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GLP-1 Plays a Limited Role in Improved Glycemia Shortly After Roux-en-Y Gastric Bypass: A Comparison With Intensive Lifestyle Modification

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Rapid glycemic improvements following Roux-en-Y gastric bypass (RYGB) are frequently attributed to the enhanced GLP-1 response, but causality remains unclear. To determine the role of GLP-1 in improved glucose tolerance after surgery, we compared glucose and hormonal responses to a liquid meal test in 20 obese participants with type 2 diabetes mellitus who underwent RYGB or nonsurgical intensive lifestyle modification (ILM) (n = 10 per group) before and after equivalent short-term weight reduction. The GLP-1 receptor antagonist exendin$_{(9–39)}$-amide (Ex-9) was administered, in random order and in double-blinded fashion, with saline during two separate visits after equivalent weight loss. Despite the markedly exaggerated GLP-1 response after RYGB, changes in postprandial glucose and insulin responses did not significantly differ between groups, and glucagon secretion was paradoxically augmented after RYGB. Hepatic insulin sensitivity also increased significantly after RYGB. With Ex-9, glucose tolerance deteriorated similarly from the saline condition in both groups, but postprandial insulin release was markedly attenuated after RYGB compared with ILM. GLP-1 exerts important insulinotropic effects after RYGB and ILM, but the enhanced incretin response plays a limited role in improved glycemia shortly after surgery. Instead, enhanced hepatic metabolism, independent of GLP-1 receptor activation, may be more important for early postsurgical glycemic improvements.

Bariatric surgery has increased significantly as a treatment option for obese patients with type 2 diabetes mellitus (T2DM). Dysglycemia improves within days of the gastrointestinal diversionary procedure Roux-en-Y gastric bypass (RYGB), before significant weight loss occurs (1–3). Whether this phenomenon is predominantly attributable to caloric restriction or whether altered hormonal, neural, or nutrient signals play a pivotal role remains unclear. The dramatic increase in the potent insulin secretagogue GLP-1 after RYGB has been associated with improved glucose tolerance in multiple studies (4–6). GLP-1 further attenuates postprandial hyperglycemia by inhibiting glucagon secretion, suppressing endogenous glucose production (EGP), delaying gastric emptying, and promoting satiety (7,8). The enhanced postsurgical GLP-1 response is frequently hypothesized to be an important determinant of improved glucoregulation after RYGB, but causality has not been clearly established in humans.

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The physiological activity of GLP-1 has been extensively investigated through blockade of its receptor (GLP-1R) by exendin$_{(9–39)}$-amide (Ex-9) (9–11). Ex-9 completely abolishes the insulinotropic effects of GLP-1 but does not affect other hormones that stimulate insulin secretion (e.g., glucose-dependent insulinotropic polypeptide [GIP]) (12). Ex-9 was recently used to investigate the contribution of the enhanced GLP-1 response to RYGB-associated hyperinsulinism (13) and to glucose metabolism following RYGB (14–16). While these studies confirm that GLP-1 augments postprandial insulin release after RYGB, they were limited by the inclusion of individuals without T2DM (14), the lack of an appropriate control group (15), or were performed after the confounding effects of significant weight loss (16).

This study used Ex-9 to determine whether the glycemic improvements observed shortly after RYGB are primarily mediated by the enhanced postsurgical GLP-1 response compared with a control group that achieved equivalent short-term weight reduction through nonsurgical intensive lifestyle modification (ILM), which is not associated with changes in the incretin response (17–19).

RESEARCH DESIGN AND METHODS

Setting and Population

This prospective, mechanistic trial was conducted at the Hospital of the University of Pennsylvania in Philadelphia, PA, between June 2011 and April 2013. Participants were not randomly assigned to RYGB or ILM because we previously found that fewer than 16% of potential bariatric surgery patients were willing to be randomized to an intervention (20). RYGB participants were recruited from the Penn Metabolic Surgery Clinic. Controls, who were not seeking weight reduction surgery and were matched for age, BMI, and glycated hemoglobin (HbA_{1c}), were recruited from local medical practices and through advertising. Twenty obese men and women with T2DM, aged ≥18 years and with a BMI of 35 to 60 kg/m^2, were recruited for the study. Exclusion criteria included a duration of T2DM >10 years, HbA_{1c} >10.0%, daily insulin requirements >1.0 units/kg/day, use of medications known to affect weight (i.e., chronic oral or inhaled glucocorticoids, certain antipsychotic medications, or medications intended to promote weight loss) within 3 months of enrollment, significant medical conditions, and pregnancy or lactation. The study was approved by the institutional review board of the University of Pennsylvania. All participants provided written informed consent.

Treatment Groups

Roux-en-Y Gastric Bypass

All RYGB procedures were performed laparoscopically using a standardized 30-mL pouch and a 100-cm Roux limb, effectively bypassing the entire duodenum and the proximal jejunum. RYGB participants followed a typical diet after bariatric surgery, which provides 500 kcal/day for the first 4 weeks, gradually increasing to 900–1,000 kcal/day by the second 4 weeks.

ILM

Participants attended weekly 60-min group sessions for the first 16 weeks of the program, followed by every-other-week sessions for the remaining 8 weeks (24 weeks of treatment). Sessions were led by a behavioral psychologist and followed a structured program, as previously described (21,22). For the first 12 weeks, participants were prescribed a diet of 1,000–1,100 kcal/day that incorporated meal replacements (Health Management Resources, Boston, MA) to induce 10% loss of initial body weight, followed by weight maintenance procedures, as previously described (21,22).

Mixed-Meal Tolerance Test

Participants underwent mixed-meal tolerance tests (MMTTs) at baseline and on two separate occasions (within 5–10 days of each other) after equivalent weight reduction was achieved. Antihyperglycemic medications were discontinued 10 days before study visits. Rapid-acting insulin was held on the morning of study visits and long-acting insulin for the preceding 12 h. Following an overnight fast, one catheter was inserted in an antecubital vein for infusions and another was placed in a contralateral heated forearm vein for blood sampling. At $t = -120$ min, a priming dose of 5 mg/kg · fasting plasma glucose (mg/dL)/90 of 6,6$^2H_2$ glucose (99% enriched; Cambridge Isotopes Laboratories, Andover, MA) was administered over 5 min, followed by a continuous infusion (0.05 mg/kg/min) until meal ingestion to determine the rate of basal EGP. Prestimulus blood samples were taken 15, 10, 5, and 1 min before meal ingestion. At $t = 0$ min, the tracer infusion was stopped and participants consumed a liquid meal test (Boost, 240 mL, 240 kcal, 55% carbohydrate, 25% protein, and 20% fat) at a standardized rate of 20 mL/min over 12 min. Subsequent blood sampling occurred at 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 105, 120, 140, 160, and 180 min after ingestion.

