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# Early Enhancements of Hepatic and Later of Peripheral Insulin Sensitivity Combined With Increased Postprandial Insulin Secretion Contribute to Improved Glycemic Control After Roux-en-Y Gastric Bypass



**Roux-en-Y gastric bypass (RYGB) improves glycemic control within days after surgery, and changes in insulin sensitivity and  $\beta$ -cell function are likely to be involved. We studied 10 obese patients with type 2 diabetes (T2D) and 10 obese glucose-tolerant subjects before and 1 week, 3 months, and 1 year after RYGB. Participants were included after a preoperative diet-induced total weight loss of  $-9.2 \pm 1.2\%$ . Hepatic and peripheral insulin sensitivity were assessed using the hyperinsulinemic-euglycemic clamp combined with the glucose tracer technique, and  $\beta$ -cell function was evaluated in response to an intravenous glucose-glucagon challenge as well as an oral glucose load. Within 1 week, RYGB reduced basal glucose production, improved basal hepatic insulin sensitivity, and increased insulin clearance, highlighting the liver as an important organ responsible for early effects on glucose metabolism after surgery. Insulin-mediated**

**glucose disposal and suppression of fatty acids did not improve immediately after surgery but increased at 3 months and 1 year; this increase likely was related to the reduction in body weight. Insulin secretion increased after RYGB only in patients with T2D and only in response to oral glucose, underscoring the importance of the changed gut anatomy.**

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Roux-en-Y gastric bypass (RYGB) surgery induces weight loss and improves metabolic abnormalities in severely obese patients (1). In patients with type 2 diabetes (T2D), the glucose-lowering effect of RYGB is superior to that of conventional antidiabetic therapy (2,3) and often occurs within days after surgery (4). Insulin resistance of liver, skeletal muscle, and fat tissue is associated with an obese state, whereas patients with T2D additionally

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suffer from  $\beta$ -cell dysfunction and impairments of the incretin system (5).

Insulin sensitivity improves with weight loss, but whether increased insulin sensitivity plays a role in the early improvement of glycemic control after RYGB is not clear (6). Reductions in homeostasis model assessment of insulin resistance (HOMA-IR) have been demonstrated within 1 week (7–11), but assessment using a hyperinsulinemic clamp at this early time point has not been performed. At 2–3 weeks postoperatively, clamp studies have found no changes in peripheral insulin sensitivity (12,13), but improvements have been reported after 4 weeks (14) and later when weight loss is pronounced (12,13,15). HOMA-IR primarily reflects hepatic insulin resistance, while the hyperinsulinemic clamp estimates peripheral insulin sensitivity (16); this could indicate a differential effect of RYGB on hepatic and peripheral insulin sensitivity (6). Endogenous glucose production has not been assessed immediately after RYGB, but one study showed reductions after 1 month (17), whereas another did not detect changes at 2 weeks (12).

Postprandial insulin secretion increases already 1 week after RYGB (9,18) and is associated with exaggerated release of glucagon-like peptide 1 (GLP-1) (19–21). In contrast, after intravenous (IV) challenges, insulin secretion increases more gradually after RYGB in patients with T2D (14,22–24) and even declines in glucose-tolerant subjects (24,25). Improved  $\beta$ -cell function after RYGB thus may be linked to the oral rather than the IV route of administration (6). However, because insulin secretion adapts to changes in insulin sensitivity (26), assessment of  $\beta$ -cell function also requires concomitant evaluation of insulin sensitivity. Evaluation of  $\beta$ -cell function using oral and IV tests as well as clamp-derived measures of insulin sensitivity has previously been performed only 4 weeks after RYGB (14).

We studied patients with T2D and obese glucose-tolerant subjects before and 1 week, 3 months, and 1 year after RYGB, assessing hepatic and peripheral insulin sensitivity using the hyperinsulinemic clamp combined with a glucose tracer technique. A secondary aim was to evaluate insulin secretion in response to both an IV glucose-glucagon challenge and an oral glucose load.

## RESEARCH DESIGN AND METHODS

### Subjects

We recruited 10 obese patients with T2D (age  $43.6 \pm 3.4$  years; median duration of diabetes 2.5 years [range 1–11]) and 10 obese normal glucose-tolerant (NGT) subjects (age  $40.1 \pm 2.8$  years) who were scheduled for laparoscopic RYGB at Hvidovre Hospital (Hvidovre, Denmark). Before enrollment in the study, all participants fulfilled the inclusion criteria for bariatric surgery in Denmark and had completed a preoperative diet-induced total body weight loss of at least 8% as required by health authorities. After the preoperative weight loss, all patients in the T2D group had a 2-h plasma glucose

(P-glucose) of  $\geq 11.1$  mmol/L, and diabetes was controlled with diet alone ( $n = 2$ ), metformin alone ( $n = 4$ ), or metformin combined with liraglutide ( $n = 2$ ) or NPH insulin ( $n = 2$ ). Liraglutide was discontinued  $\geq 10$  days before the first study day, and all other antidiabetic agents were discontinued  $\geq 3$  days before each study day; all antidiabetic medication was discontinued from the time of surgery. One patient with diabetes for 11 years required metformin at 4–11 months postoperatively. In the NGT group, all had a 2-h P-glucose level  $< 7.8$  mmol/L and  $\text{HbA}_{1c} < 6\%$  (42 mmol/mol). Written informed consent was obtained from all participants, and the study was approved by the Municipal Ethical Committee of Copenhagen in accordance with the Declaration of Helsinki II and by the Danish Data Protection Agency.

### Study Design

On separate days before and 1 week, 3 months, and 1 year after RYGB, we performed hyperinsulinemic-euglycemic clamps and IV glucose-glucagon tests. Before and 3 months and 1 year after RYGB, oral glucose tolerance tests (OGTTs) and whole-body dual-energy X-ray absorptiometry (DEXA) scans were performed on an additional study day. All participants completed the preoperative and 3 months visits; four subjects did not complete the 1-week visit because of postoperative complications and two did not wish to participate at the 1-year visit.

Before the experiments, participants were instructed to refrain from strenuous physical activity and alcohol for 3 days and to fast overnight (10–12 h). On the day of the experiment, subjects were weighed and placed in a reclining position in a hospital bed, allowing no physical activity.

