

Long-Term Effects of Bariatric Surgery on Meal Disposal and β -Cell Function in Diabetic and Nondiabetic Patients

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Gastric bypass surgery leads to marked improvements in glucose tolerance and insulin sensitivity in obese type 2 diabetes (T2D); the impact on glucose fluxes in response to a physiological stimulus, such as a mixed meal test (MTT), has not been determined. We administered an MTT to 12 obese T2D patients and 15 obese nondiabetic (ND) subjects before and 1 year after surgery (10 T2D and 11 ND) using the double-tracer technique and modeling of β -cell function. In both groups postsurgery, tracer-derived appearance of oral glucose was biphasic, a rapid increase followed by a sharp drop, a pattern that was mirrored by postprandial glucose levels and insulin secretion. In diabetic patients, surgery lowered fasting and postprandial glucose levels, peripheral insulin sensitivity increased in proportion to weight loss (~30%), and β -cell glucose sensitivity doubled but did not normalize (compared with 21 nonsurgical obese and lean controls). Endogenous glucose production, however, was less suppressed during the MMT as the combined result of a relative hyperglucagonemia and the rapid fall in plasma glucose and insulin levels. We conclude that in T2D, bypass surgery changes the postprandial response to a dumping-like pattern and improves glucose tolerance, β -cell function, and peripheral insulin sensitivity but worsens endogenous glucose output in response to a physiological stimulus. *Diabetes* 62:3709–3717, 2013

Mounting evidence supports bariatric surgery as a powerful intervention to induce remission in patients with type 2 diabetes (T2D) (1,2) and to prevent or delay incident T2D (3). This has engendered enthusiasm for bariatric surgery as a treatment for T2D (4) and has encouraged a broadening of the BMI range as an indication for surgery in diabetic patients (5).

Although weight loss and, in the early postoperative period, caloric deficit certainly make a contribution to improve glucose tolerance, surgery itself may trigger weight-independent mechanisms eventually translating

into favorable metabolic effects. This postulate is based on early animal studies (6) and, in humans, on evidence that metabolic changes sometimes precede sizeable weight loss or are disproportionate to the amount of weight lost (7). In this regard, there is evidence that different bariatric procedures (e.g., Roux-en-Y gastric bypass [RYGB], biliopancreatic diversion, and sleeve gastrectomy) may engage putative weight-independent mechanisms to different extents or involve altogether different mechanisms (8,9).

A number of previous studies have documented the effects of the most popular bariatric operation, RYGB, on glycemic control and incretin hormones (10–24), and mechanistic studies have explored the ability of RYGB to enhance insulin action and β -cell function. The great majority of these studies have used methods based on fasting measurements (e.g., homeostasis model assessments), oral glucose tolerance test–based surrogate indices of insulin sensitivity and β -cell function, or euglycemic-hyperinsulinemic clamp settings (14,16–21). A recent study (24) has taken a more physiological approach by comparing the impact of RYGB and gastric banding on the disposition of a mixed meal, with the use of a double-tracer technique, in nondiabetic (ND) subjects studied before and shortly after the operation (~20 weeks). In the current study of morbidly obese patients with T2D, we aimed at measuring the impact of RYGB on chief physiologic determinants of meal disposal long after surgery (when body weight and metabolic adaptation have stabilized) and assessing their relation to weight loss and the attendant changes in the hormonal milieu.

RESEARCH DESIGN AND METHODS

Subjects. We studied 12 morbidly obese patients with T2D and 15 sex- and BMI-matched morbidly obese ND patients. Diabetes was newly diagnosed in three patients, whereas in the other nine patients, diabetes duration was 3.9 ± 1.2 years (range 1–10). HbA_{1c} was $7.2 \pm 0.4\%$ (55 ± 5 mmol/mol); six patients were being treated by diet alone and six by oral hypoglycemic agents (three by metformin alone and three by metformin plus a sulfonylurea). Antidiabetic medication was discontinued 1 week before the metabolic studies. These 27 subjects all underwent laparoscopic RYGB; 10 T2D and 11 ND patients were restudied 1 year after surgery. Two control groups were included, consisting of 7 lean healthy volunteers and 14 obese ND volunteers whose BMI was matched to that of the RYGB patients at 1 year postsurgery. Thus, a total of 69 complete metabolic studies were performed.

This study was approved by the local ethics committee. The nature and purpose of the study were carefully explained to all participants before they provided written consent to participate.

Study design. At baseline and follow-up, subjects received a mixed meal test (MTT) with a double-tracer protocol. In brief, after an overnight (12-h) fast, subjects were admitted to our Clinical Research Unit at 8:00 A.M., and a polyethylene cannula was inserted into an antecubital vein for the infusion of all test substances. A second catheter was inserted retrogradely into an ipsilateral wrist vein on the dorsum of the hand for blood sampling, and the hand was

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Received 25 February 2013 and accepted 14 June 2013.

DOI: 10.2337/db13-0321

This article contains Supplementary Data online at <http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db13-0321/-DC1>.

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See accompanying commentary, p. 3671.

kept in a heated box at 65°C to achieve arterialization of venous blood. Baseline blood samples were drawn to measure plasma glucose, insulin, C-peptide, glucagon, glucagon-like peptide 1 (GLP-1), and gastric inhibitory polypeptide (GIP) concentrations and tracer enrichments. The MTT consisted of 75 g glucose in 150 mL water, 40 g parmesan cheese, and one 50-g egg (509 kcal; 16% protein, 28% fat, and 56% carbohydrate). The glucose solution was drunk after the solid component of the meal was consumed over a period of 10 min. The oral glucose drink was enriched with 1-[²H]glucose in order to trace glucose absorption. A primed (28 μmol · kg⁻¹ × [fasting plasma glucose]/5) continuous (0.28 μmol · min⁻¹ · kg⁻¹) infusion of 6,6-[²H₂]glucose was started (at time -120 min, or at -180 min in the T2D patients) via the antecubital vein catheter and continued until the end of the study. During the last 20 min of the basal equilibration period (at times -20, -10, and 0 min), blood samples were obtained for the determination of plasma glucose and hormone concentrations and tracer enrichments (when isotopic steady state was achieved). After the basal equilibration period, the meal was consumed over ~10 min. Plasma samples for the determination of plasma glucose and hormone concentrations and glucose tracer enrichments were obtained at 15, 30, 45, 60, 90, 120, 150, 180, 240, and 300 min after meal ingestion.

Surgical procedure. In subjects undergoing laparoscopic RYGB, a small proximal gastric pouch of 30 mL was created with several firings of a linear stapling endocutter; the jejunum was divided 120 cm distal to the ligament of Treitz, and a 2-cm end-to-side gastrojejunostomy was performed by using a hand-sewn technique. A side-to-side jejunojunction was then created, 150 cm distal to the gastrojejunostomy (17).

Analytical procedures. Plasma glucose was measured by the glucose-oxidase technique (Analox GM-9) and plasma insulin and C-peptide by electrochemiluminescence (on a COBAS e411 instrument; Roche, Indianapolis, IN). Plasma triglycerides and serum HDL cholesterol were assayed in duplicate by standard spectrophotometric methods on a Synchron Clinical System CX4 (Beckman Instruments, Fullerton, CA). 6,6-[²H₂]glucose and 1-[²H]glucose enrichments were measured by gas chromatography–mass spectrometry as described previously (25). Plasma glucagon was assayed by radioimmunoassay (Millipore Corporation, Billerica, MA). Plasma GLP-1 and GIP were assayed by radioimmunoassay as described previously (26). No significant cross-reactivity was declared by kit manufacturers. The sensitivity ranges are 0.003 nmol/L and 1.39 pmol/L for the C-peptide and insulin assay, respectively, 18.5 pg/mL for glucagon, and 1.5 pmol/L for GLP-1.

