Clinical Science

Contribution of endogenous glucagon-like peptide-1 to changes in glucose metabolism and islet function in people with type 2 diabetes four weeks after Roux-en-Y gastric bypass (RYGB)☆

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ABSTRACT

Glucagon-Like Peptide-1 (GLP-1) is an insulin secretagogue which is elevated after Roux-en-Y Gastric Bypass (RYGB). However, its contribution to glucose metabolism after RYGB remains uncertain.
Aims: We tested the hypothesis that GLP-1 lowers postprandial glucose concentrations and improves β-cell function after RYGB.
Materials and Methods: To address these questions we used a labeled mixed meal to assess glucose metabolism and islet function in 12 obese subjects with type 2 diabetes studied before and four weeks after RYGB. During the post-RYGB study subjects were randomly assigned to receive an infusion of either saline or Exendin-9,39 a competitive antagonist of GLP-1 at its receptor. Exendin-9,39 was infused at 300 pmol/kg/min for 6 h. All subjects underwent RYGB for medically-complicated obesity.
Results: Exendin-9,39 resulted in increased integrated incremental postprandial glucose concentrations (181 ± 154 vs. 582 ± 129 mmol per 6 h, $p = 0.02$). In contrast, this was unchanged in the presence of saline (275 ± 88 vs. 315 ± 66 mmol per 6 h, $p = 0.56$) after RYGB. Exendin-9,39 also impaired β-cell responsivity to glucose but did not alter Disposition Index (DI).
Conclusions: These data indicate that the elevated GLP-1 concentrations that occur early after RYGB improve postprandial glucose tolerance by enhancing postprandial insulin secretion.

1. Introduction

Roux-en-Y Gastric Bypass (RYGB) is commonly performed and can result in remission of type 2 diabetes [1]. This seems to be contingent on the duration and severity of the disease prior to the procedure [2] as β-cell function seems to be relatively unchanged by RYGB [3], in some but not all [4], studies. Glucagon-Like Peptide-1 (GLP-1) has been posited as a hormonal mediator of diabetes remission, since postprandial GLP-1 concentrations markedly increase after RYGB [5] and infusion or injection of GLP-1 enhances insulin secretion and suppresses glucagon release [6]. The possibility that RYGB might improve β-cell function compared to other bariatric interventions [4] would suggest that endogenous GLP-1 is an important contributor to the changes in glucose metabolism that occur in people with type 2 diabetes after RYGB.

Caloric restriction similar to that experienced early after RYGB also improves glucose metabolism in subjects with type 2 diabetes [7] and is associated with decreased hepatocellular fat, increased hepatic insulin sensitivity and decreased fasting endogenous glucose production (EGP) [8,9]. An improvement in β-cell function [7] occurs notwithstanding postprandial GLP-1 concentrations far lower than those observed after RYGB [10]. Jorgensen et al. utilized Exendin-9,39, a competitive antagonist of GLP-1 at its receptor, to block the effects of endogenous GLP-1 after RYGB [11] concluding that GLP-1 is important to the remission of type 2 diabetes. However, this study contrasts with other studies utilizing Exendin-9,39 in post-RYGB subjects which resulted in modest effects on postprandial glucose and insulin concentrations [12,13]. More recently, Vetter et al. reported that identical weight loss with and without RYGB had similar effects on glucose metabolism independently of GLP-1 [14]. However, it is possible that early in the post-operative course after RYGB, GLP-1 may have an effect on β-cell function which is difficult to discern from the effects of caloric restriction in the first months after surgery [7].

Table 1
Anthropometric characteristics of the participants in each group, before and after RYGB.