### Hyperinsulinemic-Euglycemic Clamp

Catheters were placed in an antecubital vein for infusion and in a dorsal vein in the hand for blood sampling, with the hand placed in a heated box for arterialization. After collecting three fasting samples, a primed continuous basal infusion (0.036 mg/kg/min) of  $[6,6\text{-}^2\text{H}_2]$ -glucose (99 atom percent enrichment; Cambridge Isotope Laboratories, Andover, MA; sterilized and packed in vials at the Central Pharmacy of the Capital Region, Herlev, Denmark) was started and continued for 120 min using a precision infusion pump (P2000; IVAC Medical Systems, Hampshire, U.K.). The priming dose was adjusted for the ambient fasting P-glucose ( $3.6 \text{ mg/min} \times \text{fasting P-glucose [mmol/L]} \times 1/5$ ). Urine was sampled during the basal infusion period in the T2D group and tested for traces of glucose (Multistix7; Siemens, Berlin, Germany). After 120 min, the clamp was initiated (4-h primed continuous insulin infusion of 40 mU/m<sup>2</sup>/min Actrapid; Novo Nordisk, Bagsværd, Denmark). Insulin was dissolved in saline, to which was added blood from the participant. P-glucose was allowed to drop to 5.5 mmol/L

before initiation of a variable infusion of 20% glucose (w/v) enriched with [6,6-<sup>2</sup>H<sub>2</sub>]-glucose (median enrichment 2.16% [interquartile range 2.10–2.24]), while glucose infusion was initiated at clamp start if P-glucose was  $\leq 5.5$  mmol/L. The basal infusion of [6,6-<sup>2</sup>H<sub>2</sub>]-glucose was decreased to 25% upon initiation of glucose infusion. Blood was sampled every 10 min during the last 30 min of the basal and clamp periods and every 20 min during the remainder of the clamp, whereas P-glucose was assessed every 5 min. Stable tracer-to-tracee ratios (TTRs) were obtained in the basal and clamp periods with mean coefficient of variation (CV)  $\pm$  SD of  $4.8 \pm 2.2\%$  and  $2.9 \pm 2.1\%$ , respectively, and P-glucose was kept stable for the last 30 min of the clamp (CV  $3.1 \pm 1.4\%$ ); there were no differences in CVs between pre- and postoperative days or between groups. Biopsies of abdominal subcutaneous fat and tissue from the vastus lateralis muscle were obtained during the basal period and after 4 h of insulin infusion using a modified Bergström needle with suction under local anesthesia. Results from biopsies will be presented elsewhere.

### OGTT

Catheters were placed in antecubital veins in both arms. At  $t = 0$  min, a bolus of 50% glucose (w/v) was injected over 1 min. The volume of the bolus was fixed for the individual patient throughout the study:  $(20 - \text{fasting preoperative P-glucose [mmol/L]}) \times (\text{height}^2 [\text{m}^2]) \times (2.1 [\text{mL/m}^2/\text{mmol/L}])$ . At  $t = 2$  min, a bolus of 1 mg glucagon (Novo Nordisk) was injected. Blood was sampled at  $t = -10, -5, 0, 2, 6, 8, 10,$  and 12 min.

### Oral Glucose Tolerance Test

Participants ingested 75 g of glucose dissolved in 250 mL of water within 5 min. Blood samples were obtained from a catheter in an antecubital vein at  $t = 0, 15, 30, 60, 90,$  and 120 min. Two participants did not complete the postoperative OGTTs because of vomiting. Otherwise, the test was well tolerated after surgery, except for mild degrees of nausea during the first hour.

### DEXA

Body composition was assessed by DEXA scanning (Discovery A, S/N 83487; Hologic Inc., Bedford, MA) using the Apex 2.3 software package.

### Surgical Procedure

Surgery was performed as previously described (9).

### Preoperative Weight Loss

Data on preoperative weight loss was collected retrospectively from patient records.

### Postoperative Diet

From the day after the operation patients were on a liquid diet of approximately 1,200 kcal/day until 14 days postoperatively, when the diet gradually changed toward solid foods.

### Analytic Procedures

Blood collected in prechilled EDTA tubes (for analysis of glucagon and fatty acids [FAs]), EDTA tubes with dipeptidyl peptidase-4 inhibitor (valine-pyrrolidide; final concentration 0.01 mmol/L, for GLP-1, glucose-dependent insulinotropic polypeptide [GIP], and glucagon from OGTTs), and heparin tubes (for TTR) was immediately centrifuged, while clot-activator tubes (for insulin and C-peptide) were left for 30 min before centrifugation. Eppendorph tubes containing EDTA were immediately centrifuged and used for analysis of P-glucose using YSI model 2300 STAT plus (YSI, Yellow Springs, OH). Serum C-peptide and insulin were analyzed using AutoDELFLIA fluoroimmunoassay (Wallac OY, Turku, Finland), HbA<sub>1c</sub> was measured using high-pressure liquid chromatography (Tosoh Bioscience, Tokyo, Japan), and plasma FAs (NEFA C kit; Wako Chemicals GmbH, Neuss, Germany) were measured using enzymatic colorimetric methods (Hitachi 912 automatic analyzer; Boehringer, Mannheim, Germany). Glucagon, total GLP-1, and total GIP were analyzed as previously described (9). TTR was analyzed using mass spectrometry as previously described (27).

### Calculations and Statistical Analysis

Rate of appearance (Ra) and rate of disappearance (Rd) of glucose were calculated from the last 30 min of the basal and clamp periods using Steele's equation (28). One patient was excluded from analysis of basal Ra because of preoperative glucosuria. Mean serum C-peptide concentration in the basal period was used to assess basal hepatic insulin sensitivity ( $\text{HISI}_{\text{basal}} = 10^6 / [\text{Ra}_{\text{basal}} \times \text{C-peptide}_{\text{basal}}]$ ), whereas mean serum insulin in the clamp period was used to calculate insulin-adjusted glucose disposal ( $\text{Rd}_{\text{clamp}} / \text{insulin}_{\text{clamp}}$ ). Suppression of Ra, FAs, and glucagon was calculated as the difference between basal and clamp levels and expressed as a percentage of the basal level. Hepatic insulin clearance at fasting was calculated as the ratio of fasting serum C-peptide to insulin, whereas clearance during the clamp was adjusted for endogenous insulin secretion ( $\text{CI}_{\text{clamp}} = \text{insulin infusion rate} / (\text{insulin}_{\text{clamp}} - [\text{C-peptide}_{\text{clamp}} \times \text{insulin}_{\text{basal}} / \text{C-peptide}_{\text{basal}}])$ ) (29).

Incremental areas under the curve (iAUCs) were calculated using the trapezoidal rule subtracting fasting levels. Early insulin secretion was estimated using insulinogenic index (IGI) from OGTTs ( $\text{IGI} = \Delta \text{C-peptide}_{0-30} / \Delta \text{glucose}_{0-30 \text{ min}}$ ), and the acute insulin response from IV glucose-glucagon tests was calculated as the mean serum C-peptide from 6–12 min subtracting fasting levels. Indices of insulin secretion (IGI and acute insulin response, respectively) were related to insulin sensitivity ( $\text{Rd}_{\text{clamp}} / \text{insulin}_{\text{clamp}}$ ) by calculating disposition indices (oral and IV, respectively) (26).