Data analysis. Fat-free mass was estimated with the use of electric bioimpedance on a Tanita scale; of note, this method has been validated in very obese individuals against the deuterated water technique (27). Endogenous glucose production (EGP) in the fasting state and throughout the absorptive period and rate of appearance of the oral glucose component (RaO) of the meal were calculated from the time course of the plasma tracer/tracee ratio of 6,6-[²H₂]glucose and 1-[²H]glucose by two-compartment modeling, as previously described (25). Insulin sensitivity was calculated as the mean metabolic clearance rate of glucose (MCR_G) during the 5 h of the MTT (from 6,6-[²H₂]glucose kinetics) divided by the mean plasma insulin concentration over the same time interval.

β-Cell function was quantitated by mathematical modeling of the plasma C-peptide response, as previously described (28). In brief, the model consists of three blocks: 1) a model for fitting the glucose concentration profile, the

purpose of which is to smooth and interpolate plasma glucose concentrations; 2) a model describing the dependence of insulin (or C-peptide) secretion on glucose concentration; and 3) a model of C-peptide kinetics, i.e., the two-exponential model proposed by Van Cauter et al. (29), in which the model parameters are individually adjusted to the subject's anthropometric data. The main parameter of the model is β-cell glucose sensitivity (β-GS), which is calculated as the mean slope of the dose-response function (i.e., relationship between insulin secretion rates [ISRs] and plasma glucose concentrations during corresponding times of the MTT). The model also yields an estimate of glucose rate sensitivity, which is the insulin secretory response to the rate of change in plasma glucose concentrations, and total insulin output, which is the total amount of insulin released over the 5 h of the meal (28). Plasma insulin clearance (MCR_I) was estimated as the ratio of fasting ISR to the fasting plasma insulin concentration.

The prehepatic insulin-to-glucagon molar concentration ratio was estimated by the following formula: {ISR(*t*)/hPF + [I(*t*)]/[G(*t*)] × [1 + MCR_{GIG}/hPF]}, where ISR(*t*) is the ISR at time *t*, hPF is hepatic plasma flow, [I(*t*)] and [G(*t*)] are the measured peripheral plasma concentrations of insulin and glucagon at time *t*, and MCR_{GIG} is the metabolic clearance rate of glucagon. hPF was estimated by multiplying the cardiac index (3.2 L · min⁻¹ · m⁻²) (30) by a plasma-to-blood ratio of 0.6, and by assuming that hepatic blood flow is 30% of cardiac index (=0.576 L · min⁻¹ · m⁻²) (31). MCR_{GIG} was taken to be 0.537 L · min⁻¹ · m⁻² (32).

The product of mean EGP and mean plasma insulin levels during the meal was taken as an index of hepatic insulin resistance. Areas under time-concentration curves (AUCs) were calculated by the trapezium rule.

Statistical analysis. All data are given as the mean ± SEM; parameters that were nonnormally distributed are presented as the median (interquartile range). Mann-Whitney test was used to compare group values, whereas surgery-induced changes were tested by the Wilcoxon signed rank test. Time series were analyzed by ANOVA for repeated measures; for these tests, variables with skewed distribution were log transformed. Group differences over time series were analyzed by two-way ANOVA for repeated measures. Simple associations were tested by calculating the Spearman rank correlation coefficient (*ρ*). Statistical analyses were performed using JMP7.0 and StatView5.0. *P* ≤ 0.05 was considered statistically significant.

RESULTS

Baseline. The four subject groups were matched for sex and age; T2D patients were older and had moderate fasting hyperglycemia (Table 1). After meal ingestion, plasma glucose excursions were similar in lean and obese controls and ND surgical patients, whereas T2D patients showed marked hyperglycemia (Fig. 1A). Fasting and postprandial insulin concentrations were higher in all three obese groups than in lean controls (Fig. 1B and Table 2). Fasting ISR generally increased with obesity as did the total insulin output over the 5 h of the test (Fig. 1C and Table 2). In T2D

TABLE 1
Anthropometric and metabolic parameters

			ND		T2D			
	Lean cts	Obese cts	Presurgery	<i>P</i>	1 year	Presurgery	<i>P</i>	1 year
<i>n</i>	7	15	11		11	10		10
HbA _{1c} (%)	—	—	—	—	—	7.1 ± 0.5	0.008	5.4 ± 0.1
Body weight (kg)	63 ± 2	94 ± 4§	146 ± 6§*	0.003	99 ± 5§	139 ± 9§*	0.005	91 ± 6§
Weight change (kg)	—	—	—	—	-46.0 ± 5.2	—	—	-48.1 ± 3.3
Weight change (%)	—	—	—	—	-31.3 ± 2.9	—	—	-34.6 ± 1.1
BMI (kg · m ⁻²)	23 ± 1	34 ± 1§	52.8 ± 1.8§*	0.003	36.1 ± 1.6§	51.6 ± 2.6§*	0.005	33.7 ± 1.8§
Fat-free mass (kg)	44 ± 1	54 ± 2§	70.0 ± 3.6§*	0.003	58.3 ± 3.2§	75.2 ± 6.4§*	0.009	61.4 ± 3.7§
Fat mass (kg)	19 ± 2	40 ± 3§	75.8 ± 3.9§*	0.003	41.0 ± 3.5§	64.2 ± 6.3§*#	0.005	29.9 ± 4.1
Fat mass (%)	29 ± 2	42 ± 2§	52.3 ± 1.6§*	0.003	40.9 ± 2.0§	45.9 ± 2.9§*	0.005	31.8 ± 3.1*#
Fasting [G] (mmol/L)	5.2 ± 0.2	5.3 ± 0.1	5.47 ± 0.13	0.02	5.05 ± 0.13	8.79 ± 0.87§*#	0.005	4.97 ± 0.16
Fasting [I] (pmol/L)	54 (28)	81 (38)§	143 (40)§*	0.003	44 (18)*	163 (145)§	0.005	60 (25)*
Fasting C-peptide (nmol/L)	0.46 (0.40)	0.52 (0.24)	1.41 (0.61)§*	0.003	0.48 (0.18)	0.88 (0.54)§*	0.01	0.54 (0.24)

All data are given as the mean ± SEM; parameters that were nonnormally distributed are presented as the median (interquartile range). G, glucose; I, insulin; Lean cts, lean controls; Obese cts, obese controls. *P* vs. presurgery by Wilcoxon test. **P* ≤ 0.05, vs. obese controls. §*P* ≤ 0.05, vs. lean controls by Mann-Whitney *U* test. #*P* ≤ 0.05, ND vs. T2D pre or 1 year.