Ex-9 Administration

Lyophilized Ex-9 (Protein/Peptide Core Facility at Massachusetts General Hospital, Boston, MA), which was greater than 95% pure, was reconstituted in 0.9% saline and 0.25% human albumin and dispensed by the Penn Investigational Drug Service. Ex-9 or matching placebo infusion was administered, in random order, during two separate visits at the point of equivalent weight reduction. Both participants and study staff were blinded to the Ex-9 or placebo assignment. At 60 min before meal ingestion, an intravenous bolus of saline or Ex-9 (7,500 pmol/kg) was administered for 1 min, followed by a continuous infusion (750 pmol/kg/min) until 180 min after ingestion. This Ex-9 infusion rate, which has been used in prior bariatric studies (13,16), blocks the insulinotropic effects of supraphysiologic infusions of GLP-1 almost completely (23).

Biochemical Analysis

All samples were collected on ice in tubes containing EDTA. Protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO)

with dipeptidyl peptidase-4 inhibitor (Millipore, Billerica, MA) was immediately added and the samples were centrifuged at 4°C, separated, and frozen at −80°C. Plasma glucose was determined in duplicate by the glucose oxidase method using an automated glucose analyzer (YSI 2300; Yellow Springs Instruments, Yellow Springs, OH). Plasma immunoreactive insulin, C-peptide, and glucagon were measured in duplicate by double-antibody radioimmunoassays (Millipore) at the Penn Diabetes Research Center (DRC). Active GLP-1 and GLP-2 and total GIP were measured in duplicate by ELISA (Millipore) at the Penn DRC and the Translational Core Laboratory of the Children's Hospital of Philadelphia, respectively. Enrichment of $6,6^2H_2$ glucose was measured using gas chromatography–mass spectrometry at the Metabolic Tracer Resource of the Penn Institute for Diabetes, Obesity and Metabolism.

Calculations

Hormonal and metabolic responses to the meal challenge were determined by calculating the integrated area over baseline (incremental area under the curve [iAUC]) by the trapezoidal rule, with the mean of the four baseline values subtracted using Origin software (version 8.5; OriginLab Corp., Northhampton, MA).

Measures of insulin secretion and sensitivity were derived from data collected during the MMTT. The insulin secretion index (ISI), a standard measure of β-cell function in response to a liquid meal test, was calculated as the ratio of the insulin to glucose response (insulin $iAUC_{0–120}$/glucose $iAUC_{0–120}$) (24). The Matsuda index of insulin sensitivity (MISI), which provides an estimate of whole-body insulin sensitivity that is highly correlated with measures from the euglycemic clamp, was calculated using the equation 10,000/sqrt (basal glucose × basal insulin × mean postprandial glucose × mean postprandial insulin) (25). MISI derived from a standard liquid meal test has been validated against the minimal model of insulin sensitivity (26). The disposition index (DI), a measure of β-cell compensation that relates insulin secretion to the prevailing insulin sensitivity, was calculated as the product of ISI and MISI (27). The DI derived from a standard liquid meal test has been validated against the DI derived from the frequently sampled intravenous glucose tolerance test (28). In addition, the DI derived from a standard liquid meal test has been specifically validated in individuals with T2DM after RYGB (29).

Basal rates of EGP were calculated using Steele's steady-state equation (30). Hepatic insulin sensitivity (HIS) was calculated using the equation 1,000/([basal EGP] × [basal insulin]) (25). Hepatic insulin clearance was calculated as the molar ratio of fasting serum C-peptide to insulin (CI) (31).

Outcomes

Outcome variables were measured at *1*) baseline (before weight loss); *2*) after equivalent weight reduction (−10% of initial body weight) with administration of saline or Ex-9 in random order; and *3*) 5–10 days later with the infusion that was not previously administered. Primary outcome variables were *1*) change in postprandial glucose tolerance (defined as the glucose iAUC) between groups after weight loss and *2*) change in postprandial glucose tolerance between groups under conditions of saline and GLP-1R blockade. Secondary outcome variables included the change in glucoregulatory hormonal responses to the MMTT under both conditions and calculated indices of insulin secretion and sensitivity and indices of hepatic glucose metabolism.

Statistics

Baseline characteristics between the two groups were compared using χ^2 tests for dichotomous variables and independent *t* tests (or the nonparametric equivalent) for continuous variables. Changes in glucose tolerance and other hormonal responses were compared in the intention-to-treat (ITT) population with the use of repeated measures linear mixed-effects models. Mixed-effects models use all available data without imputation and are more efficient than traditional methodologies (e.g., *t* tests) (32). A sensitivity analysis was performed to adjust for baseline differences between groups by entering the following covariates into each of these models: baseline weight, baseline HbA_{1c}, duration of diabetes, and insulin use. An additional sensitivity analysis was performed to include only participants who completed the study. All analyses were performed using SAS software (version 9.3; SAS Institute, Cary, NC), with a significance level of 0.05.

RESULTS

Baseline Characteristics of Study Participants

We recruited 20 participants (10 per group) for the study, of whom 16 completed visits at baseline and after equivalent weight reduction. Two ILM participants failed to achieve the target weight loss after 24 weeks of treatment and did not complete the remaining study visits. One RYGB participant was withdrawn after developing serious postoperative complications and a second was excluded after a pancreatic mass was found at the time of surgery, requiring partial pancreatectomy.

Baseline clinical characteristics of the study population did not differ significantly between groups (Table 1). Participants had a mean (SD) age of 52.9 (9.2) years, weight of 121.2 (21.7) kg, BMI of 42.5 (5.0) kg/m^2, HbA_{1c} of 7.5% (0.6%), duration of T2DM of 4.2 (3.1) years, and took an average of 2.1 (0.8) antidiabetes medications.

