



The Effect of Standard Versus Longer Intestinal Bypass on GLP-1 Regulation and Glucose Metabolism in Patients With Type 2 Diabetes Undergoing Roux-en-Y Gastric Bypass: The Long-Limb Study

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OBJECTIVE

Roux-en-Y gastric bypass (RYGB) characteristically enhances postprandial levels of glucagon-like peptide 1 (GLP-1), a mechanism that contributes to its profound glucose-lowering effects. This enhancement is thought to be triggered by bypass of food to the distal small intestine with higher densities of neuroendocrine L-cells. We hypothesized that if this is the predominant mechanism behind the enhanced secretion of GLP-1, a longer intestinal bypass would potentiate the postprandial peak in GLP-1, translating into higher insulin secretion and, thus, additional improvements in glucose tolerance. To investigate this, we conducted a mechanistic study comparing two variants of RYGB that differ in the length of intestinal bypass.

RESEARCH DESIGN AND METHODS

A total of 53 patients with type 2 diabetes (T2D) and obesity were randomized to either standard limb RYGB (50-cm biliopancreatic limb) or long limb RYGB (150-cm biliopancreatic limb). They underwent measurements of GLP-1 and insulin secretion following a mixed meal and insulin sensitivity using euglycemic hyperinsulinemic clamps at baseline and 2 weeks and at 20% weight loss after surgery.

RESULTS

Both groups exhibited enhancement in postprandial GLP-1 secretion and improvements in glycemia compared with baseline. There were no significant differences in postprandial peak concentrations of GLP-1, time to peak, insulin secretion, and insulin sensitivity.

CONCLUSIONS

The findings of this study demonstrate that lengthening of the intestinal bypass in RYGB does not affect GLP-1 secretion. Thus, the characteristic enhancement of GLP-1 response after RYGB might not depend on delivery of nutrients to more distal intestinal segments.

Roux-en-Y gastric bypass (RYGB) is now a recognized and recommended treatment option for patients with type 2 diabetes (T2D) (1). RYGB can cause major and

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sustained improvement of T2D, including complete remission of hyperglycemia in many cases (2,3), reduction of T2D-associated morbidity and mortality (4,5), and improved quality of life (2,3). The operation also causes profound effects on various aspects of glucose homeostasis, with dramatic improvement in insulin secretion and insulin sensitivity (6).

Mechanistic evidence shows that the glucose-lowering effects of RYGB and other gastrointestinal operations result not just from simple weight loss but directly from changes in gastrointestinal physiology (7). Although the exact mechanisms by which RYGB controls T2D remain incompletely understood, research over the last decades has identified several contributors, including changes in gut hormones, bile acids, intestinal glucose transport and metabolism, and nutrient sensing, among others (8). RYGB reduces the size of the stomach and bypasses a segment of the upper small intestine, including the duodenum and the proximal jejunum. The bypassed segment of small intestine, which carries bile and pancreatic secretions but is completely excluded from the transit of nutrients, is referred to as the biliopancreatic limb (Fig. 1). Several studies have shown that the exclusion of the small bowel from nutrient transit has weight loss-independent glucose-lowering effects of its own (7); however, the physiologic and molecular mechanisms activated by such intestinal bypass are incompletely understood.

An alternative hypothesis for the efficacy of RYGB holds that the shunt of ingested nutrients to the distal small intestine, where the highest density of neuroendocrine L-cells is found, stimulates them to secrete GLP-1 (9,10). This mechanism is supported by several lines of evidence. The enhanced secretion of GLP-1 occurs in parallel with other L-cell products, such as peptide tyrosine tyrosine (PYY) and oxyntomodulin, with synergistic effects leading to reduced food intake, weight loss, enhanced insulin secretion, and lower glycemia (11,12). GLP-1 concentration curves after RYGB are almost superimposable to insulin concentration curves, and elegant GLP-1 receptor studies demonstrated that this incretin drives, at least in part, the enhanced early postprandial insulin secretion after surgery (13). A recent study also showed that both GLP-1 and PYY responses correlate with increased nutrient delivery to the distal intestine in mice (14). Clinical studies have suggested that increasing the length of intestinal bypass in RYGB could further improve control of T2D (15–17), possibly via potentiation of the GLP-1 response resulting in even greater insulin secretion than standard RYGB. Moreover, operations like the one-anastomosis gastric bypass or biliopancreatic diversion that impose a longer length of intestinal bypass compared with RYGB induce higher rates of T2D remission than RYGB in observational (18) and randomized clinical trials (2) respectively.

Clinical studies including randomized comparison of RYGB outcomes with different limb lengths are scarce in current literature. Previous reports tested variants of the RYGB procedure that also lengthened the alimentary limb and did not control for interference from on-going therapies with glucose-lowering medications or weight loss (15,19–23). Understanding exactly how surgery produces its effects on GLP-1 provides a unique opportunity to elucidate elusive aspects of gut hormone regulation and disease pathophysiology. This knowledge will enable the optimization of clinical efficacy of metabolic surgery and could also help identify new targets for future therapeutics of T2D and obesity.