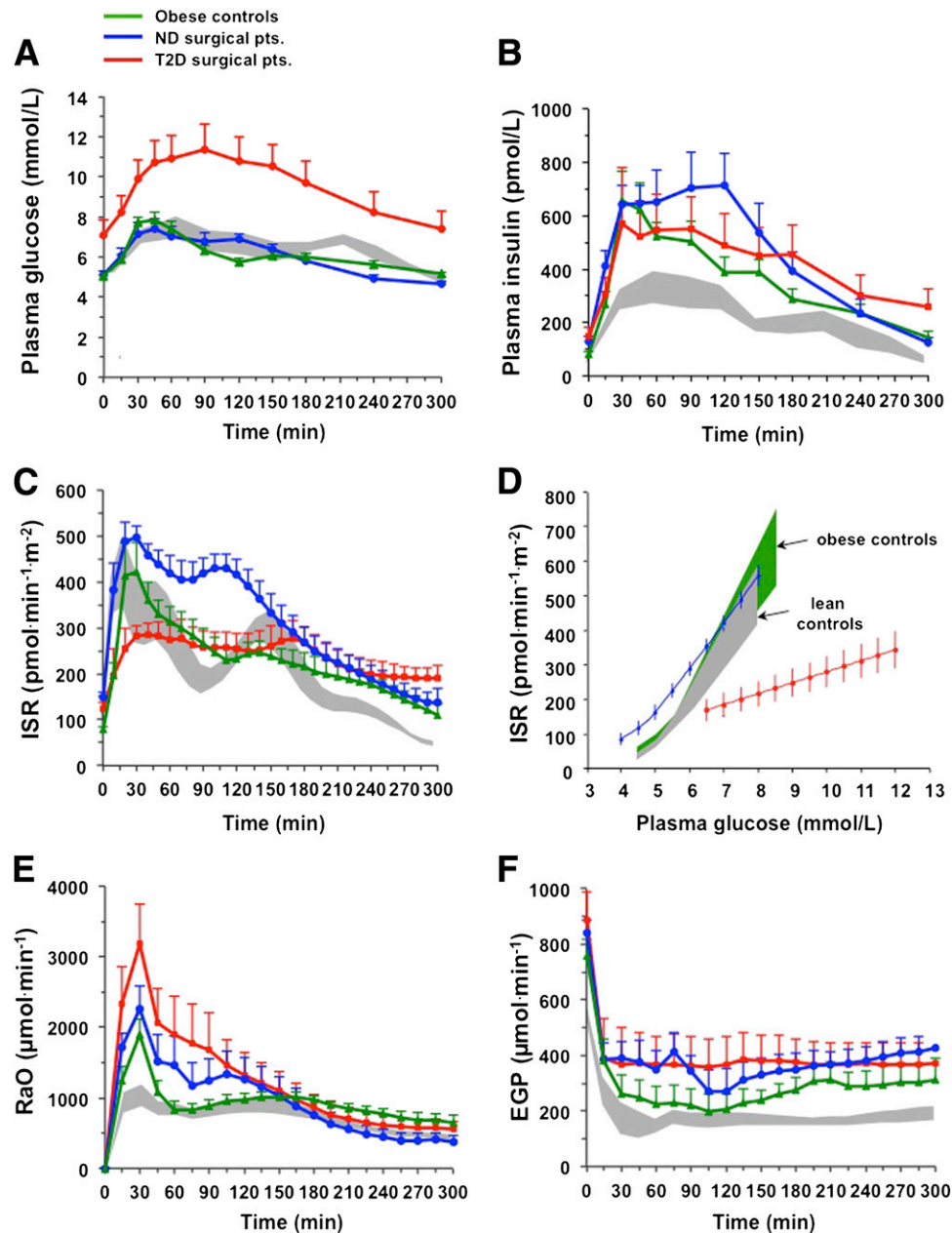


FIG. 1. Plasma glucose (A) and insulin concentrations (B), ISRs (C), and insulin secretion dose response (D), RaO (E), and EGP (F) in the four groups of study subjects at baseline. The gray shaded areas are the mean \pm SEM for the lean control group, and the green shaded area is the mean \pm SEM for the obese control group. Pts, patients.

patients, total insulin output was not different from that of lean controls despite the hyperglycemia. Consequently, β -GS was markedly impaired in this group (Fig. 1D and Table 2).

On the MTT, the RaO was similar in all groups, both in time course (Fig. 1E) and amount (averaging 51, 52, 50, and 62 g over 5 h in lean control subjects, obese control subjects, ND surgical patients, and T2D surgical patients, respectively) (Table 2). In contrast, fasting EGP was higher in each obese group (700 [277], 771 [242], and 764 [395] $\mu\text{mol}/\text{min}$) than in lean controls (580 [51] $\mu\text{mol} \cdot \text{min}^{-1}$) and remained higher during the MTT despite the postmeal hyperinsulinemia ($\text{AUC}_{\text{EGP}} = 79$ [46], 100 [56], and 100 [97] $\text{mmol} \cdot \text{min}$ vs. 61 [15] of controls). The difference in EGP time course between each of the obese groups and lean controls

(confirmed by repeated-measures ANOVA, $P < 0.001$ for all) was evident after the initial nadir (Fig. 1F). In the whole dataset, both fasting EGP and AUC_{EGP} were positively related to BMI ($r = 0.48$, $P = 0.001$, and $r = 0.30$, $P = 0.05$, respectively). Plasma glucose clearance rose during the MTT in all groups; its mean value over 5 h was reduced in both surgical groups, especially in T2D patients, in comparison with lean controls despite the hyperinsulinemia (Table 2). As a consequence, insulin sensitivity, as the insulin-mediated MCR_{G} , was lower in both obese groups and severely impaired in T2D patients (Table 2).

In all obese groups, fasting plasma glucagon was increased, whereas the meal-induced glucagon increments were blunted. Fasting GLP-1 and GIP levels were increased only in T2D patients, as was AUC_{GIP} (Table 2).

TABLE 2
Glucose metabolism and insulin secretion

	Lean cts	Obese cts	ND			T2D		
			Presurgery	<i>P</i>	1 year	Presurgery	<i>P</i>	1 year
Mean glucose [G] (mmol/L)	6.1 ± 0.2	6.1 ± 0.1	6.0 ± 0.2	NS	5.9 ± 0.1	9.3 ± 0.9*#	0.005	6.4 ± 0.3
AUC _G (mol/L)	1.80 (0.17)	1.84 (0.21)	1.76 (0.26)	NS	1.79 (0.17)	2.58 (0.99)§*#	0.01	1.87 (0.19)
Mean insulin [I] (pmol/L)	181 (81)	353 (208)§	493 (269)§	NS	416 (274)§	353 (581)§	0.02	320 (247)
AUC _I (nmol)	56 (15)	92 (65)§	148 (81)§	NS	125 (82)§	106 (113)§	0.03	96 (60)
Mean C-peptide (nmol/L)	1.59 (0.71)	1.41 (1.10)	2.90 (1.03)§*	0.02	1.80 (0.71)	1.85 (1.23)	NS	1.74 (0.91)
AUC _{C-pep} (μmol)	0.48 (0.21)	0.42 (0.33)	0.87 (0.31)§*	0.01	0.54 (0.21)	0.56 (0.37)*	NS	0.52 (0.27)
Fasting ISR (pmol · min ⁻¹ · m ⁻²)	54 (28)	74 (25)	147 (69)§*	0.004	57 (28)	104 (62)	0.02	73 (35)
IS (nmol · m ⁻²)	64 (23)	59 (37)	100 (29)§*	0.03	68 (30)	66 (28)#	NS	65 (44)
MCR _I (L · min ⁻¹ · m ⁻²)	1.66 (1.25)	0.95 (0.32)§	1.17 (0.73)	NS	1.50 (0.67)*	1.00 (0.73)	0.02	1.37 (0.26)*
β-GS (pmol · min ⁻¹ · m ⁻² · mM ⁻¹)	96 (60)	110 (92)	122 (41)	0.02	91 (19)	33 (25)§*#	0.007	61 (38)§*#
Rate sensitivity (nmol · m ⁻²)	4.3 (3.0)	4.1 (5.0)	5.2 (4.9)	0.04	15.0 (13.3)	2.6 (3.2)§*#	0.01	10.9 (8.4)*
Fasting EGP (μmol · kg _{FFM} ⁻¹ · min ⁻¹)	13.3 (1.7)	13.1 (4.0)	12.1 (0.4)	NS	11.9 (0.6)	11.9 (0.9)	NS	13.3 (0.8)
AUC _{EGP} (mmol · kg _{FFM} ⁻¹)	1.41 (0.22)	1.39 (0.87)	1.57 (0.80)	0.008	2.18 (0.89)§*	1.36 (1.39)	(0.09)	2.04 (1.15)§
AUC _{RaO} (mmol)	283 (79)	290 (28)	288 (59)	NS	264 (52)	343 (81)	NS	332 (242)
MCR _G (mL · min ⁻¹ · kg _{FFM} ⁻¹)	4.4 (1.1)	4.2 (0.4)	3.6 (1.1)§*	NS	3.6 (0.9)	2.3 (0.8)§*#	<0.01	3.4 (1.2)
MCR _C /[I] (mL · min ⁻¹ · kg _{FFM} ⁻¹ · nM ⁻¹)	24.4 (8.4)	10.5 (12.0)§	7.6 (5.2)§	NS	9.1 (9.2)§	6.7 (3.6)§*	0.008	11.3 (9.2)§
Fasting glucagon (pg · mL ⁻¹)	38 (17)	69 (33)§	69 (40)§	0.006	50 (23)*	69 (49)#	0.03	32 (16)*
AUC _{Glucagon} (ng · mL ⁻¹)	17.1 (4.4)	22.1 (6.2)§	24.6 (7.8)	NS	49.7 (25.7)§	23.2 (17.5)	NS	29.1 (21.9)
ΔAUC _{Glucagon} (ng · mL ⁻¹)	2.8 (3.7)	0.6 (3.9)§	0.6 (7.4)§	0.006	10.5 (8.4)§*	0.3 (3.0)§	0.007	5.7 (4.5)*
Fasting GIP (pmol/L)	4.5 (5.5)	10.0 (7.0)	9.0 (12.5)	NS	7.0 (4.0)	11.5 (10.0)§	NS	9.5 (11.0)
AUC _{GIP} (nmol · L ⁻¹)	12.5 (11.1)	7.2 (3.6)	6.6 (3.6)	NS	6.7 (3.3)§	11.3 (7.5)§#	NS	8.0 (9.8)
ΔAUC _{GIP} (nmol · L ⁻¹)	11.9 (12.3)	3.6 (4.4)§	4.1 (3.1)	NS	4.5 (3.2)	8.3 (7.2)	NS	5.7 (6.0)
Fasting GLP-1 (pmol/L)	11.0 (12.0)	7.0 (4.5)	9.0 (7.0)	NS	11.0 (7.0)*	12.5 (12.0)§#	NS	11.5 (7.0)*
AUC _{GLP-1} (nmol · L ⁻¹)	6.3 (3.0)	4.5 (2.2)	6.2 (3.6)	0.008	16.8 (9.3)§*	4.7 (3.7)	0.02	12.1 (18.5)*