Achievement of Target Weight Loss

ILM participants lost a mean of 12.6 (1.2) kg (−10.2% of initial body weight) versus 12.0 (1.2) kg (−9.9%) in the RYGB group (P = 0.95). Target weight loss was achieved more rapidly after RYGB than ILM (58.9 [12.1] vs. 85.5 [24.4] days, respectively; P = 0.02). Both groups lost minimal weight between the second and third study visits, which occurred at a mean of 6.6 (1.8) days apart. All ILM participants continued to require antidiabetes medications after weight loss, whereas the majority of the

Table 1—Baseline characteristics of participants

	ILM (n = 10)	RYGB (n = 10)	P value
Age (years)	51.8 (11.6)	54.0 (6.6)	0.61
Sex (%)			0.14*
Male	5 (50.0)	1 (10.0)	
Female	5 (50.0)	9 (90.0)	
Race (%)			0.63*
White	8 (80.0)	6 (60.0)	
Black	2 (20.0)	4 (40.0)	
Weight (kg)	122.0 (20.6)	120.5 (23.9)	0.88
BMI (kg/m^2)	41.8 (3.8)	43.2 (6.0)	0.53
Waist circumference (cm)	134.6 (13.5)	129.0 (18.0)	0.44
HbA_{1c} (%)	7.5 (0.7)	7.5 (0.6)	0.95
Duration of T2DM (years)	3.1 (2.7)	5.2 (3.3)	0.13
Antidiabetes medications (n)**	2.5 (0.2)	1.9 (0.2)	0.08
Diabetes medications (%)			0.21*
Oral medications only	9 (90.0)	6 (60.0)	
Insulin only	0 (0.0)	3 (30.0)	
Oral medication and insulin	1 (10.0)	1 (10.0)	

Data are presented as means (SD) unless otherwise indicated. $P > 0.05$ for all comparisons between groups. *Fisher's exact test used. **Four participants in the ILM group took dipeptidyl peptidase-4 inhibitor intravenously at the baseline visit and two discontinued the medication before the second study visit. One participant in the RYGB group took a GLP-1 mimetic at the baseline visit, which was discontinued before the second study visit.

RYGB group (5 of 8) had discontinued all antidiabetes medications after surgery (P = 0.03).

Postprandial Glucose and Hormonal Responses After Equivalent Weight Loss

Glucose and hormonal curves are shown in Figs. 1 and 2. The change in fasting glucose and hormonal concentrations did not differ significantly between groups after equivalent weight reduction (Table 2). These parameters tended to decrease significantly from baseline in both groups, with the exception of fasting GLP-1 and GLP-2 concentrations (which were essentially unchanged).

Peak glucose concentrations were also similar in both groups after weight loss, but the shape and the temporal patterning of the glucose tolerance curve markedly differed (Fig. 1). Peak glucose values were achieved earlier in the RYGB group and returned to baseline values more rapidly.

Surprisingly, the change in postprandial glucose tolerance over the 180-min period after meal ingestion did not significantly differ between groups with equivalent weight loss (P = 0.11). Although the glucose response to meal ingestion decreased significantly from baseline with ILM (P = 0.003), it was only modestly reduced following RYGB.

To account for the rapid absorption of glucose following RYGB, the glucose response to meal ingestion also was evaluated from time 0 to 120 min and from time 120 to 180 min. The glucose iAUC was essentially unchanged from baseline in the RYGB group over the first 120 min, reflecting the rapid and exaggerated peak in postprandial glucose. In contrast, the glucose iAUC decreased significantly in the ILM group over the first 120 min (P = 0.026). Despite the change in the temporal patterning of the glucose response following RYGB, the glucose iAUC decreased similarly in both groups over time 120 to 180 min.

As expected, the GLP-1 response increased significantly after RYGB but was essentially unchanged with ILM ($P <$ 0.001 for the comparison between groups). The GLP-2 response also was significantly enhanced after RYGB ($P <$ 0.001 for the comparison between groups). In contrast, the GIP response did not significantly differ between groups.

The insulin response to meal ingestion increased similarly in both groups following equivalent weight reduction (P = 0.84 for the comparison between groups). Postprandial insulin release increased by 18.3% following RYGB compared with 10.9% after ILM. Similarly, the C-peptide response increased in both groups (P = 0.49 for the comparison between groups). However, the postprandial C-peptide response seemed to increase from baseline to a greater extent within the RYGB group (25.9%; P = 0.002) versus the ILM group (6.8%; P = 0.17). Thus, the temporal patterning of the glucose and hormonal responses improved to a greater extent after RYGB, although this was not reflected in the iAUCs. Paradoxical to the robust GLP-1 response, postprandial glucagon release was increased nearly fourfold following surgery but decreased after ILM ($P <$ 0.001 for the comparison between groups).

Postprandial Glucose and Hormonal Responses With GLP-1R Blockade

The change in fasting glucose and hormonal concentrations did not differ significantly between groups with