Data are expressed as means  $\pm$  SEMs unless otherwise specified. Postoperative changes were analyzed by ANOVA in a linear mixed effects model using time from surgery and group as fixed effects and individual subjects as random effect. Logarithmic transformation was used if

distribution was skewed. Post hoc comparisons of group differences at a given point in the study were performed using unpaired *t* tests. Pearson test was used to evaluate correlations. Before the study, we estimated that eight patients would allow detection of within-group postoperative changes in insulin sensitivity of 20% (power 0.80, level of significance 0.05) based on data from patients with T2D (30). Statistical analyses were performed in R software version 2.11.1 (www.r-project.org).

## RESULTS

### Weight and Body Composition

Before the study, participants in the two groups achieved a similar required diet-induced total weight loss of  $-9.2 \pm 1.2\%$  (Fig. 1). Weight loss was accelerated after RYGB and initially comparable in the two groups, but at 1 year weight loss was larger in the NGT group. Postoperative weight loss was mostly fat mass (percentage of total weight loss at 3 months: T2D group  $65.5 \pm 3.1\%$ , NGT group  $71.4 \pm 1.6\%$ ;  $P = 0.10$  between groups; at 1 year: T2D group  $67.1 \pm 3.3\%$ , NGT group  $76.8 \pm 2.5\%$ ;  $P = 0.03$ ), but fat-free mass (FFM) also was reduced by 9–10% at 3 months and 11–13% at 1 year (Table 1).

### Glycemic Control and Insulin Metabolism

Patients with T2D improved glycemic control postoperatively (Table 1); fasting P-glucose declined by  $\sim 20\%$  at 1 week and was  $<5.6$  mmol/L in 6 of 10 and in 5 of 9 patients at 3 months and 1 year, respectively. HbA<sub>1c</sub> also was reduced, reaching values  $<6\%$  ( $<42$  mmol/mol) in 7 of 10 patients at 3 months and 8 of 9 patients at 1 year, whereas 2-h P-glucose after OGTT declined by  $>50\%$

postoperatively (Figure 3A and Table 1). The NGT group experienced postoperative reductions of 5–10% in fasting P-glucose and of  $\sim 30\%$  in 2-h P-glucose; HbA<sub>1c</sub> was unchanged.

In both groups, fasting serum insulin decreased—and did so to a larger degree than C-peptide—pointing to a significant increase in fasting insulin clearance from 1 week after RYGB. Clearance of exogenous insulin during the clamp increased similarly in the two groups from 1 week after RYGB, and clamp insulin concentration was reduced by 20–30% after surgery (Table 2).

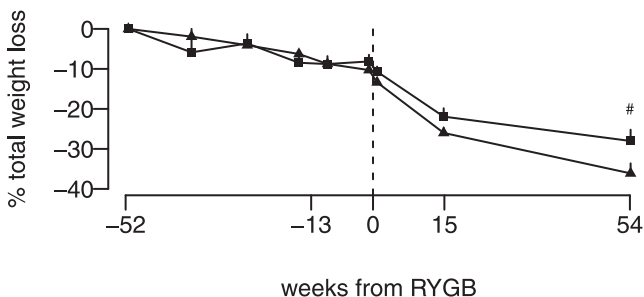
### Endogenous Glucose Production

Preoperative basal glucose production (Ra basal) tended to be higher in patients with T2D than in subjects with NGT ( $P = 0.09$ ), whereas basal hepatic insulin sensitivity did not differ significantly between groups (Table 2). Basal glucose production decreased equally at 1 week after RYGB in the two groups, and basal hepatic insulin sensitivity increased 50% because of concomitant reductions in basal serum C-peptide. While basal glucose production remained reduced in the T2D group, it was no longer significantly changed at 3 months and 1 year in the NGT group. Nevertheless, basal hepatic insulin sensitivity increased 1.5- to 2-fold in both groups.

Glucose production during the clamp was incompletely suppressed before surgery in patients with T2D (Ra<sub>clamp</sub> was  $>0$  mg/min;  $P < 0.01$ ) and was not significantly changed at 1 week, but it had declined at 3 months ( $P = 0.06$ ) and was completely suppressed by 1 year (Ra<sub>clamp</sub> not different from 0 mg/min;  $P = 0.30$ ). Subjects with NGT experienced no changes in glucose production during the clamp; it was almost completely suppressed at all time points (Ra<sub>clamp</sub> not different from 0 mg/min;  $P > 0.05$  at all time points).

### Glucose Disposal and FAs

Glucose disposal (Rd<sub>clamp</sub>) in patients with T2D was lower than in subjects with NGT before surgery and was unchanged at 1 week after surgery but increased by  $\sim 50\%$  after 3 months and by  $\sim 75\%$  after 1 year (Table 2). Glucose disposal in the NGT group decreased by  $\sim 30\%$  at 1 week, was unchanged at 3 months, and increased by  $\sim 60\%$  after 1 year compared with preoperative values. Lower insulin concentration during the clamp seemed to explain the reduced glucose disposal in the NGT group at 1 week: glucose disposal adjusted for clamp insulin concentration (Rd/I<sub>clamp</sub>) was not significantly changed at 1 week in either group. The adjusted glucose disposal tended to be lowest before surgery in patients with T2D (Rd/I<sub>clamp</sub>  $P = 0.07$ ; Rd/I<sub>ffm</sub>  $P = 0.05$ ) and increased significantly in both groups at 3 months and 1 year. The change in glucose disposal correlated with the weight loss at 1 year in the total group of participants ( $\Delta$ Rd<sub>clamp</sub>  $r = 0.49$ ,  $P = 0.04$ ;  $\Delta$ Rd/I<sub>ffm</sub>  $r = 0.45$ ,  $P = 0.06$ ), while the correlations were not significant at 3 months.



BMI	pre-diet	-1	+1	+15	+54
T2D	42.4 $\pm$ 1.7	38.9 $\pm$ 1.6**	37.3 $\pm$ 1.8**	33.1 $\pm$ 1.5**	30.8 $\pm$ 1.7**
NGT	44.9 $\pm$ 1.0	40.2 $\pm$ 0.8**	37.9 $\pm$ 0.9**	33.2 $\pm$ 1.1**	28.5 $\pm$ 1.5**

**Figure 1**—Preoperative and postoperative total body weight loss (%) in patients with T2D (black squares) and NGT (black triangles) undergoing RYGB at  $t = 0$ . Values are mean  $\pm$  SEM. Changes in BMI were analyzed with mixed-effects ANOVA (time,  $P < 0.01$ ; group,  $P = 0.52$ ; time  $\times$  group,  $P = 0.01$ ). # $P < 0.05$  for difference in response between groups, \*\* $P < 0.01$  for the change from pre-diet level within the group.