All data are given as the mean ± SEM; parameters that were nonnormally distributed are presented as the median (interquartile range). AUC, area under the curve; β-GS, β-cell glucose sensitivity; EGP, endogenous glucose production; ISR, insulin secretion rate; ΔAUC, incremental AUC; IS, total insulin output; Lean cts, lean controls; MCR_G, metabolic clearance rate of glucose; MCR_I, insulin clearance; Obese cts, obese controls; RaO, rate of appearance of oral glucose. *P* vs. presurgery by Wilcoxon test. **P* ≤ 0.05, vs. obese controls. §*P* ≤ 0.05, vs. lean controls by Mann-Whitney test. #*P* ≤ 0.05, ND vs. T2D pre or 1 year. The *P* value in parentheses shows that it is near the statistical significance.

After surgery. At 1 year, ND and T2D patients had lost 31 and 35% of their initial body weight, respectively, in an approximate ratio of 1:3 between fat-free mass and fat mass (Table 1). In T2D patients, HbA_{1c} had dropped by an average 1.5%, and all patients were off antidiabetic drug treatment; of them, two patients had impaired glucose tolerance and one of these two also had impaired fasting glucose (5.9 mmol/L). Four patients experienced dumping-like symptoms (tachycardia, sweating, nausea, and diarrhea) after meal ingestion.

On the MTT, the postsurgery plasma glucose and insulin profiles were grossly altered in both ND and T2D subjects, with a large excursion peaking at 60 min followed by a sharp drop to basal levels or below (Fig. 2). In the T2D group, the glucose and insulin AUCs were significantly smaller than preoperatively (Table 2). The time course of insulin secretion ran parallel to that of plasma glucose (Fig. 3); fasting insulin secretion decreased in both groups and total insulin output decreased in the ND group (Table 2). When viewing insulin secretion in the context of the corresponding plasma glucose levels, β-GS was significantly reduced in the ND group postsurgery (although still within the range of the obese control group), and ~100% increased in T2D patients (although still largely below control values) (Fig. 3). The small decline in β-GS in ND subjects could be due to chronically reduced carbohydrate intake or could simply be time related. Insulin sensitivity improved significantly in both groups (by two-way ANOVA from repeated measures, *F* = 10.5, *P* < 0.005) to levels

similar to those of the obese controls. The estimates of insulin sensitivity fell along the overall relationship describing the association of insulin resistance with BMI, and the surgically induced changes in insulin sensitivity were correlated with the corresponding changes in body weight (Table 2 and Fig. 4).

In both ND and T2D subjects postsurgery, the rate of appearance of oral glucose was similar in total amount (Table 2) but distorted in time course, in a manner resembling the plasma glucose curves closely (Fig. 5). Thus, most oral glucose was absorbed during the first hour after meal ingestion, as the AUC calculated in the first 60 min showed (AUC pre- vs. postsurgery: 122 [63] vs. 186 [37] mmol in ND and 129 [81] vs. 190 [94] mmol in T2D, *P* < 0.04 vs. postsurgery for both). EGP was unchanged in ND as well as T2D patients in the fasting state but rebounded significantly above presurgery values during meal absorption, particularly over the second half of the postcibal period when plasma glucose concentrations were back to basal or below. The EGP time course was increased in both groups (with no significant difference between T2D and ND patients, *P* = 0.74), whether expressed in absolute terms (Fig. 5) or normalized by fat-free mass (*F* = 133.9, *P* < 0.0001) (Table 2).

Fasting glucagon levels dropped in both ND and T2D, but the response to the meal (as the incremental AUC) was greatly enhanced in both groups (Table 2 and Fig. 6). After surgery, the estimated prehepatic insulin-to-glucagon molar concentration ratio was higher than presurgery (especially

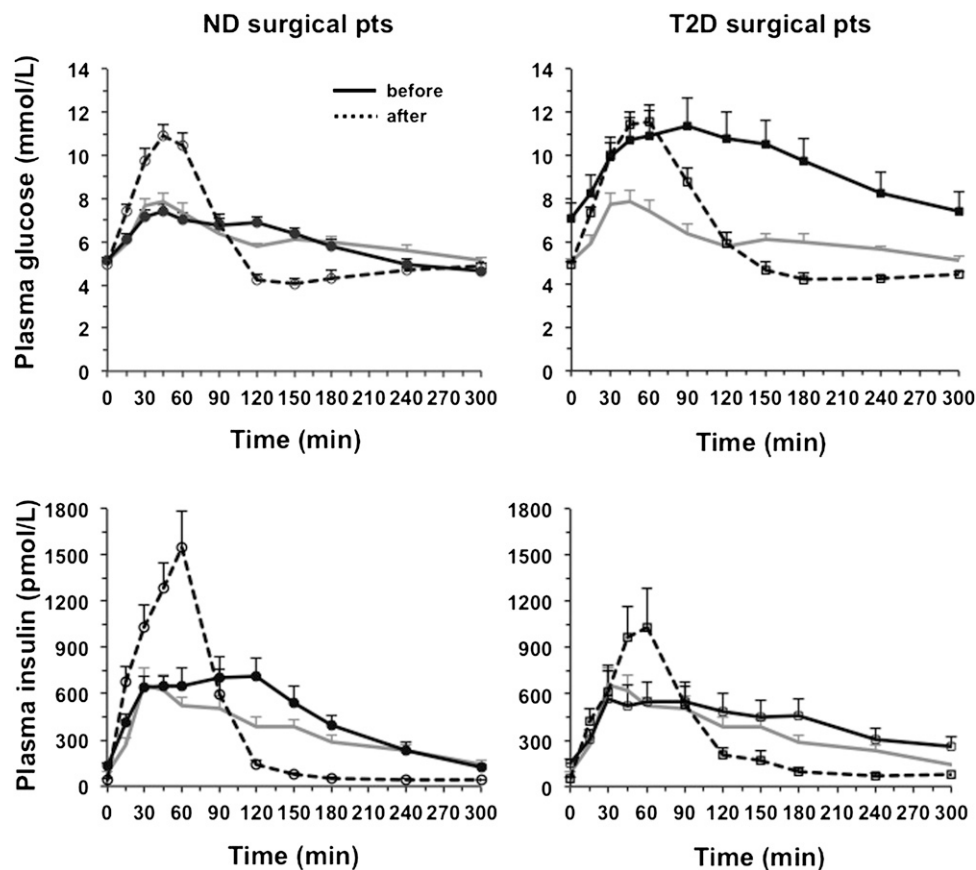


FIG. 2. Plasma glucose and insulin concentrations in the patients (pts) before and after RYGB. The corresponding data for the obese control group are shown by the gray line.

in T2D patients) for the first 80–100 min but then fell below preoperative levels for the remainder of the MTT in both groups (Supplementary Fig. 1).

