12.36 ± 10.68	0.195

Data are the mean ± SD. P-values represent differences between remitters and non-remitters, determined using independent sample *t* test or the Mann-Whitney *U* test.

[‡] P-values calculated from corresponding log values.

AUC, area under the curve; BCGS, β -cell glucose sensitivity; GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide-1; iAUC, incremental AUC; ICR, insulin clearance rate; ISI, Matsuda insulin sensitivity index; ISR, insulin secretion rate; tAUC, total AUC.