We used a reductionist approach and hypothesized that if the shunt of nutrients into the distal intestine is the predominant mechanism behind the changes in GLP-1 regulation after RYGB, a longer length of intestinal bypass, and, therefore, a shorter transit for nutrients to the distal small intestine, would potentiate the postprandial peak in GLP-1, translating into higher insulin secretion and, thus, additional improvements in glucose tolerance.

To investigate this hypothesis, we conducted a mechanistic study comparing two variants of RYGB that differ in the length of intestinal bypass (Fig. 1). We compared standard limb RYGB with a biliopancreatic limb of 50 cm versus a long limb RYGB with a substantially longer biliopancreatic limb of 150 cm. In the standard RYGB, even a 50-cm proximal intestinal bypass has been shown to markedly increase postprandial GLP-1 responses. We therefore hypothesized that a tripling of the length of the bypass would enhance GLP-1 responses even further.

The primary end point of this trial was GLP-1 response to meal stimulation within the first 2 weeks after surgery. This outcome reflected our core hypothesis and was best suited to answer our mechanistic question. We hypothesized that the long limb RYGB would shunt nutrients to more distal parts of the small intestine which have greater L-cell density (24), resulting in a higher or earlier peak GLP-1 concentration. We also hypothesized that this phenomenon would take place early after surgery and be independent of intestinal adaptation,

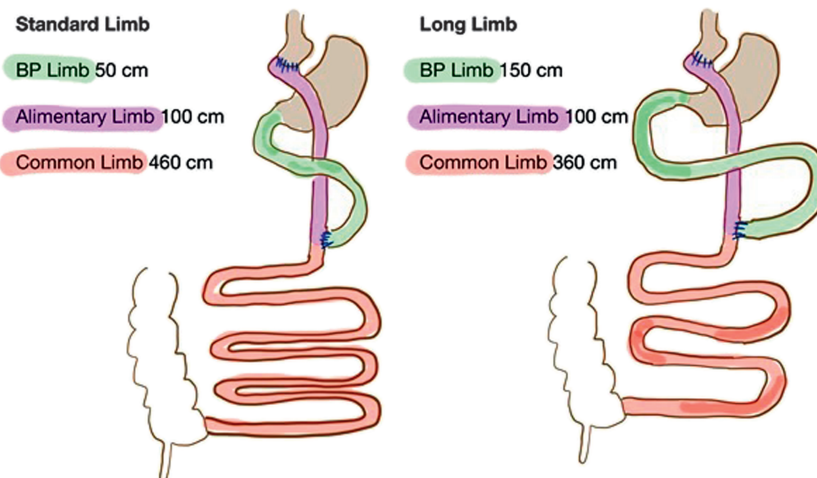


Figure 1—Schematic drawing of the standard limb and the long limb RYGB including the median small intestinal lengths as measured intraoperatively. BP, biliopancreatic.

i.e., before any compensatory changes in L-cell number take place (14). Peak postprandial GLP-1 concentrations have been shown to be the most reproducible marker of GLP-1 response after RYGB (25). The postprandial GLP-1 response also has clinical relevance as it correlates with the rate of T2D remission after RYGB (26). Secondary end points included fasting and postprandial glucose excursions, measures of insulin sensitivity, and glycemic control and weight loss within the first year after surgery. To rule out possible confounding effects of weight loss, patients were also studied at baseline and after an equivalent weight loss of 20% in both groups.

RESEARCH DESIGN AND METHODS

Study Design

This was a mechanistic study. Fifty-three patients with T2D and obesity due to undergo RYGB surgery were recruited from two obesity surgery centers and randomized at a ratio of 1:1 to either a 150-cm (long limb) or 50-cm (standard limb) RYGB while keeping the alimentary limb constant at 100 cm (Fig. 1). Both the patient and the clinical/research teams (except the operating surgeon) were blinded to treatment disposition. The detailed protocol can be accessed as Supplementary Material.

Inclusion and Exclusion Criteria

Key inclusion criteria included an age of 18–70 years, a diagnosis of T2D treated with at least one glucose-lowering medication, BMI ≥ 30 kg/m², and eligibility for metabolic surgery based on the UK National Institute for Health and Care Excellence guidance 189. Key exclusion criteria were any surgical, medical, or psychological contraindications to metabolic surgery; pregnancy; and breastfeeding.

Ethics Approval

The trial was approved by the West London Research Ethics Committee (London, U.K.; reference 15/LO/0813) and registered in the International Standard Randomized Controlled Trial Registry (ISRCTN 15283219). Written informed consent was obtained from all patients prior to participation.

Intervention and Follow-up

Patients were assessed by the multidisciplinary clinical team as part of routine National Health Service care preoperatively, at 10–14 days, and at 3, 6, and 12 months after surgery, unless clinical need dictated more frequent consultations. Operations were performed laparoscopically by five surgeons who followed a standard operating protocol agreed before the trial commenced (Supplementary Material). The procedures were filmed to enable independent assessment of the consistency of the surgical technique among the operating surgeons. The total length of the small intestine was measured from the ligament of Treitz to the ileocecal valve. This was performed using set distance markers on laparoscopic graspers and running the bowel segment by segment along the antimesenteric border.