The GLP-1, but not the GIP, response was increased in ND as well as T2D; the time profile of the GLP-1 and GIP responses showed a sharper early rise and a rapid drop thereafter (Fig. 6).

MCR₁, calculated as the ratio of fasting insulin output to fasting peripheral plasma insulin concentrations, was slightly reduced in the obese groups as compared with the lean controls and was significantly increased postsurgery in both ND and T2D (two-way ANOVA).

DISCUSSION

Before the operation, the metabolic response to the mixed meal in the ND obese subjects was characterized by insulin resistance of glucose disposal and insulin hypersecretion, with preserved β -GS and rate sensitivity, i.e., the metabolic picture of obesity. In the T2D patients, insulin resistance was worse, and both β -GS and rate sensitivity were markedly impaired, thereby accounting for the hyperglycemia. Whereas the rate of appearance of oral glucose was similar in time course and total amount in all groups, in obese and T2D patients, EGP was higher at baseline and was incompletely suppressed during the meal, in some proportion to the degree of obesity, despite the hyperinsulinemia, thereby manifesting liver insulin resistance. In the T2D patients, the blunted rise in the prehepatic insulin-to-glucagon ratio during the first 2 h postmeal (Supplementary Fig. 1) likely contributed to the elevated EGP.

One year after RYGB, at a time when drastic weight loss had occurred and body weight had stabilized, fasting plasma glucose was similar in ND and diabetic patients, but in the latter, 2-h glucose concentrations were significantly higher (5.93 ± 0.53 vs. 4.23 ± 0.31 mmol/L, $P = 0.02$), and mean glucose levels during the 5-h meal tended to be higher (Table 2), than in ND surgical patients. Insulin sensitivity improved in both surgical groups to levels close to those of the BMI-matched control group, i.e., in rough proportion to the weight loss and without reaching the values of the lean control subjects (Fig. 4). This result, obtained by tracer analysis of a dynamic test such as the MTT, confirms the findings of steady-state (clamp) measurements of insulin sensitivity (17), namely, that RYGB does not potentiate insulin action beyond, or independently of, the effect on weight loss. β -GS worsened slightly in the ND surgical patients, whereas it doubled in the diabetic patients, in whom, however, it remained markedly inferior to that of BMI-matched ND subjects. This outcome prevailed despite the large increase in GLP-1 response, a consistent change after bariatric surgery (10–17), even as long as 10 years after surgery (33), which is quantitatively related to enhanced insulin secretion (19). In both groups, rate sensitivity improved postsurgery, likely reflecting the rapid plasma glucose excursions as well as, at least in part, the heightened GLP-1 surge (Supplementary Fig. 2A). The latter finding is compatible with evidence that a rapid rather than delayed delivery of insulin improves glucose tolerance irrespective of the degree of insulin resistance (34), and may explain why meal tolerance in our patients was preserved 1 year after surgery despite the abnormal β -GS.

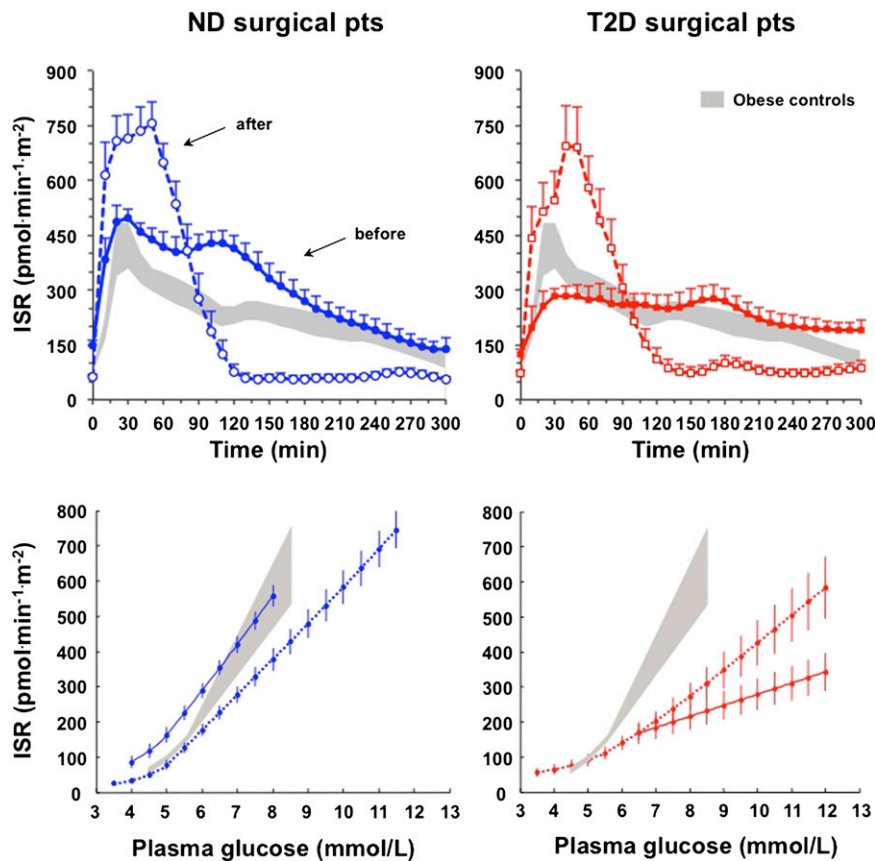


FIG. 3. ISR and dose-response function in the patients (pts) before and after RYGB. The corresponding data for the obese control group are shown by the shaded areas.

Thus, in T2D patients, recovery of both insulin sensitivity and glucose sensitivity was sizeable but incomplete, leaving behind a trace of glucose intolerance. These results confirm previous findings in both ND and T2D subjects, using a liquid formula meal and homeostasis model assessment of insulin resistance as a surrogate index of insulin resistance (35), or an oral glucose tolerance test (18). Further increments in both functions may occur if more weight is lost or as removal of the toxic effects of hyperglycemia continues; on the other hand, diabetes may relapse if weight is regained, if insulin resistance otherwise worsens, or if the disease itself should progress. In addition, as previously shown, in T2D patients, the outcome of glucose tolerance at 1 year post-RYGB may be better or worse than in the present series of patients depending on the initial degree of β -cell dysfunction (18).