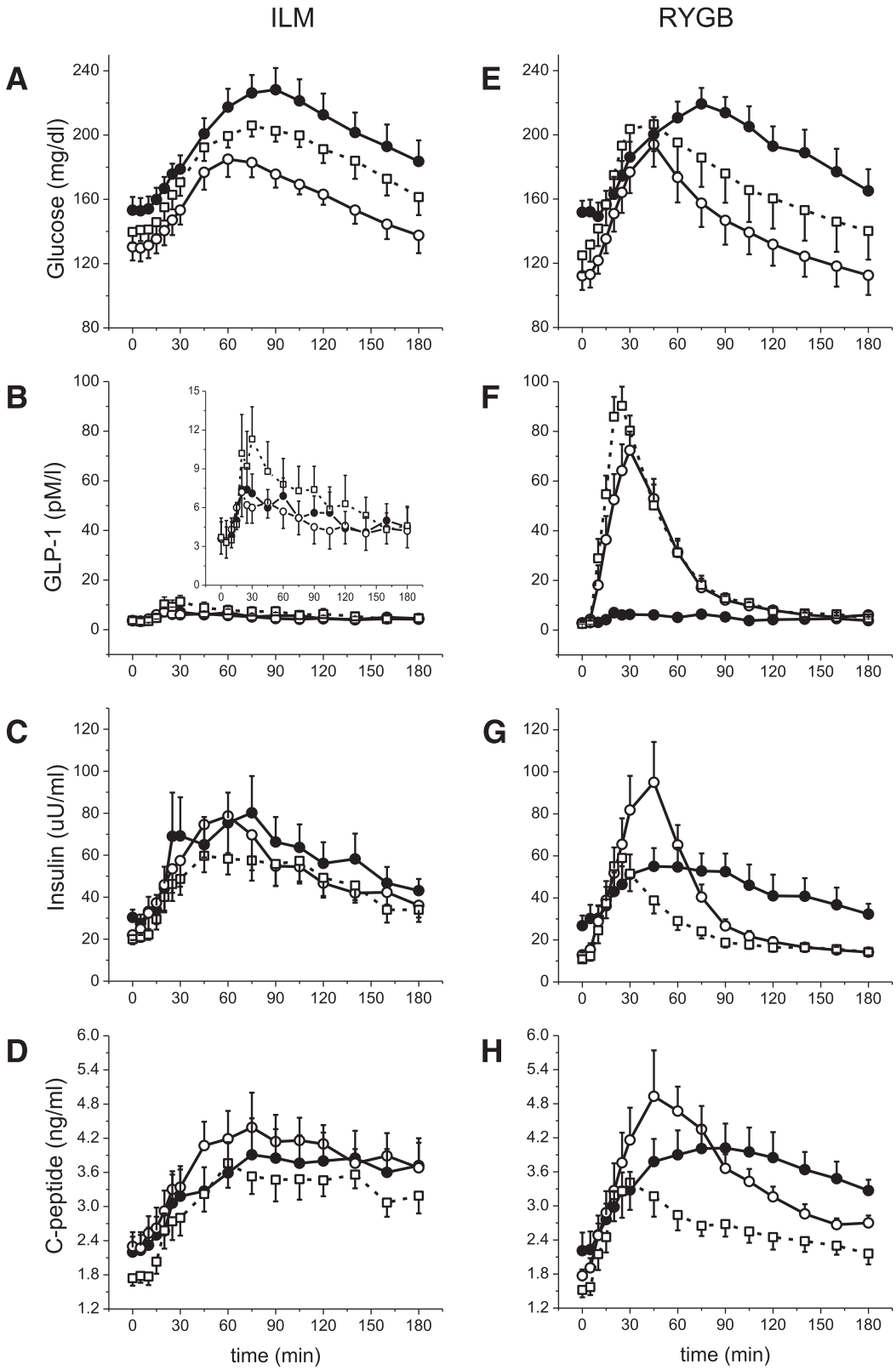

Figure 1—Glucose, GLP-1, insulin, and C-peptide responses to meal ingestion. ILM (panels *A–D*) and RYGB responses (panels *D–G*) are shown at baseline (black circles), after equivalent reduction of 10% of initial body weight with saline (white circles), and after equivalent weight reduction with GLP-1R blockade (white squares). Data are presented as means (SEMs).

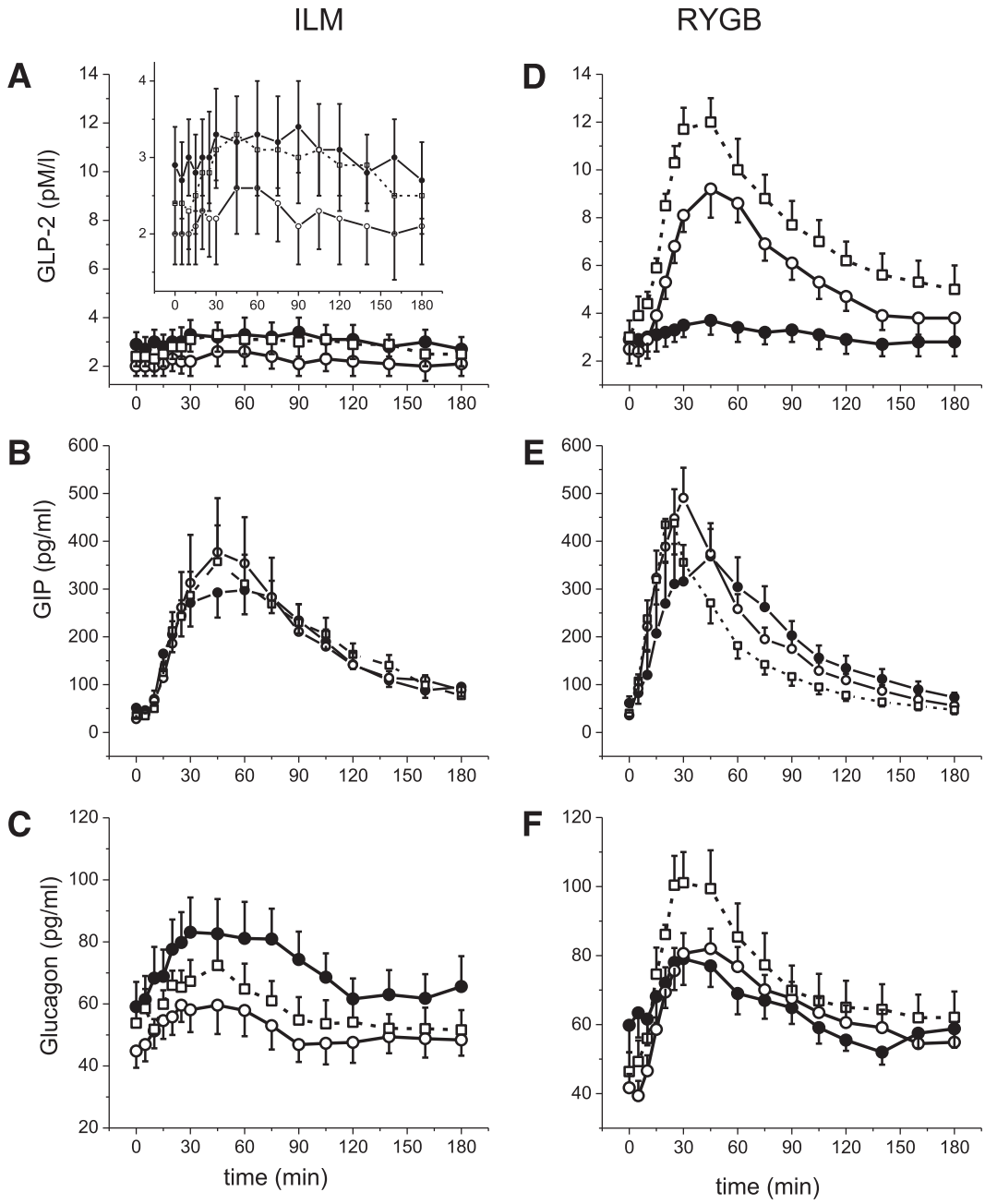

Figure 2—GLP-2, GIP, and glucagon responses to meal ingestion. ILM (panels *A–C*) and RYGB responses (panels *D–F*) are shown at baseline (black circles), after equivalent reduction of 10% of initial body weight with saline (white circles), and after equivalent weight reduction with GLP-1R blockade (white squares). Data are presented as means ± SEMs.

administration of Ex-9 (Table 3). However, fasting glucose concentrations increased significantly in both groups.