**Table 1—Weight, FFM, glycaemic control, and fasting measurements of insulin and C-peptide in patients with T2D and NGT before and 1 week, 3 months, and 1 year after RYGB**

	T2D group				NGT group				Mixed effect model ANOVA		
	Before	1 Week	3 Months	1 Year	Before	1 Week	3 Months	1 Year	Time	Group	Time × group
Participants (male/female)	10 (4/6)	8 (4/4)	10 (4/6)	9 (4/5)	10 (3/7)	8 (3/5)	10 (3/7)	9 (3/6)	—	—	—
Weight (kg)	121.5 ± 8.9	118.1 ± 9.5*	103.3 ± 7.8**	96.0 ± 8.2**	116.9 ± 4.9	112.3 ± 6.2	96.6 ± 4.8**	82.6 ± 4.8**	<0.01	0.52	0.06
FFM (kg)	73.3 ± 6.9	—	66.8 ± 6.0**	64.9 ± 6.4**	64.4 ± 4.1	—	58.2 ± 3.7**	56.3 ± 3.5**	<0.01	0.25	0.97
Fasting P-glucose (mmol/L)	8.3 ± 0.6	6.6 ± 0.4**	5.7 ± 0.2**	5.6 ± 0.2**	5.1 ± 0.1††	4.8 ± 0.1††	4.6 ± 0.1*††	4.7 ± 0.1*††	<0.01	<0.01	<0.01
HbA <sub>1c</sub> (%)	7.0 ± 0.3	—	5.9 ± 0.2**	5.7 ± 0.2**	5.4 ± 0.1††	—	5.3 ± 0.1†	5.3 ± 0.1†	<0.01	<0.01	<0.01
HbA <sub>1c</sub> (mmol/mol)	53 ± 3.3	—	41 ± 2.2**	39 ± 2.2**	36 ± 1.1††	—	34 ± 1.1†	34 ± 1.1†	<0.01	<0.01	<0.01
2-h P-glucose after OGTT (mmol/L)	15.2 ± 1.0	—	7.7 ± 0.7**	5.9 ± 0.5**	6.3 ± 0.3††	—	4.4 ± 0.3**††	4.2 ± 0.4**††	<0.01	<0.01	<0.01
Fasting S-insulin (pmol/L)	97 ± 13	89 ± 18*	51 ± 8**	41 ± 6**	77 ± 9	57 ± 6*	30 ± 3**	30 ± 3**	<0.01	0.12	0.32
Fasting S-C-peptide (pmol/L)	1,256 ± 113	1,287 ± 217	925 ± 103**	859 ± 104**	1,054 ± 64	905 ± 77	593 ± 38**†	544 ± 45**†	<0.01	0.02	0.62
Fasting CI	13.1 (11.8–16.0)	14.7* (13.6–16.9)	17.9** (16.6–22.4)	20.7** (19.2–24.4)	13.2 (12.2–16.5)	14.9* (14.0–18.6)	18.4** (17.8–20.9)	17.8** (16.6–20.1)	<0.01	0.86	0.04

Values are mean ± SEM. Clearance of insulin during fasting (fasting CI), fasting serum C-peptide-to-serum insulin ratio) is reported as median (interquartile range) because of skewed distribution. \*P < 0.05 and \*\*P < 0.01 for the change from preoperative level within the group (post hoc estimates from mixed effect model). †P < 0.05 and ††P < 0.01 for differences between the groups at a given study session (post hoc unpaired t test). P-, plasma; S-, serum.

**Table 2—Endogenous glucose production, glucose disposal, FAs, and glucagon in patients with T2D and NGT before and 1 week, 3 months, and 1 year after RYGB: results from the basal state and the hyperinsulinemic-euglycemic clamp**