As found by Bradley et al. (24) in ND subjects, RYGB drastically changed the shape of the glucose and hormonal responses to the meal, with a markedly biphasic pattern reminiscent of a dumping syndrome. Glucose fluxes confirmed that this was the consequence of the altered delivery of gastric contents to the peripheral circulation. A somewhat unexpected consequence of the altered pattern of transit of alimentary glucose was the reduced suppression of endogenous glucose release during the meal. Under euglycemic clamp conditions, hepatic and peripheral insulin resistance are typically somewhat interrelated and change consensually (36). A mixed meal, however, creates a hormonal makeover by stimulating both insulin and glucagon secretion, especially in diabetic patients in whom glucagon responses are exaggerated (37). In our surgical

patients, both fasting insulin and fasting glucagon concentrations fell significantly postsurgery; correspondingly, fasting EGP did not change after the operation. After the meal, the prehepatic insulin-to-glucagon molar ratio normally rises in a time course roughly parallel to that of

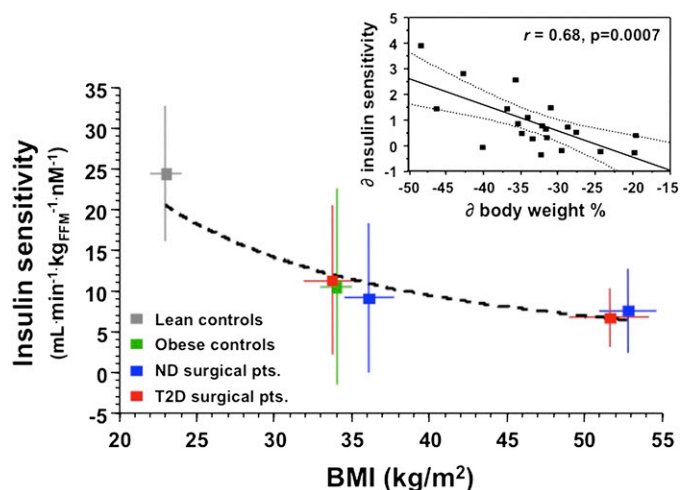


FIG. 4. Insulin sensitivity (as the ratio of mean glucose clearance to mean insulin concentration during a 5-h MTT) plotted against the corresponding BMI value in the four study groups. The points to the far right are those for the surgical groups before the operation. Plots are median (interquartile range). The dotted line is a power function fit of the plots. The inset shows the relationship between the change in insulin sensitivity and the percent change in body weight in the surgical patients (pts); the linear fit and the 95% CI are shown.

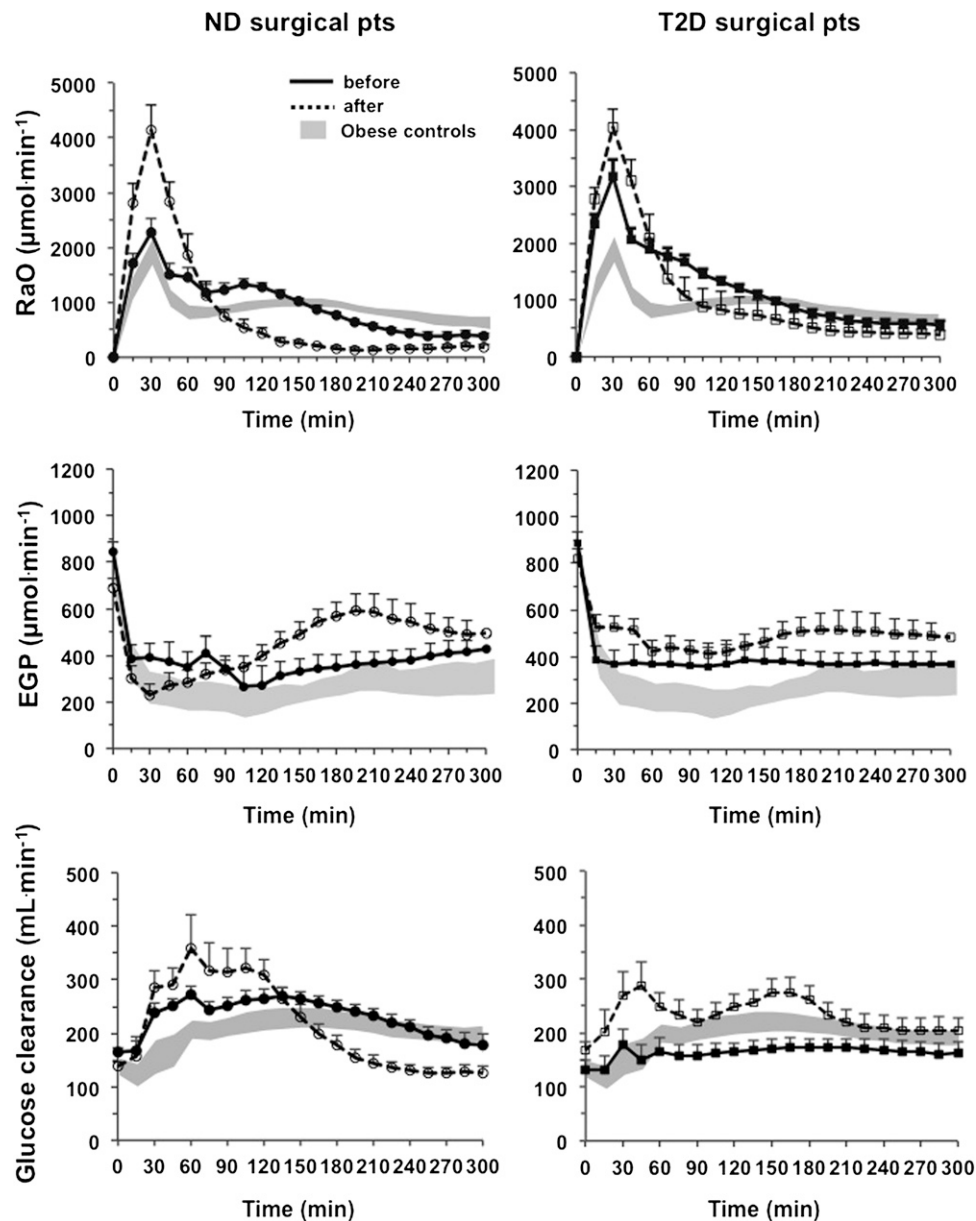


FIG. 5. Rate of appearance of oral glucose (RaO), endogenous glucose (EGP), and glucose clearance rate in the patients (pts) before and after RYGB. The corresponding data for the obese control group are shown by the shaded areas. By two-way ANOVA, the time course of EGP is significantly higher postsurgery than presurgery ($F = 27.6$, $P < 0.0001$) in both ND and T2D.

plasma glucose concentrations and ISRs; in T2D patients, the ratio shows the blunted initial rise followed by a sustained increase typical of the insulin secretory response (Supplementary Fig. 1). After surgery, however, plasma glucagon rose sharply and remained above basal levels throughout the absorption period both in ND and T2D patients (Fig. 6), such that the prehepatic insulin-to-glucagon ratio, after peaking at ~ 1 h postmeal, dropped rapidly to levels below presurgery (Supplementary Fig. 1), in phase with the lower plasma glucose levels. Thus, the raised postmeal EGP was the integrated liver response to lower insulin, higher glucagon, and falling glucose levels. The correlation between the calculated index of hepatic insulin resistance and the glucagon response supports the role of the relative hyperglucagonemia (Supplementary Fig. 2B). It should be noted that in the cited study in ND subjects (24), the time course of EGP was similar to ours

but the glucagon response was rather flat and unchanged from presurgery. This difference may be due to the use of a smaller meal (~ 300 kcal with only 9 g of protein compared with 500 kcal in the current study) and/or the shorter time interval from surgery (22 weeks), a time at which patients are typically still losing weight. The stimulus for the meal-induced hyperglucagonemia 1 year after surgery, which has been noted before (15), remains undetermined. Diminished paracrine control of α -cell activity by insulin (38) seems unlikely as the glucagon excursions in our patients were essentially synchronous with those of plasma insulin. Potential mechanisms are enhanced postprandial neural stimulation (39), overstimulation of glucagon release by GIP (40) or GLP-2 (41), and cosecretion of glucagon and GLP-1/GIP by intestinal cells (42). In our data, the postsurgery time courses of glucagon and GIP were in phase, lending some support to an effect of GIP on α -cells. It