Changes in the glucose and hormonal responses to meal ingestion after GLP-1R blockade also did not differ significantly between groups. Postprandial glucose tolerance deteriorated significantly relative to the saline condition in both groups, with a 44.4% increase in the glucose response after ILM (P = 0.003) and a 41.5% increase following RYGB (P = 0.005). Postprandial insulin release was markedly attenuated following RYGB (−44.4%; P = 0.01), whereas it decreased marginally after ILM. Similarly, the C-peptide response decreased significantly with GLP-1R blockade following RYGB but was only modestly reduced after ILM (P = 0.04 for the comparison between groups). The postprandial GLP-1 and GLP-2 responses further increased in both groups with administration of Ex-9, whereas the glucagon response was modestly increased only in the RYGB group.

Changes in Indices of Insulin Secretion and Sensitivity

β-Cell function, as indicated by ISI, improved similarly in both groups after equivalent weight reduction (Fig. 3).

Table 2—Anthropometric and metabolic data pre- and post-10% weight loss

	ILM (n = 10)			RYGB (n = 10)			
	Baseline	After 10% weight loss	Δ with 10% weight loss	Baseline	After 10% weight loss	Δ with10% weight loss	P value
Anthropometric and metabolic measures							
Weight (kg)	122.0 (6.5)	109.4 (6.3)	−12.6 (1.2)++	120.5 (7.6)	108.5 (6.3)	−12.0 (1.2)++	0.76
BMI (kg/m^2)	41.8 (1.2)	37.3 (1.4)	−4.5 (0.4)++	43.2 (1.9)	39.1 (1.4)	−4.1 (0.4)++	0.54
Waist circumference (cm)	134.6 (4.3)	126.6 (4.9)	−8.0 (0.8)++	129.0 (5.7)	121.1 (4.9)	−7.9 (1.2)++	0.94
HbA_{1c} (%)	7.5 (0.2)	6.4 (0.1)	−1.1 (0.2)++	7.5 (0.2)	6.4 (0.1)	−1.1 (0.2)++	0.88
Fasting measures							
Glucose (mg/dL)	152.2 (7.8)	129.9 (8.5)	−22.2 (9.2)*	155.0 (6.4)	113.4 (8.5)	−41.6 (9.2)++	0.15
GLP-1 (pmol/L)	3.6 (1.0)	3.6 (0.8)	0.0 (0.4)	2.7 (0.4)	2.6 (0.8)	−0.1 (0.4)	0.79
GLP-2 (pmol/L)	2.9 (0.5)	2.0 (0.5)	−0.9 (0.5)	2.9 (0.6)	2.5 (0.5)	−0.4 (0.5)	0.45
GIP (pg/mL)	51.7 (5.2)	28.1 (4.6)	−23.6 (10.2)*	57.6 (12.0)	35.8 (4.6)	−21.8 (10.2)*	0.90
Insulin (μU/mL)	28.3 (3.3)	21.7 (1.9)	−6.5 (4.1)	28.1 (5.0)	13.1 (1.9)	−15.0 (4.1)+	0.16
C-peptide (ng/mL)	2.2 (0.3	2.3 (0.2)	0.1 (0.3)	2.2 (0.3)	1.8 (0.2)	−0.4 (0.3)	0.25
Glucagon (pg/mL)	63.7 (7.2)	46.9 (4.4)	−16.8 (47)+	61.5 (6.3)	42.5 (4.4)	−19.0 (4.7)++	0.75
Glucose and hormonal responses to MMTT (iAUC)							
Glucose (mg · dL^{-1} · min^{-1})	8,403.4 (979.9)	5,320.5 (711.1)	−3,082.9 (896.2)+	6,484.9 (991.4)	5,558.1 (711.1)	−926.8 (896.2)	0.11
0–120 min	5,827.0 (585.7)	4,206.5 (613.6)	−1,620.5 (601.2)*	4,989.4 (585.7)	5,062.6 (613.6)	73.3 (601.2)	0.06
120–180 min	2,576.4 (501.9)	1,121.2 (285.5)	−1,455.2 (446.4)+	1,535.5 (501.9)	495.1 (285.5)	−1,040.5 (446.4)*	0.52
GLP-1 (pmol · L^{-1} · min^{-1})	321.6 (84.7)	236.0 (276.0)	−85.6 (284.3)	362.2 (93.1)	3,393.8 (276.0)	3,031.6 (284.3)++	<0.001
GLP-2 (pmol · L^{-1} · min^{-1})	39.7 (19.9)	59.1 (70.0)	19.3 (64.2)	43.9 (35.1)	558.0 (70.0)	514.0 (64.2)++	<0.001
GIP (pg · mL^{-1} · min^{-1})	24,155.1 (2,373.6)	30,462.0 (5,866.7)	6,307.4 (5,255.0)	22,178.6 (3,966.1)	27,506.0 (5,932.7)	5,327.3 (5,257.2)	0.90
Insulin (μU · mL^{-1} · min^{-1})	4,796.6 (1,297.4)	5,317.4 (960.5)	520.8 (851.1)	4,177.2 (983.0)	4,940.5 (960.5)	763.2 (851.1)	0.84
C-peptide (ng · mL^{-1} · min^{-1})	242.4 (49.0)	258.9 (42.9)	16.5 (47.9)	245.9 (49.0)	309.7 (42.9)	63.8 (47.9)+	0.49
Glucagon (pg · mL^{-1} · min^{-1})	1,960.5 (307.2)	1,254.9 (270.2)	−705.6 (615.3)	1,051.8 (637.2)	4,101.8 (270.2)++	3,050.0 (615.3)++	<0.001

Data are presented as means (SEs). The data for the two intervention groups are model-based estimates for the ITT population. P values refer to the delta between groups. ++$P < 0.001$ compared with baseline; *$P < 0.05$ compared with baseline; +$P < 0.01$ compared with baseline.