The management of glucose-lowering medications was performed by a single consultant diabetologist (A.D.M.) who was blinded to treatment allocation. Glucose-lowering medications were discontinued during the 12-month follow-up depending on HbA_{1c} concentrations and capillary glucose measurements, and when considered clinically safe. Diabetes remission was defined based on a variation of the American Diabetes Association criteria (27) as an HbA_{1c} <48 mmol/mol and fasting glucose <5.6 mmol/L in the absence of glucose-lowering medication for a minimum of 12 months. Micronutrient supplementation was based on British Obesity and Metabolic Surgery Society guidance (28).

Mechanistic Visits

Mechanistic assessments took place at three time points: preoperatively, at 10–14 days after surgery to examine the effects of the interventions before substantial weight loss has taken place, and when 20% of weight loss was achieved to remove weight loss as a confounding variable. Five days prior to the mechanistic visits, all glucose-lowering medications were discontinued, and intermediate-acting insulin was used as a rescue treatment, if necessary. Patients were asked to refrain from alcohol and strenuous physical activity for 48 h before the visit. They were admitted to the Imperial or King's National Institute for Health Research clinical research facilities in the evening and

consumed a standardized meal. The next morning, they underwent a two-stage euglycemic hyperinsulinemic clamp with stable isotope labeled [6, 6-²H₂] glucose using a validated protocol (29). Stage 1 consisted of insulin infusion at 0.5 mU kg⁻¹ min⁻¹ (low dose) for 120 min to measure the insulin sensitivity of endogenous glucose production; stage 2 consisted of insulin infusion at 1.5 mU kg⁻¹ min⁻¹ (high dose) for 120 min to measure the insulin sensitivity of peripheral glucose uptake. On the morning of the third and final day of their visit, they underwent a mixed meal tolerance test. Blood samples were obtained before and for 180 min following a liquid meal (Ensure Compact, 300 kcal in 125 mL; 17% protein, 35.1% fat, and 47.9% carbohydrates).

Sample Analysis

Plasma/serum samples were stored at –80°C until further analysis. Glucose was measured on the ARCHITECT c8200 platform using a hexokinase method, insulin using ARCHITECT i2000SR immunoassay, active GLP-1, and PYY using a customized multiplexed Magpix immunoassay. Glucose isotopic enrichment was measured by Gas chromatography–mass spectrometry on a HP 5971A MSD (Agilent Technologies, Wokingham, Berks, U.K.). R_a and R_d from plasma were calculated using non-steady-state equations proposed by Steele and modified for stable isotopes (30).

Sample Size Calculations

The majority of published studies have shown that peak active GLP-1 concentrations are approximately twofold greater after standard limb RYGB (6,31) compared with preoperative values. We estimated that that peak active GLP-1 levels after long limb RYGB will be tripled at 10–14 days after surgery. We powered this trial to detect a statistically significant difference in peak active GLP-1 of 10.0 pmol/L between the group means assuming a SD of 10.8 pmol/L within each group. With a sample size of 20 completers in each arm, our statistical power was 80% to detect this difference at $\alpha = 0.05$.

Statistical Analyses

A detailed statistical analysis plan is available with the Supplementary Material. In summary, continuous variables were summarized using the number of (nonmissing) data points as mean and SD if found to follow a normal distribution. Continuous variables not found to be normally distributed were summarized by the number of data points as median and interquartile range (IQR). Categorical variables were summarized by frequency and percentage (based on the nonmissing sample size) of values in each category. All the analyses presented in this report were based on the full analysis population, which consisted of patients in the groups to which they were randomized, regardless of deviation from the protocol or whether they received the allocated surgery. Patients with completely missing data at the outcome time point were excluded from this data set for the particular outcome for which they had missing data. The analysis of the primary outcome was performed using ANCOVA. In the analysis, the peak of active GLP-1 concentration at the early mechanistic postoperative visit at 10–14 days was considered as the outcome measure, while baseline peak of active GLP-1 was included as a covariate. The baseline adjusted difference in outcome values between groups was reported along with a corresponding 95% CI.

Secondary outcomes measured on a continuous scale, with a baseline measurement, were analyzed using a similar approach to that outline for the primary efficacy outcome. The data from each postoperative time point were analyzed in a separate analysis. For continuous secondary outcomes where there was no baseline measurement, the two groups were compared using the unpaired *t* test. Alternatively, the Mann-Whitney *U* test was used if the assumptions of the *t* test were not met. Binary and nominal outcomes were compared between the two study groups using either the χ^2 test or Fisher's exact test if the number of responses in some categories was low. Ordinal outcomes were analyzed using the Mann-Whitney *U* test. Statistical significance was defined as a *P* value of *P* < 0.05. Association between outcomes were performed using Pearson correlation. Alternatively, Spearman's rank correlation was used if

the Pearson correlation assumptions were not met. Within-group comparisons were performed using the mixed model analysis. The data analyses were performed using the statistical software packages Stata (version 15.1), SPSS (version 20 or later), and GraphPad PRISM (version 6 or later).