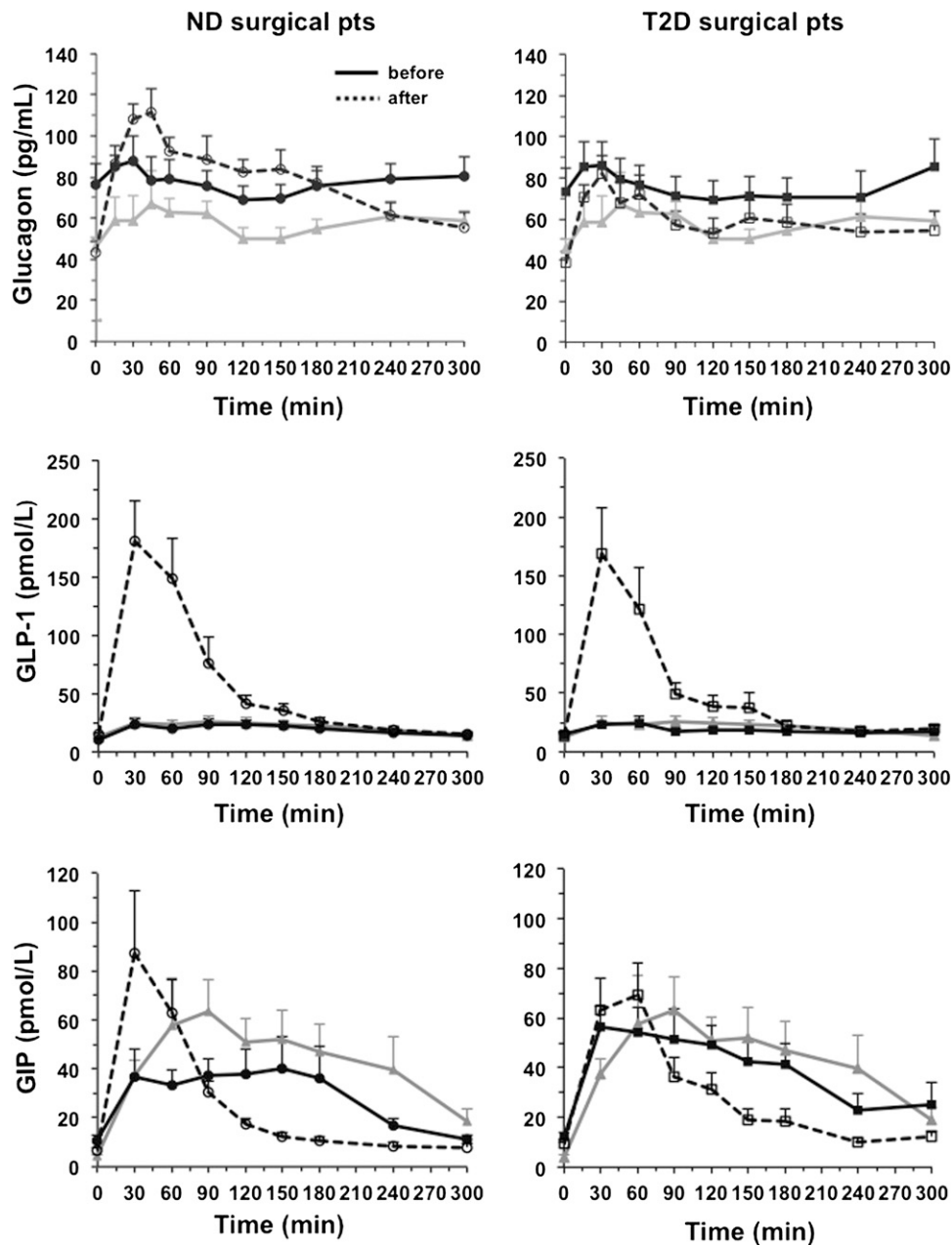


FIG. 6. Plasma glucagon, GLP-1, and GIP response to the meal in the patients (pts) before and after RYGB. The corresponding data for the obese control group are shown by the gray lines.

should also be considered that the standard glucagon assay has limitations in terms of its ability to discriminate between pancreatic glucagon and other sources (43).

Although gastric emptying was not measured in the present studies, our previous work in post-RYGB patients (using a scintigraphic technique) has confirmed that the operation causes accelerated gastric emptying even 14–26 months later (44); this result is fully compatible with the accelerated appearance of oral glucose in the present series.

In summary, the long-term outcome of RYGB is a comparable weight reduction and a proportional improvement in insulin sensitivity in ND and well-controlled, recent-onset diabetic patients. In both, delivery of oral glucose to the peripheral circulation is maintained in quantity but strikingly changed in time course, with a “dumping” pattern resulting from the modified anatomy. Glucose tolerance is preserved in ND and much improved in diabetic patients; in the latter,

a detectable degree of glucose intolerance persists. In diabetic patients, β -cell function improves presumably as a combined result of reversal of glucose toxicity, lower insulin secretory burden, and incretin-mediated potentiation, but β -GS remains subnormal. This may predispose patients to recurrent diabetes. The surgery-induced change in glucose delivery triggers not only a heightened GLP-1 response but also an exaggerated glucagon response synchronous with the glucose excursions, which likely contributes to maintain euglycemia through elevated rates of EGP.

ACKNOWLEDGMENTS

This work was aided in part by a grant from the Italian Ministry of Education, University and Research (PRIN 2010329EKE).

No potential conflicts of interest relevant to this article were reported.

S.C. designed the study, performed the *in vivo* studies, analyzed the data, and wrote the manuscript. E.M. performed the *in vivo* studies and contributed to the discussion. A.G. and D.C. analyzed the tracer data. J.J.H. was responsible for hormone measurements and contributed to the discussion. B.A. and M.N. performed the *in vivo* studies. S.B. was responsible for laboratory measurements. M.A. operated on the patients. A.M. analyzed the β -cell function data. E.F. contributed to the design of the study, analyzed the data, and wrote the manuscript. S.C. and E.F. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

The authors are grateful to Dr. Laura Manca (University of Pisa) for help with the statistical analysis.