Table 3—Metabolic data with saline and Ex-9 infusion at the point of 10% weight loss

	ILM (n = 10)			RYGB (n = 10)			
	Saline	Ex-9	Δ with Ex-9	Saline	Ex-9	Δ with Ex-9	P value
Fasting measures							
Glucose (mg/dL)	129.9 (8.5)	139.3 (8.2)	9.4 (3.5)*	113.4 (8.5)	128.4 (8.3)	15.0 (3.7)++	0.28
GLP-1 (pmol/L)	3.6 (0.8)	3.7 (1.0)	0.1 (0.4)	2.6 (0.8)	2.7 (1.0)	0.2 (0.4)	0.81
GLP-2 (pmol/L)	2.0 (0.5)	2.4 (0.5)	0.4 (0.2)	2.5 (0.5)	2.9 (0.5)	0.4 (0.2)	0.98
GIP (pg/mL)	28.1 (4.6)	35.6 (7.1)	7.5 (5.7)	35.8 (4.6)	40.8 (7.4)	4.9 (6.1)	0.76
Insulin (μU/mL)	21.7 (1.9)	19.5 (2.0)	−2.2 (1.9)	13.1 (1.9)	10.7 (2.0)	−2.4 (2.0)	0.95
C-peptide (ng/mL)	2.2 (0.4)	1.9 (0.1)	−0.4 (0.1)*	1.8 (0.2)	1.5 (0.1)	−0.3 (0.1)	0.34
Glucagon (pg/mL)	46.9 (4.4)	55.4 (4.4)	8.5 (2.1)++	42.5 (4.4)	48.2 (4.5)	5.7 (2.2)*	0.37
Glucose and hormonal response to MMTT (iAUC)							
Glucose (mg · dL^{-1} · min^{-1})	5,320.5 (711.1)	7,683.4 (948.1)+	2,362.9 (681.6)+	5,558.1 (711.1)	7,863.3 (973.8)	2,305.2 (716.9)+	0.95
0–120 min	4,206.5 (613.6)	5,453.2 (570.1)	1,246.7 (492.8)*	5,062.6 (613.6)	6,472.3 (589.7)	1,409.7 (515.4)*	0.82
120–180 min	1,121.2 (285.5)	2,230.6 (443.7)	1,109.4 (291.7)+	495.1 (285.5)	1,374.3 (452.2)	879.3 (304.5)+	0.59
GLP-1 (pmol · L^{-1} · min^{-1})	236.0 (276.0)	525.9 (209.4)	289.9 (454.3)	3,393.8 (276.0)	3,844.3 (214.3)	450.6 (456.6)	0.81
GLP-2 (pmol · L^{-1} · min^{-1})	59.1 (70.0)	79.8 (75.7)	20.8 (114.0)	558.0 (70.0)	802.1 (85.1)	244.1 (120.5)	0.20
GIP (pg · mL^{-1} · min^{-1})	30,462.0 (5,866.7)	28,903.0 (2,913.6)	−1,559.5 (3,782.8)	27,506.0 (5,932.7)	20,149 (3,010.5)	−7,356.8 (3,849.1)	0.30
Insulin (μU · L^{-1} · min^{-1})	5,317.4 (960.5)	4,787.2 (540.8)	−530.3 (801.6)	4,940.5 (960.5)	2,720.3 (566.7)	−2,220.2 (819.2)*	0.16
C-peptide (ng · mL^{-1} · min^{-1})	258.9 (42.9)	245.8 (24.6)	−13.2 (31.8)	309.7 (42.9)	194.8 (24.6)	−114.8 (31.8)+	0.04
Glucagon (pg · mL^{-1} · min^{-1})	1,254.8 (270.2)	854.6 (468.4)	−400.2 (558.3)	4,101.8 (270.2)	4,699.5 (496.0)	597.7 (581.7)	0.23

Data are presented as means (SEs). The data for the two intervention groups are model-based estimates for the ITT population of 20 participants. P values refer to the delta between groups. *$P < 0.05$ compared with baseline; +$P < 0.01$ compared with baseline; ++$P < 0.001$ compared with baseline.

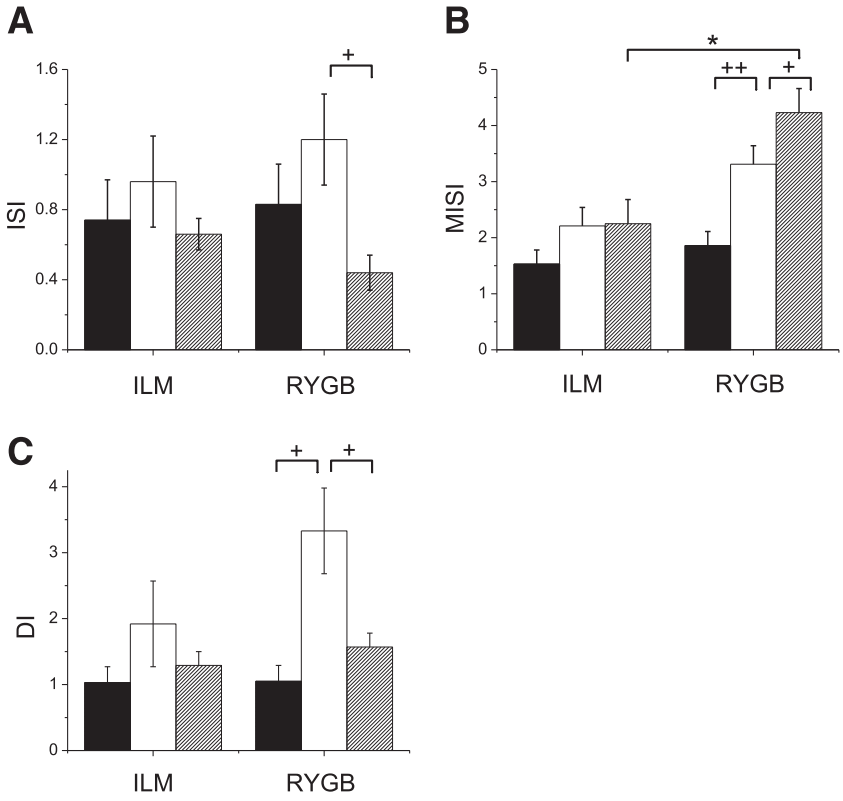

Figure 3—Calculated responses of insulin secretion and sensitivity in ILM and RYGB. Black bars indicate values at baseline (before weight loss); white bars indicate values after weight loss (−10% of initial body weight) with saline; gray hatched bars indicate values after weight loss (−10% of initial body weight) with Ex-9. Bar graphs of the area under the curve (AUC) for the ISI are shown in panel *A*, the MISI in panel *B*, and the DI in panel *C*. The data for the two intervention groups are model-based estimates for the ITT population. Data are presented as means ± SEMs. *$P < 0.05$; +$P < 0.01$; ++$P < 0.001$.