RESULTS

Participants

A total of 53 participants were recruited into the study; 27 patients were randomized to the standard limb RYGB group, and 26 patients were randomized to the long limb RYGB group. For unexpected intraoperative anatomical reasons, one patient in the standard limb group underwent a vertical sleeve gastrectomy, and one patient in the long limb group underwent a one-anastomosis gastric bypass. These patients were excluded from the mechanistic analyses but were included in the clinical analyses as per intention to treat. There were no significant differences in the rates of surgical complications between the two groups (Supplementary Table 5).

Baseline Characteristics

There were no significant differences in baseline characteristics between the two groups (Table 1 and Supplementary Table 8). The majority of the patients were middle-aged white European females. The mean BMI was 42 ± 6 kg/m² in the standard limb and 43 ± 8 kg/m² in the long limb group. Patients in the standard limb group had a mean HbA_{1c} of 73 ± 17 mmol/mol, median duration of T2D of 8 (IQR 6–10) years, and were taking a median number of 3 (2–3) glucose-lowering medications (Table 1). Patients in the long limb group had an HbA_{1c} of 76 ± 16 mmol/mol, median duration of T2D of 8 (6–9) years, and were taking a median number of 3 (2–3) glucose-lowering medications. There were no differences in the mechanistic measurements at baseline between the two groups.

Primary Outcome Measure

Compared with baseline, patients in both groups exhibited a significant increase in the postprandial peak of active GLP-1 concentrations at 2 weeks after surgery (Fig. 2). There were also

significant increases in the postprandial peak of active GLP-1 concentrations and area under the curve (AUC) compared with baseline within both groups at the point of 20% weight loss (Supplementary Table 6). However, there were no significant differences between the standard and long limb groups in terms of the GLP-1 response at any time point (Fig. 2 and Supplementary Table 1). There were also no differences between groups in the time to GLP-1 peak which was 30 min.

Secondary Outcomes

Glucose Tolerance and Insulin Secretion

Fasting and total postprandial glucose concentrations (AUC) at the mixed meal tolerance test were significantly reduced compared with baseline within both groups at the 2 week and at matched 20% weight loss (Supplementary Table 6), but there were no significant differences between the groups at any time point (Fig. 2 and Supplementary Table 1). There were small but statistically significant differences in incremental glucose AUC between the groups, with lower concentrations in the long limb compared with the standard limb group (Supplementary Table 1). The peak concentration of postprandial insulin at the mixed meal tolerance test was significantly increased within both groups at 2 weeks and at matched 20% weight loss compared with baseline (Supplementary Table 6). However, there were no significant differences between the groups at any time point (Fig. 2 and Supplementary Table 1). The total AUC of the postprandial insulin concentration did not change significantly either within or between groups (Supplementary Table 6).

Insulin Sensitivity

The *R*_a during the low-dose insulin infusion of the euglycemic hyperinsulinemic clamp decreased significantly within both groups at 2 weeks and at matched 20% weight loss compared with baseline (Supplementary Table 6), indicating substantially improved hepatic insulin sensitivity both early and after substantial weight loss. However, there were no significant differences between the groups at any time point (Fig. 3 and Supplementary Table 2). The *R*_d during the high-dose insulin infusion of the euglycemic hyperinsulinemic clamp, a

Table 1—Key clinical parameters at baseline and at 1 year after intervention

Characteristic	At baseline		1 year postoperatively		P value*
	Long limb group (n = 26)	Standard limb group (n = 27)	Long limb group (n = 26)	Standard limb group (n = 26)	
Sex, female	18 (69)	16 (59)			
Ethnicity					
White	18 (69)	23 (85)			
Asian	6 (23)	2 (7.5)			
Afro-Caribbean	2 (8)	2 (7.5)			
Age (years)	48 ± 9	49 ± 10			
Weight (kg)	121 ± 28	117 ± 18	87 ± 24	82 ± 13	0.36
BMI (kg/m ²)	43 ± 8	42 ± 6	31 ± 7	29 ± 5	0.43
Total body weight loss (%)			29 ± 8	30 ± 8	0.52
Waist circumference (cm)	128 ± 14	129 ± 12	99 ± 16	97 ± 12	0.39
Neck circumference (cm)	44 ± 6	44 ± 4	37 ± 5	37 ± 4	0.87
Total body fat percentage (%)	44 ± 6	43 ± 7	30 ± 9	27 ± 8	0.32
Total body fat free mass (kg)	66 ± 15	63 ± 13	56 ± 12	55 ± 9	0.30
Duration of T2D (years)	8 (6–9)	8 (6–10)			
Number of glucose-lowering medications	3 (2–3)	3 (2–3)	0 (0–0)	0 (0–0)	NS
HbA _{1c} (mmol/mol)	76 ± 16	73 ± 17	41 ± 5	43 ± 10	0.20
Rate of T2D remission			20 (77)	16 (62)	0.23

Data are n (%), median (IQR), or mean ± SD. Statistical tests used: ANCOVA, unpaired *t* test, logistic regression, Fisher exact test. *P* values compare long limb versus standard limb outcomes at 1 year postoperatively using ANCOVA and the baseline observation of interest as the covariate.

measure of peripheral insulin sensitivity, increased significantly within both groups at 2 weeks and at matched 20% weight loss compared with baseline (Supplementary Table 6). However, there were no significant differences between the groups at any time point (Fig. 3 and Supplementary Table 2). The results did not change when R_a and R_d were corrected for the prevailing serum insulin concentrations during the clamp.