	T2D group				NGT group				Mixed effect model ANOVA			
	Before	1 Week	3 Months	1 Year	Before	1 Week	3 Months	1 Year	Time	Group	Time $\times$ group	
	-7 (-8 to -4)	7 (6-7)	98 (92-109)	386 (374-392)	-4 (-9 to -3)	6 (5-7)	104 (96-114)	379 (365-397)				
<b>Basal period</b>												
Ra (mg/min)	207 $\pm$ 15	174 $\pm$ 11**	176 $\pm$ 14**	170 $\pm$ 11**	175 $\pm$ 6	157 $\pm$ 9*	166 $\pm$ 12	163 $\pm$ 8	<0.01	0.26	0.22	
HISI	4.8 $\pm$ 1.0	7.7 $\pm$ 2.8*	9.0 $\pm$ 1.9**	8.7 $\pm$ 1.6**	6.0 $\pm$ 0.4	9.2 $\pm$ 1.2*	11.9 $\pm$ 1.2**	12.6 $\pm$ 1.1**	<0.01	0.16	0.39	
P-FA ( $\mu$ mol/L)	705 $\pm$ 56	841 $\pm$ 36*	741 $\pm$ 52	519 $\pm$ 31**	635 $\pm$ 67	787 $\pm$ 41*	670 $\pm$ 50	567 $\pm$ 71**	<0.01	0.65	0.38	
P-Glucagon (pmol/L)	10.7 $\pm$ 3.4	10.3 $\pm$ 2.9	7.4 $\pm$ 2.1**	8.1 $\pm$ 2.0**	7.4 $\pm$ 1.3	13.4 $\pm$ 1.9**	6.6 $\pm$ 0.8	7.8 $\pm$ 1.5	<0.01	0.99	<0.01	
C-peptide-to-glucagon ratio	284 $\pm$ 65	189 $\pm$ 64	221 $\pm$ 49	160 $\pm$ 47*	183 $\pm$ 35	66 $\pm$ 10**	95 $\pm$ 12*	83 $\pm$ 14*	<0.01	0.02	0.78	
<b>Clamp period</b>												
Ra (mg/min)	55 $\pm$ 13	44 $\pm$ 8	30 $\pm$ 7	9 $\pm$ 8**	31 $\pm$ 15	14 $\pm$ 15	21 $\pm$ 12	22 $\pm$ 15	0.04	0.39	0.08	
Suppression of Ra (%)	70 $\pm$ 5	71 $\pm$ 5	83 $\pm$ 4	98 $\pm$ 5**	82 $\pm$ 9	92 $\pm$ 9	87 $\pm$ 8	88 $\pm$ 9	0.04	0.45	0.04	
Rd (mg/kg/min)	3.5 $\pm$ 0.5	3.5 $\pm$ 0.5	5.4 $\pm$ 0.5**	6.2 $\pm$ 0.7**	5.6 $\pm$ 0.6††	3.9 $\pm$ 0.4**	6.4 $\pm$ 0.6	9.1 $\pm$ 1.1**†	<0.01	0.04	0.03	
Rd/I ( $\mu$ g/kg/min per pmol/L)	7.7 $\pm$ 1.1	9.2 $\pm$ 1.6	14.9 $\pm$ 1.8**	16.7 $\pm$ 2.6**	11.4 $\pm$ 1.6	9.5 $\pm$ 1.1	17.3 $\pm$ 1.8*	25.9 $\pm$ 5.6**	<0.01	0.22	0.23	
Rd/I <sub>firm</sub> ( $\mu$ g/kg <sub>firm</sub> /min per pmol/L)	14.0 $\pm$ 2.2	—	24.7 $\pm$ 3.1**	25.8 $\pm$ 4.0**	22.1 $\pm$ 3.1†	—	30.5 $\pm$ 2.9**	38.7 $\pm$ 6.5**	<0.01	0.11	0.12	
P-Glucose (mmol/L)	5.4 $\pm$ 0.09	5.4 $\pm$ 0.04	5.5 $\pm$ 0.03	5.5 $\pm$ 0.04	5.4 $\pm$ 0.04	5.6 $\pm$ 0.07	5.5 $\pm$ 0.07	5.5 $\pm$ 0.05	0.27	0.17	0.45	
S-Insulin (pmol/L)	469 $\pm$ 30	395 $\pm$ 25**	380 $\pm$ 25**	394 $\pm$ 30**	527 $\pm$ 34	415 $\pm$ 28**	379 $\pm$ 23**	395 $\pm$ 33**	<0.01	0.49	0.48	
S-C-peptide (pmol/L)	622 $\pm$ 101	528 $\pm$ 103	509 $\pm$ 84	533 $\pm$ 85	1,001 $\pm$ 70††	696 $\pm$ 79**	626 $\pm$ 82**	650 $\pm$ 57**	<0.01	0.05	0.04	
CI (mL/min/kg)	13.9 $\pm$ 0.9	16.4 $\pm$ 1.2*	18.0 $\pm$ 1.2**	17.9 $\pm$ 1.2**	12.9 $\pm$ 0.8	16.2 $\pm$ 1.3*	18.7 $\pm$ 1.5**	20.3 $\pm$ 2.3**	<0.01	0.85	0.46	
Suppression of P-FA (%)	80 $\pm$ 3	69 $\pm$ 4**	89 $\pm$ 2**	95 $\pm$ 1**	86 $\pm$ 2	86 $\pm$ 3††	93 $\pm$ 1**	95 $\pm$ 1**	<0.01	0.02	<0.01	
Suppression of P-Glucagon (%)	51 $\pm$ 6	49 $\pm$ 10	55 $\pm$ 10	65 $\pm$ 6	70 $\pm$ 7†	78 $\pm$ 5††	71 $\pm$ 6	75 $\pm$ 6	0.46	0.01	0.55	

Values are mean  $\pm$  SEM. Days from surgery are expressed as median (interquartile range). Basal period values are the mean values from the last 30 min of the basal period; clamp period values are the mean values from the last 30 min of the clamp period. CI, clearance rate of insulin during clamp; HISI, hepatic insulin sensitivity index; Rd/I, Rd adjusted for clamp insulin concentration; Rd/I<sub>firm</sub>, Rd adjusted for clamp insulin concentration and FFM; S-, serum; P-, plasma; FA, fatty acids. \* $P$  < 0.05 and \*\* $P$  < 0.01 for the change from preoperative level within the group (post hoc estimates from mixed effect model). † $P$  < 0.05 and †† $P$  < 0.01 for differences between the groups at a given study session (post hoc unpaired  $t$  test).

Fasting plasma FA concentration increased by ~20% at 1 week, returned to preoperative values at 3 months, and was slightly decreased after 1 year in both groups (Fig. 2A, Table 2). Suppression of FAs in plasma during the clamp did not differ significantly between groups preoperatively and increased similarly at 3 months and 1 year. At 1 week, suppression of FAs was reduced in the T2D group and unchanged in the NGT group.

### $\beta$ -Cell Function

Insulin secretion in response to oral glucose was markedly enhanced postoperatively in patients with T2D, with twofold increases in IGI and iAUC of serum C-peptide and fourfold increased oral disposition index (Fig. 3B and 3C and Table 3). Peak serum C-peptide increased slightly in both groups, and time to peak was significantly reduced ( $P < 0.01$ ). IGI was unchanged after surgery in subjects with NGT, whereas iAUC of C-peptide and the oral disposition index increased moderately at 3 months and 1 year, respectively.

Insulin secretion after IV glucose-glucagon was unchanged postoperatively in patients with T2D (Fig. 4, Table 3) regardless of disease duration (data not shown), but the IV disposition index increased at 3 months and 1 year because of increased insulin sensitivity. In subjects with NGT, the C-peptide response to IV glucose-glucagon declined after surgery, but the IV disposition index was unchanged. Disposition index in the NGT group remained higher than in the T2D group after both oral and IV challenges.

### Glucagon, GLP-1, and GIP

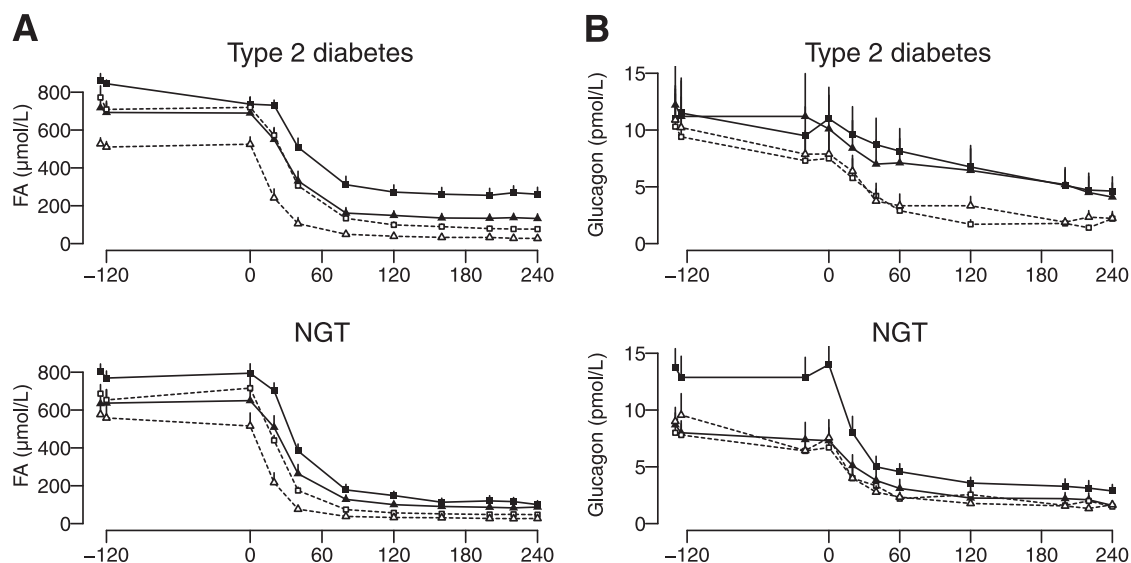
In patients with T2D, basal P-glucagon was unchanged at 1 week and decreased at 3 months and 1 year after