Whole-body insulin sensitivity, as estimated by MISI, also improved in both groups with weight loss ($P = 0.13$ for comparison between groups). However, MISI increased modestly from baseline after ILM ($P = 0.07$) but was significantly increased after RYGB ($P = 0.0006$). DI also increased in both groups after equivalent weight reduction ($P = 0.13$ for the comparison between groups). DI increased modestly following ILM but significantly increased by threefold after RYGB ($P = 0.002$).

With GLP-1R blockade, ISI decreased in both treatment groups ($P = 0.59$ for the comparison between groups). ISI decreased significantly from baseline in the RYGB group ($P = 0.002$) but was only modestly decreased in the ILM group. The change in MISI differed significantly between groups with administration of Ex-9 ($P = 0.04$). MISI was essentially unchanged in the ILM group with GLP-1R blockade but increased significantly after RYGB ($P = 0.004$). DI also decreased in both groups with GLP-1R blockade ($P = 0.11$ for the comparison between groups). DI decreased modestly in the ILM group but was significantly decreased following RYGB ($P = 0.002$).

Changes in Indices of Hepatic Glucose Metabolism

Greater suppression of basal EGP was observed after RYGB relative to ILM ($P = 0.043$), as shown in Fig. 4. Coincident with the reduction in basal rate of EGP, HIS was significantly improved after RYGB compared with ILM ($P = 0.02$). Hepatic insulin clearance, as estimated by the CI ratio, increased in both treatment groups with weight reduction ($P = 0.52$ for the comparison between groups). However, the CI ratio increased only modestly after ILM but increased significantly following RYGB ($P = 0.01$).

With GLP-1R blockade, basal EGP and HIS were essentially unchanged in both groups. Hepatic insulin clearance significantly decreased in both groups with GLP-1R blockade, although again, the difference between groups was not significant ($P = 0.86$).

Adjusted Analyses and Sensitivity Analyses

Findings did not differ after adjusting for baseline weight, HbA_{1c}, duration of T2DM, and insulin use or after an analysis of completers was performed.

DISCUSSION

The rapid and large increase in GLP-1 occurring shortly after RYGB has led to speculation that this incretin hormone may be an important mediator of early improvements in glycemia. Here we show that, despite the exaggerated postsurgical GLP-1 response, postprandial glucose tolerance improved similarly after RYGB relative to a control group that achieved equivalent short-term weight loss through ILM. Furthermore, blockade of the

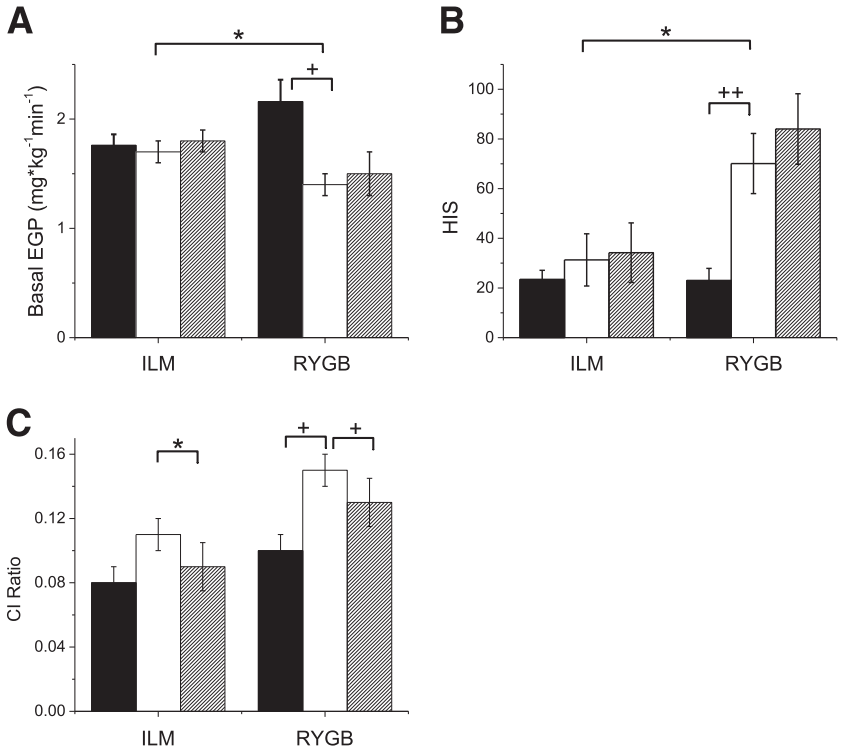

Figure 4—Calculated responses of hepatic glucose metabolism in ILM and RYGB. Black bars indicate values at baseline (before weight loss); white bars indicate values after weight loss (−10% of initial body weight) with saline; gray hatched bars indicate values after weight loss (−10% of initial body weight) with Ex-9. Bar graphs of the area under the curve (AUC) for basal EGP are shown in panel *A*, HIS in panel *B*, and hepatic insulin clearance (CI ratio) in panel *C*. The data for the two intervention groups are model-based estimates for the ITT population. Data are presented as means ± SEMs. *$P < 0.05$; +$P < 0.01$; ++$P < 0.001$.

GLP-1R resulted in similar deterioration of postprandial glucose tolerance in both groups, providing evidence against a greater contribution of GLP-1 action to improved glucose tolerance following surgery as compared with ILM.