Clinical Outcomes

Glycemic Control and Weight Loss

Both groups experienced significant improvement of T2D after surgery as indicated by all measures of glycemic control. HbA_{1c} levels and fasting glycemia reduced significantly in both groups compared with baseline (Supplementary Fig. 1A and Supplementary Table 7). However, there were no significant differences in HbA_{1c} concentrations between the standard limb and long limb at any time point postoperatively including at 12 months (standard limb 43 ± 10 mmol/mol vs. long limb 41 ± 5 mmol/mol, *P* = 0.20) (Table 1). There were no statistically significant differences in the percentage of patients achieving remission of hyperglycemia at 12 months between groups, either in the

intention-to-treat or per-protocol analysis (standard limb 62% vs. long limb 77%, *P* = 0.23) (Table 1, Supplementary Fig. 1C, and Supplementary Table 8). The usage of glucose-lowering medications decreased similarly in both groups (Supplementary Table 3 and Supplementary Fig. 2).

At 2 weeks after surgery, patients in both groups lost a similar amount of total body weight (standard limb 6.2 ± 2.3% vs. long limb 6.1 ± 1.6%, *P* = 0.97). As per protocol, both groups were studied again at matched 20% weight loss; this occurred at a mean of 4.5 months after surgery (standard limb 21.5 ± 2.8% vs. long limb 20.6 ± 2.7%). There were no significant differences in total body weight loss percentage between the groups at any time point postoperatively including at 12 months (standard limb 30 ± 8% vs. long limb 29 ± 8%, *P* = 0.52) (Supplementary Fig. 1B).

Small Intestinal Length

The median total small intestinal length in the standard limb group was 615 cm (range 320–740 cm), and in the long limb group, it was 610 cm (range 520–910 cm; *P* = 0.10). The median common channel length in the standard limb group was 465 cm (range 170–590

cm), and in the long limb group, it was 360 cm (range 250–660 cm; *P* = 0.12). The median biliopancreatic limb/total small intestinal length ratio in the standard limb group was 8% (range 7–16%), and in the long limb group it was 25% (range 16–29%; *P* < 0.001) (Supplementary Table 4). However, there were no significant correlations between the biliopancreatic limb/total small intestinal length ratio and GLP-1 peak concentration or AUC (Supplementary Table 4).

CONCLUSIONS

The findings of this mechanistic study show that increasing the length of intestinal bypass in RYGB is not associated with a greater postprandial GLP-1 and insulin secretion in humans. Despite incorporating a threefold longer biliopancreatic limb resulting in delivery of nutrients to more distal segments of the small intestine compared with the standard technique, the long limb RYGB did not produce any measurable difference in fasting or postprandial peak GLP-1 concentrations, time to peak concentrations, and total GLP-1 AUC. Indeed, the postprandial curves of GLP-1 response in the two groups were superimposable. While GLP-1 was chosen as the primary end point of this study to