RYGB, whereas basal P-glucagon was unchanged postoperatively in subjects with NGT except for a transient increase at 1 week (Fig. 2B, Table 2). The ratio of basal C-peptide to glucagon concentration declined 1 week after surgery in subjects with NGT and remained low at 3 months and 1 year; it was largely unchanged in patients with T2D, except for a decline 1 year postoperatively. Postoperative changes in the ratio of C-peptide to glucagon thus were not related to changes in basal glucose production. Glucagon suppression during the clamp was highest in the NGT group and did not change postoperatively in either group. Preoperative glucagon secretion was suppressed in response to oral glucose in both groups (iAUCs were negative), but after surgery postprandial glucagon response increased (Fig. 3D, Table 4).

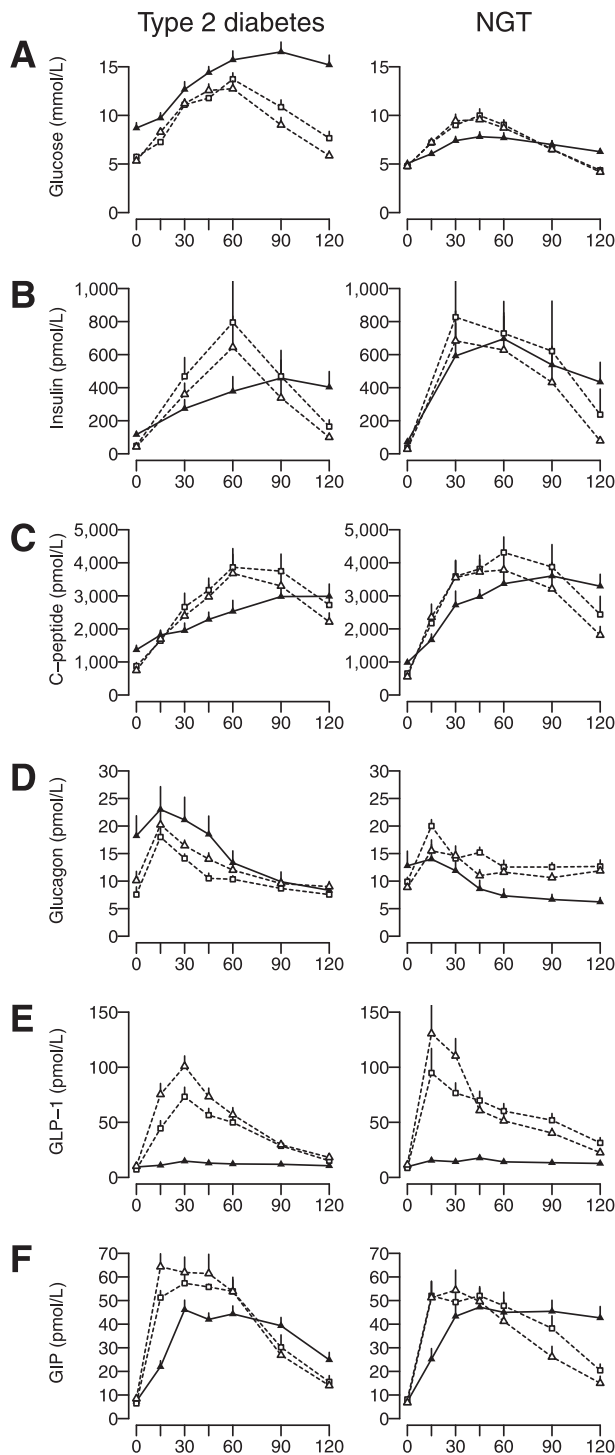
After RYGB, GLP-1 secretion was exaggerated in both groups, with a 5-fold increased peak concentration and 12-fold increased iAUC after oral glucose (Fig. 3E, Table 4). Postprandial GIP secretion was largely unchanged, although at 1 year peak GIP increased significantly in patients with T2D (Fig. 3F, Table 4) and occurred earlier in subjects with NGT ( $P < 0.01$ ). Fasting concentrations of incretin hormones were unaltered postoperatively.

### DISCUSSION

In this study, changes in insulin sensitivity and  $\beta$ -cell function were assessed 1 week after RYGB and throughout the first postoperative year in patients with T2D and obese NGT subjects. Before surgery, patients with T2D were more insulin resistant than glucose-tolerant subjects, but differences in insulin secretion were



**Figure 2**—Plasma FAs (A) and glucagon (B) in the fasting state and during hyperinsulinemic-euglycemic clamp (initiated at  $t = 0$  min) in patients with T2D (upper panels) and NGT (lower panels) before (solid line, black triangles) and 1 week (solid line, black squares), 3 months (dotted line, white squares), and 1 year (dotted line, white triangles) after RYGB. Values are mean  $\pm$  SEM.



**Figure 3**—Plasma glucose (A), serum insulin (B), serum C-peptide (C), plasma glucagon (D), plasma total GLP-1 (E) and plasma total GIP (F) in response to an OGTT in patients with T2D (left) and NGT (right) before (solid line, black triangles) and 3 months (dotted line, white squares) and 1 year (dotted line, white triangles) after RYGB. Values are mean + SEM.

more pronounced, with >50% lower insulin secretion responses in patients with T2D.

After RYGB, our main finding was an early increase in hepatic insulin sensitivity at 1 week in both study groups,

as indicated by reduced basal glucose production and increased basal hepatic insulin sensitivity index. Hepatic insulin clearance and clearance of exogenous insulin were furthermore increased at 1 week, and because insulin clearance has been suggested to be initiated by receptor-mediated endocytosis, this could indicate a common mechanism responsible for the early improvement in hepatic insulin action and clearance (31). In contrast, peripheral insulin sensitivity was not improved after 1 week, but glucose disposal and suppression of FAs increased in both groups after 3 months and 1 year, pointing toward improvements in muscle and fat tissue insulin sensitivity.