Two recent studies suggest that factors independent of the enhanced incretin response, including caloric restriction and weight loss, may play a predominant role in short-term improvements in glycemia after RYGB. Despite the exaggerated postsurgical GLP-1 response, similar reductions in postprandial glucose tolerance were reported in individuals who underwent RYGB compared with those who consumed an isocaloric diet (200–300 kcal/day) that simulated postoperative intake (33) or those who achieved equivalent weight reduction by gastric banding (34). While these studies suggest that GLP-1 may play a smaller role in improved glycemia after RYGB than has been hypothesized, the direct effects of GLP-1 on glucose tolerance were not investigated.

Recent studies that have used GLP-1R blockade confirm that the enhanced postsurgical GLP-1 response is an important determinant of postprandial insulin release and improved β-cell function after RYGB (13–16). In these studies, as well as in the current study, administration of Ex-9 significantly attenuated the β-cell response to nutrients following RYGB. Postprandial glucose tolerance was not severely worsened with Ex-9 in individuals who underwent RYGB, and a similar degree of deterioration was observed in the control groups (which included lean or weight-matched participants). This supports our finding that the effects of GLP-1 on glycemia are not heightened after RYGB.

As previously reported, indices of insulin secretion and sensitivity in response to a liquid meal test improved within weeks of RYGB (15). However, these indices also improved in the ILM group, underscoring the important contribution of caloric restriction and weight loss. Whole-body insulin sensitivity, which reflects both hepatic and peripheral insulin sensitivity (25), improved significantly from baseline within the RYGB group but not within the ILM group. This phenomenon was largely driven by improvements in HIS, as described below.

Several recent studies corroborate our findings of the important contribution of enhanced hepatic glucose metabolism to early glycemic improvements following RYGB (35–37). Similar to the current study, rapid improvements in HIS and reductions in EGP have been reported as early as within 1 month after RYGB (35,36). However, these studies lacked a control group treated solely with caloric restriction, making it difficult to assess the relative contribution of caloric deficit to changes in hepatic parameters. A major strength of our study is the use of a control group with matched weight reduction. In

the current study, hepatic insulin sensitivity improved to a greater extent after equivalent weight reduction achieved by RYGB than ILM. This effect was driven by a marked decrease in the rate of basal EGP, a primary determinant of fasting glucose concentrations (38), after RYGB; the reduction in fasting plasma insulin concentrations was not significantly different between groups.

Several recent studies also have demonstrated rapid and significant improvements in hepatic insulin clearance following RYGB (35,37). In the current study, hepatic insulin clearance was enhanced following RYGB, but this increase was not significantly different from that observed in the ILM group after equivalent weight reduction. Because caloric restriction plays a significant role in improved hepatic glucose metabolism, it is certainly plausible that the overall caloric intake was lower in the RYGB group (36). However, other factors that are differentially affected by RYGB (e.g., GLP-2 [39], bile acids [40]) may also account for the greater improvements observed after surgery. In addition, hepatic fat content may decrease more quickly after RYGB than ILM, which may also affect hepatic glucose metabolism.

The paradoxical increase in postprandial glucagon release after RYGB may partially account for the attenuated improvement in postprandial glucose tolerance observed after RYGB relative to ILM. This increase was unexplained by the glucagonotropic gut hormone GIP (41) but may be mediated, in part, by the enhanced GLP-2 response, which is known to stimulate glucagon release (42). Increased neural or nutrient stimulation of islets (13,43) or stimulation of the secretion of gut-derived glucagon (44) may also contribute to the enhanced postsurgical glucagon response. Administration of Ex-9 further worsened hyperglucagonemia following RYGB, suggesting that GLP-1 may restrain an even more exaggerated glucagon response following surgery. Tolerance to GLP-1 as a result of tachyphylaxis or downregulation of the GLP-1R may also have developed after RYGB, which may have further attenuated improvements in glycemia (45).

Our study also had several limitations. The trial was not randomized, and slight or unmeasured clinical differences between groups at baseline may have confounded results. While the small number of participants may also have limited our ability to detect differences between groups, slight differences (particularly with respect to the duration of T2DM and the number of antidiabetes medications) potentially provide the greatest physiologic impact. Given the marked difference in the temporal patterning of the glucose response between groups as a consequence of the altered gastrointestinal anatomy following RYGB, the utility of glucose iAUC as a measure of glucose tolerance may be limited. However, we found no difference in glucose tolerance between groups when the glucose iAUC was measured over the first 120 min or during the final 60 min of the postprandial period. We also did not measure caloric intake and body composition (specifically hepatic fat content), which are important determinants of hepatic glucose metabolism. However, ILM participants recorded daily caloric intake in food diaries to corroborate their targeted intake (1,000 kcal/day). The RYGB participants did not quantify their caloric intake, but prior studies report an average intake of 800–1,000 kcal/day 2–3 months after surgery (46), which was approximately the time at which participants returned for their after RYGB assessments. We also did not measure EGP during the meal challenge, limiting our ability to draw conclusions about the dynamic relationship between the enhanced GLP-1 and glucagon responses and their effects on EGP.

In conclusion, we demonstrated that although GLP-1 plays an important role in glucose-mediated insulin secretion after RYGB, the robust postsurgical GLP-1 response does not seem to play a pivotal role in short-term improvements in glycemia after surgery in individuals with T2DM. Postprandial glucose tolerance decreased similarly between participants who achieved equivalent weight reduction by RYGB or nonsurgical ILM. Blockade of the GLP-1R caused comparable deterioration in the glycemic response in both groups, suggesting that GLP-1 action contributes equally to glucoregulation after RYGB or ILM. Despite the paradoxical hyperglucagonemia that was observed after RYGB, HIS was markedly enhanced in the surgical group relative to the ILM condition, an effect independent of GLP-1R activation, suggesting that hepatic mechanisms may play an important role in early improvements in glycemia after surgery. However, sustained increases in GLP-1 after surgery may have an important effect on long-term glucoregulation and β-cell function, and further study is warranted.

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Author Contributions. M.L.V., T.A.W., K.L.T., and M.R.R. designed the study. M.L.V. collected and analyzed the data and wrote the manuscript. Z.F.K., R.C., and S.R. collected the data. R.H.M. provided statistical consultation. R.H.M., J.L.C., and A.I. performed the statistical analyses. K.M., G.K., and N.N.W. referred participants. T.A.W., K.L.T., Z.F.K., R.C., S.R., R.H.M., K.M., G.K., N.N.W., and M.R.R. performed a critical review of the manuscript for important intellectual content. M.L.V. is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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