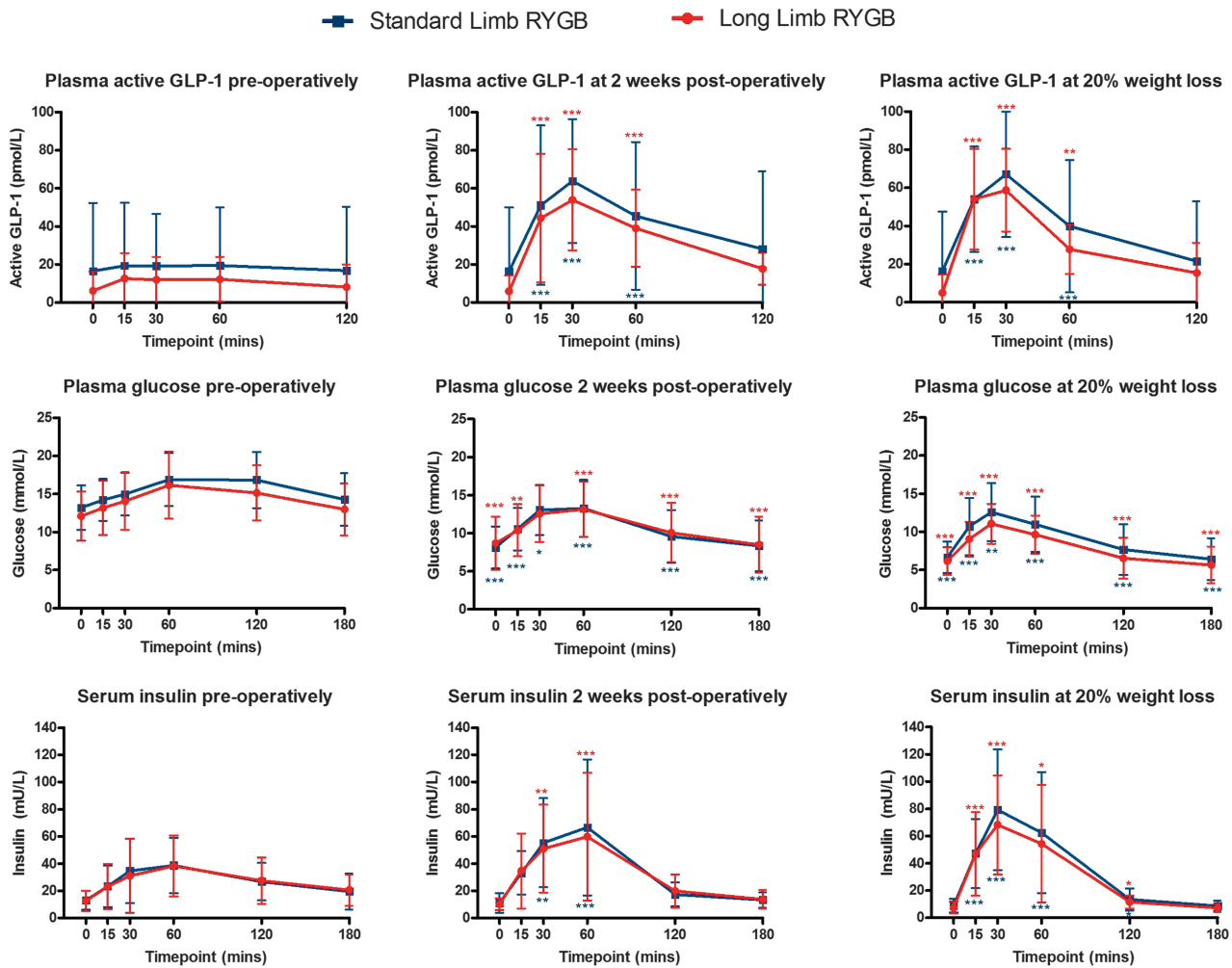


Figure 2—GLP-1, glucose, and insulin responses during the mixed meal tolerance test. Data are plotted as means \pm SD. A mixed effects model analysis with Bonferroni adjustment was used for multiple comparisons. Stars in blue and red indicate statistical significance in the within-group comparison of the standard limb and long limb groups, respectively, to baseline. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

test a physiologic hypothesis, we also did not observe any other differences in other measures of glucose homeostasis that have clinical relevance, i.e., fasting and postprandial glucose and insulin concentrations.

These findings challenge the widespread belief that the shunt of nutrient to more distal segments of the small intestine is the dominant mechanism by which RYGB enhances GLP-1 response (14). On the basis of this mechanism, the threefold longer bypass of the long limb RYGB used in this study should have elicited at least differences in time to peak or peak concentrations of GLP-1 compared with the standard procedure. One plausible explanation for our unexpected findings may be that there may be no linear relationship between GLP-1

secretion and the number of L cells exposed to ingested nutrients, as previously suggested (14). As enteroendocrine L cells are also located in the proximal intestine, the delivery of nutrients beyond a critical point in the jejunum may not result in further enhancement of the GLP-1 response. An alternative mechanism is that RYGB may change yet unknown mechanisms involved in the physiologic regulation of GLP-1 that depend on the integrity of the anatomy and physiology of the proximal small intestine. The anti-incretin framework postulates the existence of a homeostatic mechanism in which nutrient-stimulated anti-GLP-1 signals from the proximal small intestine compensate for the action of GLP-1 secreted in the distal small intestine to defend against postprandial

hyperinsulinemic hypoglycemia (32,33). Consistent with this model, bypass of the proximal small intestine might reduce the stimulation of factors, e.g., ketone bodies arising from the intestine (34), that tonically inhibit L-cell secretion, thus resulting in enhanced GLP-1 and, thus, insulin, response. This mechanism would explain why GLP-1 response is enhanced by a variety of procedures that disrupt the anatomy of the proximal small intestine and, conversely, why increasing the length of the bypass beyond a critical point, as in the long limb RYGB used in this study, does not produce appreciable differences in GLP-1 secretion. A third explanation of our findings is that RYGB may change yet unknown mechanisms involved in the physiologic regulation of