Insulin secretion in response to oral glucose increased in patients with T2D, whereas insulin secretion was unchanged after IV stimulation. In subjects with NGT, the time course of insulin secretion after oral glucose changed with a higher and earlier peak C-peptide, but the IGI was unchanged. In response to IV glucose-glucagon, insulin secretion declined in subjects with NGT.

Immediate improvement in hepatic insulin sensitivity without changes in peripheral sensitivity is the typical response to calorie restriction in obese subjects regardless of glucose tolerance and is associated with an early decrease in liver fat (30,32–34). Thus, we suggest that the increase in hepatic insulin sensitivity and insulin clearance 1 week after RYGB observed in our study could be the result of postoperative calorie restriction, perhaps due to a rapid decrease in hepatic fat content. In fact, changes in basal glucose production and basal concentrations of glucose and insulin have been reported to be even larger after 1 week of strict dieting (600 kcal/day) (30); hence, calorie restriction per se is sufficient to cause changes of this magnitude. Furthermore, several studies have reported comparable changes in HOMA-IR after RYGB and calorie restriction (7,13,15,22,35,36), although a few studies found larger improvements in HOMA-IR after RYGB than after restrictive surgery (37,38) or diet alone (38,39). Better compliance to the diet in patients undergoing RYGB surgery could possibly explain some, if not all, of these differences.

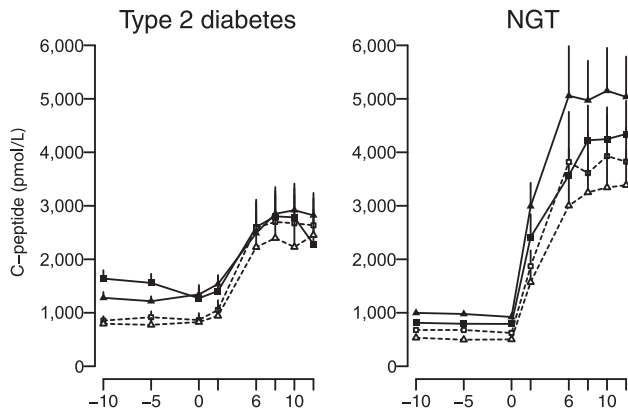
Glucose disposal during the clamp was largely unchanged 1 week after RYGB, except for a decline in  $R_d$  in the NGT group that could be attributed to the lower insulin concentration during the clamp brought about by increased insulin clearance. Also, at 1 week after surgery, glucose disposal could still be influenced by postoperative stress (40,41), possibly counteracting a beneficial effect of surgery. However, lack of improvement in glucose disposal is in line with other clamp studies performed 4 weeks after RYGB when surgical stress has abated (17,42). Elevated FA levels also act to reduce glucose disposal (43,44) and were seen in both groups 1 week after RYGB, in accordance with previous studies early after RYGB (12,22,42) and calorie restriction (22). Notably, improved hepatic insulin sensitivity is not seen in response to increased FAs (43) or surgical stress (41).



**Table 3—Insulin secretion in response to OGTT and IV glucose-glucagon test in patients with T2D and NGT before and 1 week, 3 months, and 1 year after RYGB**

	T2D group				NGT group				Mixed effect model ANOVA	Time × Group
	Before	1 week	3 months	1 year	Before	1 week	3 months	1 year		
<b>OGTT</b>										
Days from surgery	−17 (−21 to −12)	—	106 (99–112)	387 (373–395)	−21 (−31 to −15)	—	103 (97–116)	371 (370–398)	—	—
iAUC C-peptide (nmol/L × min)	125 ± 25	—	257 ± 40**	236 ± 38**	237 ± 35	—	321 ± 54**	286 ± 57	<0.01	0.18
Peak C-peptide (pmol/L)	3,061 ± 409	—	4,044 ± 584*	3,710 ± 599	3,720 ± 390	—	4,783 ± 625**	4,255 ± 637	<0.01	0.38
IGI (pmol/L/mM)	121 (81–262)	—	237** (230–496)	260** (149–404)	705†† (572–824)	—	741†† (544–745)	747†† (472–885)	<0.01	<0.01
Dl <sub>total</sub> × 10 <sup>−3</sup>	0.9 (0.5–1.1)	—	4.5** (2.9–4.8)	3.9** (3.5–4.5)	7.3†† (6.2–8.7)	—	12.8†† (10.3–13.9)	13.8**†† (8.2–20.1)	<0.01	<0.01
<b>IV glucose-glucagon</b>										
Days from surgery	−5 (−8 to −4)	9 (7–9)	98 (93–102)	387 (383–402)	−5 (−6 to −4)	9 (9–10)	99 (93–108)	376 (369–385)	—	—
ΔP-Glucose <sub>0–2 min</sub> (mmol/L)	13.2 ± 1.2	13.5 ± 1.1	12.4 ± 1.1	10.7 ± 2.3	14.5 ± 1.2	15.8 ± 1.4	14.4 ± 1.3	13.3 ± 1.8	0.12	0.21
AIIR (pmol/L)	1,049 (969–1,885)	926 (879–1,754)	1,351 (868–2,498)	1,029 (973–1,778)	3,614††† (3,042–4,159)	2,912* (1,581–4,479)	2,308** (1,617–3,613)	1,866** (1,541–3,571)	0.04	0.01
Dl <sub>iv</sub> × 10 <sup>−3</sup>	10.4 (6.8–12.7)	10.6 (9.1–13.6)	26.1** (17.4–28.5)	21.9** (19.3–25.9)	37.4†† (28.8–59.1)	29.6† (18.7–40.9)	40.8†† (32.4–49.9)	37.6† (30.2–112.8)	<0.01	<0.01

Values are mean ± SEM. Days from surgery, IGI, acute insulin response (AIIR), and disposition index (DI) are expressed as medians (interquartile ranges) because of skewed distribution. \**P* < 0.05 and \*\**P* < 0.01 for the change from preoperative level within the group (post hoc estimates from mixed effect model). †*P* < 0.05 and ††*P* < 0.01 for differences between the groups at a given study session (post hoc unpaired *t* test).



**Figure 4**—Serum C-peptide in response to an IV glucose-glucagon test in patients with T2D (left) and NGT (right) before (solid line, black triangles) and 1 week (solid line, black squares), 3 months (dotted line, white squares), and 1 year (dotted line, white triangles) after RYGB. Values are mean ± SEM.