REFERENCES

- Buchwald H, Estok R, Fahrenbach K, et al. Weight and type 2 diabetes after bariatric surgery: systematic review and meta-analysis. *Am J Med* 2009; 122:248–256, e5
- Vetter ML, Cardillo S, Rickels MR, Iqbal N. Narrative review: effect of bariatric surgery on type 2 diabetes mellitus. *Ann Intern Med* 2009;150: 94–103
- Carlsson LM, Peltonen M, Ahlin J, et al. Bariatric surgery and prevention of type 2 diabetes in Swedish obese subjects. *N Engl J Med* 2012;367:695–704
- Pories WJ, Swanson MS, MacDonald KG, et al. Who would have thought it? An operation proves to be the most effective therapy for adult-onset diabetes mellitus. *Ann Surg* 1995;222:339–350; discussion 350–352
- Dixon JB, le Roux CW, Rubino F, Zimmet P. Bariatric surgery for type 2 diabetes. *Lancet* 2012;379:2300–2311
- Rubino F, Marescaux J. Effect of duodenal-jejunal exclusion in a non-obese animal model of type 2 diabetes: a new perspective for an old disease. *Ann Surg* 2004;239:1–11
- Rubino F, R'bib SL, del Genio F, Mazumdar M, McGraw TE. Metabolic surgery: the role of the gastrointestinal tract in diabetes mellitus. *Nat Rev Endocrinol* 2010;6:102–109
- Ferrannini E, Mingrone G. Impact of different bariatric surgical procedures on insulin action and beta-cell function in type 2 diabetes. *Diabetes Care* 2009;32:514–520
- Stefater MA, Wilson-Pérez HE, Chambers AP, Sandoval DA, Seeley RJ. All bariatric surgeries are not created equal: insights from mechanistic comparisons. *Endocr Rev* 2012;33:595–622
- le Roux CW, Welbourn R, Werling M, et al. Gut hormones as mediators of appetite and weight loss after Roux-en-Y gastric bypass. *Ann Surg* 2007; 246:780–785
- Korner J, Bessler M, Inabnet W, Taveras C, Holst JJ. Exaggerated glucagon-like peptide-1 and blunted glucose-dependent insulinotropic peptide secretion are associated with Roux-en-Y gastric bypass but not adjustable gastric banding. *Surg Obes Relat Dis* 2007;3:597–601
- Laferrère B, Heshka S, Wang K, et al. Incretin levels and effect are markedly enhanced 1 month after Roux-en-Y gastric bypass surgery in obese patients with type 2 diabetes. *Diabetes Care* 2007;30:1709–1716
- Rodieux F, Giusti V, D'Alessio DA, Suter M, Tappy L. Effects of gastric bypass and gastric banding on glucose kinetics and gut hormone release. *Obesity (Silver Spring)* 2008;16:298–305
- Laferrère B, Teixeira J, McGinty J, et al. Effect of weight loss by gastric bypass surgery versus hypocaloric diet on glucose and incretin levels in patients with type 2 diabetes. *J Clin Endocrinol Metab* 2008; 93:2479–2485
- Vidal J, Nicolau J, Romero F, et al. Long-term effects of Roux-en-Y gastric bypass surgery on plasma glucagon-like peptide-1 and islet function in morbidly obese subjects. *J Clin Endocrinol Metab* 2009;94:884–891
- Kashyap SR, Daud S, Kelly KR, et al. Acute effects of gastric bypass versus gastric restrictive surgery on beta-cell function and insulinotropic hormones in severely obese patients with type 2 diabetes. *Int J Obes (Lond)* 2010;34:462–471
- Camastra S, Gastaldelli A, Mari A, et al. Early and longer term effects of gastric bypass surgery on tissue-specific insulin sensitivity and beta cell function in morbidly obese patients with and without type 2 diabetes. *Diabetologia* 2011;54:2093–2102
- Nannipieri M, Mari A, Anselmino M, et al. The role of beta-cell function and insulin sensitivity in the remission of type 2 diabetes after gastric bypass surgery. *J Clin Endocrinol Metab* 2011;96:E1372–E1379
- Salehi M, Prigeon RL, D'Alessio DA. Gastric bypass surgery enhances glucagon-like peptide 1-stimulated postprandial insulin secretion in humans. *Diabetes* 2011;60:2308–2314
- Muscelli E, Mingrone G, Camastra S, et al. Differential effect of weight loss on insulin resistance in surgically treated obese patients. *Am J Med* 2005; 118:51–57
- Falkén Y, Hellström PM, Holst JJ, Näslund E. Changes in glucose homeostasis after Roux-en-Y gastric bypass surgery for obesity at day three, two months, and one year after surgery: role of gut peptides. *J Clin Endocrinol Metab* 2011;96:2227–2235
- Schauer PR, Kashyap SR, Wolfski K, et al. Bariatric surgery versus intensive medical therapy in obese patients with diabetes. *N Engl J Med* 2012;366: 1567–1576
- Yip K, Heinberg L, Giegerich V, Schauer PR, Kashyap SR. Equivalent weight loss with marked metabolic benefit observed in a matched cohort with and without type 2 diabetes 12 months following gastric bypass surgery. *Obes Surg* 2012;22:1723–1729
- Bradley D, Conte C, Mittendorfer B, et al. Gastric bypass and banding equally improve insulin sensitivity and β cell function. *J Clin Invest* 2012; 122:4667–4674
- Gastaldelli A, Casolaro A, Pettiti M, et al. Effect of pioglitazone on the metabolic and hormonal response to a mixed meal in type II diabetes. *Clin Pharmacol Ther* 2007;81:205–212
- Muscelli E, Mari A, Natali A, et al. Impact of incretin hormones on β -cell function in subjects with normal or impaired glucose tolerance. *Am J Physiol Endocrinol Metab* 2006;291:E1144–E1150
- Strain GW, Wang J, Gagner M, Pomp A, Inabnet WB, Heymsfield SB. Bioimpedance for severe obesity: comparing research methods for total body water and resting energy expenditure. *Obesity (Silver Spring)* 2008;16:1953–1956
- Mari A, Camastra S, Toschi E, et al. A model for glucose control of insulin secretion during 24 h of free living. *Diabetes* 2001;50(Suppl. 1):S164–S168
- Van Cauter E, Mestrez F, Sturis J, Polonsky KS. Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes* 1992;41:368–377
- Taylor HL, Tiede K. A comparison of the estimation of the basal cardiac output from a linear formula and the cardiac index. *J Clin Invest* 1952;31:209–216
- Cherrick GR, Stein SW, Leevy CM, Davidson CS. Indocyanine green: observations on its physical properties, plasma decay, and hepatic extraction. *J Clin Invest* 1960;39:592–600
- Fisher M, Sherwin RS, Hendler R, Felig P. Kinetics of glucagon in man: effects of starvation. *Proc Natl Acad Sci USA* 1976;73:1735–1739
- Dar MS, Chapman WH 3rd, Pender JR, et al. GLP-1 response to a mixed meal: what happens 10 years after Roux-en-Y gastric bypass (RYGB)? *Obes Surg* 2012;22:1077–1083
- Basu A, Alzaid A, Dinneen S, Caumo A, Cobelli C, Rizza RA. Effects of a change in the pattern of insulin delivery on carbohydrate tolerance in diabetic and nondiabetic humans in the presence of differing degrees of insulin resistance. *J Clin Invest* 1996;97:2351–2361
- Jørgensen NB, Jacobsen SH, Dirksen C, et al. Acute and long-term effects of Roux-en-Y gastric bypass on glucose metabolism in subjects with type 2 diabetes and normal glucose tolerance. *Am J Physiol Endocrinol Metab* 2012;303:E122–E131
- Ferrannini E, Gastaldelli A, Iozzo P. Pathophysiology of prediabetes. *Med Clin North Am* 2011;95:327–339, vii–viii
- Unger RH, Cherrington AD. Glucagonocentric restructuring of diabetes: a pathophysiologic and therapeutic makeover. *J Clin Invest* 2012;122:4–12
- Maruyama H, Hisatomi A, Orzi L, Grodsky GM, Unger RH. Insulin within islets is a physiologic glucagon release inhibitor. *J Clin Invest* 1984;74: 2296–2299
- Adrian TE, Bloom SR, Edwards AV. Neuroendocrine responses to stimulation of the vagus nerves in bursts in conscious calves. *J Physiol* 1983;344:25–35
- Meier JJ, Gallwitz B, Siepmann N, et al. Gastric inhibitory polypeptide (GIP) dose-dependently stimulates glucagon secretion in healthy human subjects at euglycaemia. *Diabetologia* 2003;46:798–801
- Meier JJ, Nauck MA, Pott A, et al. Glucagon-like peptide 2 stimulates glucagon secretion, enhances lipid absorption, and inhibits gastric acid secretion in humans. *Gastroenterology* 2006;130:44–54
- Habib AM, Richards P, Cairns LS, et al. Overlap of endocrine hormone expression in the mouse intestine revealed by transcriptional profiling and flow cytometry. *Endocrinology* 2012;153:3054–3065
- Holst JJ. Extrapancreatic glucagons. *Digestion* 1978;17:168–190
- Dirksen C, Damgaard M, Bojsen-Møller KN, et al. Fast pouch emptying, delayed small intestinal transit, and exaggerated gut hormone responses after Roux-en-Y gastric bypass. *Neurogastroenterol Motil* 2013;25:346–e255