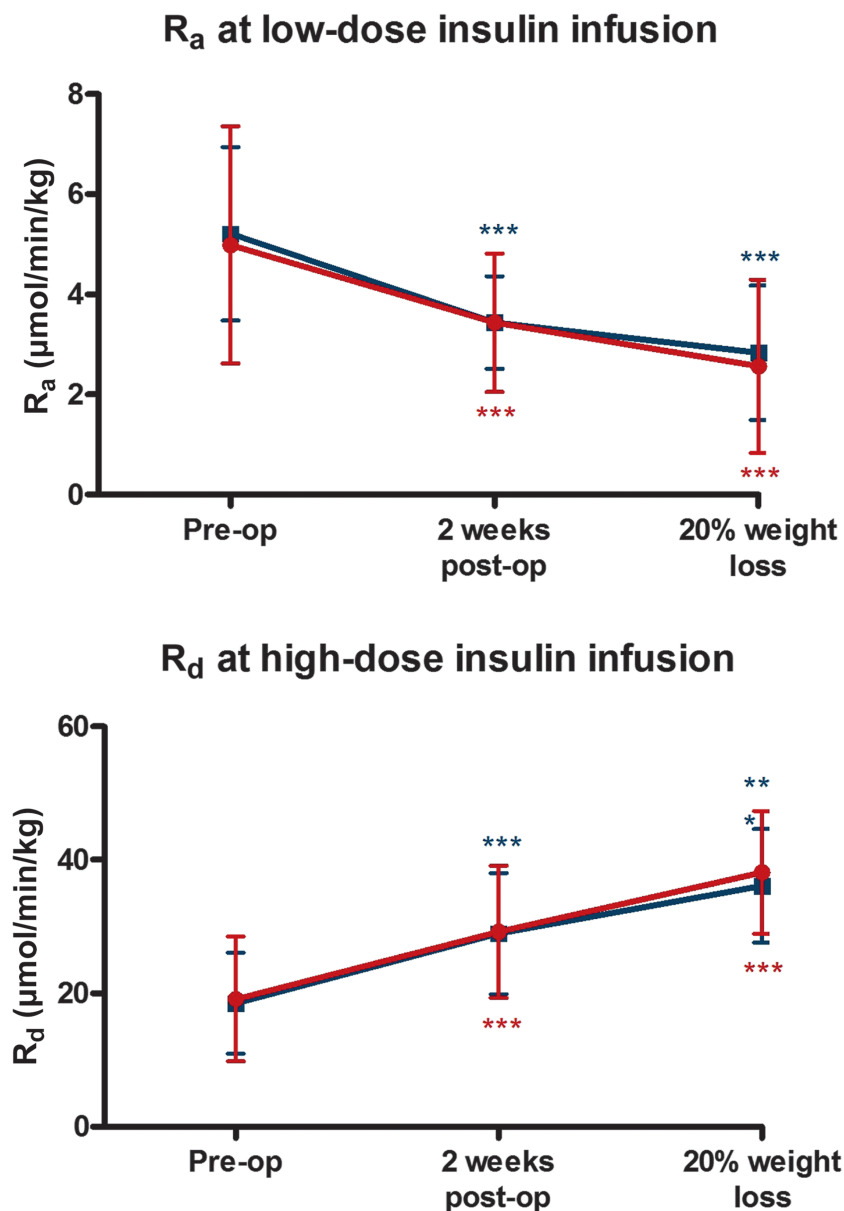


Figure 3—Measures of hepatic and peripheral insulin sensitivity during the euglycemic hyperinsulinemic clamp. R_a at the low-dose insulin infusion (measure of hepatic insulin sensitivity) and R_d at the high-dose insulin infusion (measure of peripheral insulin sensitivity). Data are plotted as means \pm SD. $N = 23$ in each group. A mixed effects model analysis with Bonferroni adjustment was used for multiple comparisons. Stars in blue and red indicate statistical significance in the within-group comparison of the standard limb and long limb groups, respectively, to baseline. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

GLP-1 that depend on the integrity of the anatomy and physiology of the stomach (35).

A previous retrospective case-control study demonstrated higher postprandial GLP-1 concentrations after long biliopancreatic limb RYGB compared with standard RYGB (36). Differences in both study design and study subjects may explain these conflicting observations. The patients in that retrospective study did not have T2D, were studied 4 years

after surgery, and underwent a slightly longer intestinal bypass (200 vs. 150 cm in our trial). It is theoretically possible, albeit unlikely, that a longer biliopancreatic limb than the one used in our trial may be associated with differences in intestinal adaptation leading to greater postprandial GLP-1 response in the long term. However, changes in GLP-1 response typically occur immediately after RYGB (37), and in this study, we did not observe any difference in GLP-1

response either 2 weeks after the operation or at the 20% weight loss time point (mean of 4.5 months postoperatively), a time interval that should allow for substantial intestinal adaptation to occur (38).