Peripheral insulin sensitivity increased at 3 months and 1 year in both groups, probably because of weight loss (45,46), as indicated by the positive correlation between changes in glucose disposal and weight loss 1 year after surgery.

Increased insulin secretion after ingestion of oral glucose in patients with T2D is in accordance with previous findings after RYGB (9,14,37) and is likely caused by the large postoperative increase in GLP-1 as demonstrated in studies using pharmacological blockade of the GLP-1 receptor (19,20). In addition, relief of gluco- and lipotoxicity has been proposed as potential contributors to improved β-cell function based previous reports of gradual improvement in insulin secretion in response to an IV glucose bolus (12,14,22–24). However, in our study, patients with T2D displayed unchanged insulin secretion in response to the IV challenge despite reductions in fasting glucose concentrations and FA levels (at 1 year). This discrepancy may be explained by the use of the combined glucose-glucagon stimulus (47), which may be less influenced by differences in fasting glucose concentrations (48,49). Of note, the concomitantly improved insulin sensitivity makes the insulin secretion less inadequate, as seen by increased IV disposition index in the T2D group at 3 months and 1 year. Insulin secretion after the IV test declined postoperatively in glucose-tolerant subjects, likely as an adaptation to improved insulin sensitivity (i.e., the IV disposition index was unchanged). Postoperative improvements in β-cell secretory capacity per se thus are not supported by this study in agreement with previous findings (37,50).

In both groups, relative increases in insulin secretion after oral glucose exceeded changes after IV stimulation whether expressed independently or as the disposition index, highlighting the importance of gut-related

**Table 4**—Glucagon, GLP-1, and GIP in response to OGTT in patients with T2D and NGT before and 3 months and 1 year after RYGB

	T2D group			NGT group			Mixed effect model ANOVA		
	Before	3 Months	1 Year	Before	3 Months	1 Year	Time	Group	Time × group
<b>Glucagon</b>									
Fasting (pmol/L)	18.2 ± 3.8	7.6 ± 1.5**	10.1 ± 1.7**	12.8 ± 2.8	9.9 ± 0.8	8.9 ± 1.3	<0.01	0.59	0.05
iAUC (pmol/L × min)	-342 ± 147	370 ± 196**	280 ± 142**	-403 ± 218	352 ± 124**	398 ± 228**	<0.01	0.64	0.77
Peak (pmol/L)	21.7 ± 4.4	16.2 ± 1.4	21.0 ± 3.1	14.7 ± 2.7	17.3 ± 1.5	17.6 ± 1.8	0.41	0.34	0.12
<b>GLP-1</b>									
Fasting (pmol/L)	9.4 ± 0.9	7.2 ± 1.2	10.2 ± 0.7	9.7 ± 0.8	8.6 ± 0.7	11.4 ± 0.8	0.01	0.23	0.79
iAUC (pmol/L × min)	333 ± 141	4,022 ± 377**	4,822 ± 538**	425 ± 108	5,232 ± 571**	5,199 ± 670**	<0.01	0.27	0.26
Peak (pmol/L)	15.0 ± 1.3	76.1 ± 8.5**	104.7 ± 9.3**	18.6 ± 2.0	91.1 ± 17.9**	146.5 ± 34.6**	<0.01	0.28	0.33
<b>GIP</b>									
Fasting (pmol/L)	7.3 ± 1.1	6.4 ± 1.0	8.4 ± 0.6	7.2 ± 1.0	8.1 ± 1.1	6.8 ± 1.0	0.81	0.96	0.16
iAUC (pmol/L × min)	3,497 ± 312	3,800 ± 386	3,600 ± 449	3,895 ± 533	3,515 ± 469	3,169 ± 523	0.64	0.86	0.41
Peak (pmol/L)	48.7 ± 3.8	59.7 ± 4.6	72.7 ± 8.2**	52.0 ± 5.4	58.2 ± 6.3	62.8 ± 9.2	<0.01	0.72	0.41

Values are mean ± SEM. \*\*P < 0.01 for the change from preoperative level within the group (post hoc estimates from mixed effect model).

potentiating factors. Increased postprandial GLP-1 has consistently been reported after RYGB (6) and is likely related to the changed gut anatomy since calorie restriction and weight loss do not substantially change GLP-1 secretion (7,13,35). GIP secretion has been reported to be unchanged after RYGB in some (8,9,35,37) but not all previous studies (14,18), whereas increased postprandial glucagon secretion is a common finding after RYGB (8,9,18,19,35), although it is somewhat paradoxical considering the high concomitant levels of GLP-1 and glucose. The increase may represent glucagon of gut origin resulting from excess postoperative stimulation of L-cells (18). At any rate, glucagon suppression during the clamp was unchanged postoperatively, confirming intact  $\alpha$ -cell responsiveness to IV glucose, insulin, or both (19).

This study has limitations. First, participants were included after a mean preoperative weight loss of 9%, which is likely to improve several metabolic parameters, especially hepatic insulin sensitivity (32). Thus, preoperative characteristics of the study participants may not be comparable to RYGB candidates or patients with T2D who were not subjected to a preoperative diet. Nevertheless, postoperative metabolic improvements were still observed, although the magnitude probably would have been greater, if participants had not been subjected to the preoperative diet. Second, the study was powered to detect postoperative changes in insulin sensitivity within the groups, and minor changes in other parameters or minor differences between groups thus may not have reached significance. Finally, we did not include a nonoperated group subjected to the same postoperative diet, which would be of major interest provided that adherence to the diet can be controlled.

In conclusion, RYGB increases basal hepatic insulin sensitivity 1 week after surgery in patients with T2D and in obese glucose-tolerant subjects. Concomitant increases in insulin clearance further highlight the liver as an important organ responsible for the early effects on glucose metabolism after surgery. Later improvements in peripheral insulin sensitivity (at 3 months and 1 year postoperatively) are likely related to the reduction in body weight. Insulin secretion increases after RYGB in patients with T2D but only in response to oral glucose, underscoring the importance of the changed gut anatomy and exaggerated GLP-1 secretion.

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**Author Contributions.** K.N.B.-M. and C.D. wrote the study protocol, identified eligible participants, conducted the study, researched and analyzed data, contributed to the discussion, and wrote the manuscript. N.B.J., S.H.J., A.K.S., P.H.A., D.L.H., D.W., L.N., and V.B.K. analyzed data, contributed to the discussion, and reviewed and edited the manuscript. J.F.P.W., B.K., J.J.H., E.A.R., and S.M. designed the study protocol, generated and analyzed data, contributed to the discussion, and reviewed and edited the manuscript. K.N.B.-M. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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