Our study demonstrated the substantial variability in the length of the small intestine (320–910 cm). We incorporated this confounder in our measurements by examining correlations between the percentage of the biliopancreatic limb length to the total small intestinal length and GLP-1 responses. There was no correlation between these two measurements both early and late after surgery. This means that GLP-1 secretion was not enhanced even in the subgroup of patients with relatively short small intestines in whom a long limb RYGB would have shunted nutrients relatively distally. Our aim was also to make our study clinically relevant. Thus, we elected to investigate a biliopancreatic length of 150 cm, which is commonly used in routine clinical practice globally without an adverse safety signal. Procedures with longer biliopancreatic diversions (e.g., biliopancreatic diversion or duodenal switch) could theoretically have resulted in enhanced GLP-1 responses, but these procedures involve resection of gastric tissue (a further confounding of gastrointestinal physiology) and are also less commonly performed due to the risk of severe macro- and micronutrient deficiencies. Furthermore, as a mechanistic investigation performed in a prospective randomized manner, our study is more robust than retrospective studies in avoiding confounding from differences in GLP-1 secretion at baseline. These considerations are reassuring about the ability of our study to detect differences, had they existed, in the effects of the two RYGB variants on GLP-1 secretion. In line with the equivalence on GLP-1 secretion, our study found no significant differences between the two groups in terms of clinical outcomes for the first year after surgery. Both patient groups, in fact, exhibited similar reduction in fasting glucose and HbA_{1c} levels, as well as weight loss at 12 months. Our findings are in line with other studies where a longer biliopancreatic limb was used for RYGB but resulted in no additional benefit in terms of reduction in HbA_{1c}, T2D remission, or weight loss (19,20,39,40). Although other

studies reached opposite conclusions, it must be noted that the majority were in fact designed to alter the length of both biliopancreatic and alimentary limbs at the same time. This must be considered when interpreting their findings.

Several aspects of the study design strengthen the reliability of the results. To our knowledge, this is the first mechanistic study that utilized a double-blind randomized controlled design to conduct a head-to-head comparison between two variants of RYGB. The entire length of the small intestine was measured during all operations. Deep metabolic phenotyping of all participants was performed after washout of glucose-lowering medications early postoperatively, allowing mitigation of pharmacologic influence on glucose metabolism. We used several clinical and biological measures of glucose metabolism, including the gold-standard method of measuring insulin sensitivity through euglycemic hyperinsulinemic clamps with stable isotopes. Moreover, performing mechanistic tests early after surgery and again when the two groups of patients had achieved the same reduction of body weight removes any confounding from weight loss. Most importantly, this is the first study to attempt to isolate the specific contribution of the length of the biliopancreatic limb on glucose metabolism. Previous studies that looked at the role of the bypass of the proximal intestine in RYGB used variants of the procedure that also lengthened other intestinal limbs, were not randomized, or did not control for interference from on-going therapies with glucose-lowering medications or weight loss (15,19–23). Given the complexity of gastrointestinal physiology, the significant redundancy of mechanisms that influence glucose and weight regulation, and the effects of weight loss and on-going drug therapies on glucose homeostasis, identifying the role of distinct anatomic changes on physiologic and clinical effects of complex procedures, such as RYGB, requires rigorous and controlled designs. We demonstrated that our novel approach is feasible in which, until recently, clinicians have empirically altered the anatomy of operations based on speculation or personal preference, rather than solid and objective mechanistic evidence.

This study has some limitations. First, the primary end point examined GLP-1 secretion, and we cannot exclude that

varying the length of intestinal bypass could influence other gut hormones or other aspects of gastrointestinal physiology involved in glucose metabolism, i.e., changes in bile acid metabolism, gut microbiota, or intestinal glucose absorption in the common limb (41). The latter mechanism could have contributed to the slightly lower glucose concentrations in the long limb group only when incremental AUCs were compared. These differences appear to be small and not reflected in any of the other glucose indices measured. Second, this was an experimental medicine study with mechanistic outcomes and not a clinical trial. Thus, it was not powered to detect significant differences in clinical outcomes, and we only extended our follow-up to 1 year after surgery. Hence, we cannot derive definitive conclusions on the relative clinical efficacy of the two variants of RYGB tested in this study. However, the lack of any meaningful difference in fasting and postprandial glucose excursions, insulin sensitivity, or insulin secretion between the two groups of this study suggests that lengthening the intestinal bypass may not be an effective way to further improve efficacy of standard RYGB in the control of T2D or obesity, at least within the first postoperative year. The discrepant findings between human (13) and animal studies (42) have created controversy regarding the role of GLP-1 in the glycemic improvements after RYGB. However, the interrogation of its contribution to glucose regulation was beyond the scope of our study. Third, we did not measure gastric emptying as it has been demonstrated that this is rapid after RYGB, and the two procedures we tested did not differ in the anatomy of the gastric pouch or gastrojejunal anastomosis. Fourth, we did not measure orocecal transit time to formally confirm the presence of more rapid nutrient delivery to the distal small intestine and cecum.

In conclusion, this mechanistic study has demonstrated that the elongation of the biliopancreatic limb of RYGB from 50 to 150 cm is not associated with enhanced GLP-1 response in patients with T2D and obesity within the first year after surgery. Alternative proximal intestinal or gastric mechanisms might be responsible for the enhancement of GLP-1. Shifting the focus to the targeting of

those mechanisms will enable the optimization of metabolic surgery and drug development for T2D and obesity.

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