	Saline → Exendin-9,39		Saline → Saline		[*]p-Value
	Baseline	Exendin-9,39	Baseline	Saline	
Sex (M/F)	2/5		1/4		
Age (years)	54 ± 2		51 ± 6		0.34
HbA$_{1c}$ (%)	6.9 ± 0.4		6.6 ± 0.4		0.58
Weight (kg)	116 ± 10 [**]$p = 4.6 \times 10^{-5}$	104 ± 9	136 ± 11 [**]$p = 0.005$	125 ± 10	0.09
Lean body mass (kg)	56 ± 5 [**]$p = 1.4 \times 10^{-5}$	51 ± 4	66 ± 7 [**]$p = 0.006$	60 ± 5	0.22
Fasting glucose (mmol/l)	8.9 ± 1.1 [**]$p = 0.01$	5.9 ± 0.4	7.5 ± 1.2 [**]$p = 0.01$	6.1 ± 0.9	0.09
Fasting insulin (pmol/l)	75 ± 13 [**]$p = 0.003$	37 ± 10	94 ± 10 [**]$p = 0.03$	58 ± 8	0.23
Fasting C-peptide (nmol/l)	1.5 ± 0.2 [**]$p = 0.001$	0.9 ± 0.2	1.6 ± 0.1 [**]$p = 0.006$	1.3 ± 0.1	0.05
Fasting glucagon (ng/l)	73 ± 6 [**]$p = 0.01$	60 ± 3	65 ± 5 [**]$p = 0.93$	65 ± 6	0.05

[*] Represents the value of an unpaired, two-tailed *t*-test for between-group differences in symmetrical percent change for a given parameter.

[**] Represents the value of a paired, two-tailed t-test for within-group differences for a given parameter.

We undertook the present series of experiments to examine the effects of endogenous GLP-1 secretion on glucose metabolism four weeks after RYGB in obese subjects with type 2 diabetes [12,15]. Subjects were studied on two occasions. The first study occurred on the day prior to bariatric surgery while the second occurred four weeks after surgery. During the study performed prior to surgery all subjects received a saline infusion. After surgery, subjects were randomized to receive either saline (Saline → Saline) or Exendin-9,39 (Saline → Exendin-9,39). We report that while blockade of the GLP-1 receptor with Exendin-9,39, impairs insulin secretion and decreases β-cell responsivity it did not alter postprandial glucose turnover. We conclude that elevated postprandial GLP-1 concentrations seen after RYGB slightly, but significantly, enhance β-cell responsivity to glucose after surgery.

2. Research Design and Methods

2.1. Subjects

After approval from the Mayo Institutional Review Board, twelve subjects with type 2 diabetes (treated with metformin alone or in combination with a sulfonylurea) undergoing RYGB gave written, informed consent to participate in the study. All subjects had no microvascular complications, were at a stable weight and did not engage in regular exercise. They were instructed to follow a weight maintenance diet (~55% carbohydrate, 30% fat, and 15% protein) prior to the first study and instructed to stop glucose-lowering medication three weeks prior to study. During this period subjects self-monitored fasting glucose concentrations twice daily and were instructed to contact the study team in the event of hyperglycemia (≥11 mmol/l) or polyuria and polydipsia. This did not occur in any of the subjects. Body composition was measured using dual-energy X-ray absorptiometry (DPX scanner; Lunar, Madison, WI).

2.2. Experimental Design

Subjects were studied on two occasions four weeks apart. On the first occasion (Pre-RYGB study) subjects were studied on the day prior to RYGB. Saline was infused from 0 to 360 min of the study (see below). On the second occasion subjects were studied 4 weeks after RYGB. At that time subjects were randomized to either receive Exendin-9,39 (1200 pmol/kg bolus followed by infusion at 300 pmol/kg/min [16]) referred to as the Saline → Exendin-9,39 group, or saline, referred to as the Saline → Saline group. The trial was registered at www.clinicaltrials.gov. Identifier: NCT01843855.

On either occasion subjects were admitted to the CRU at 1700 on the evening prior to study. Subsequently, they consumed a standardized low calorie meal (10 kcal/kg body weight: 40% carbohydrate, 30% fat, and 30% protein) tolerable to patients who have undergone restrictive upper gastrointestinal procedures and then fasted overnight. At 0630 on the following morning (−180 min), a forearm vein was cannulated (18 g needle) to allow infusions to be performed. An 18 g cannula was
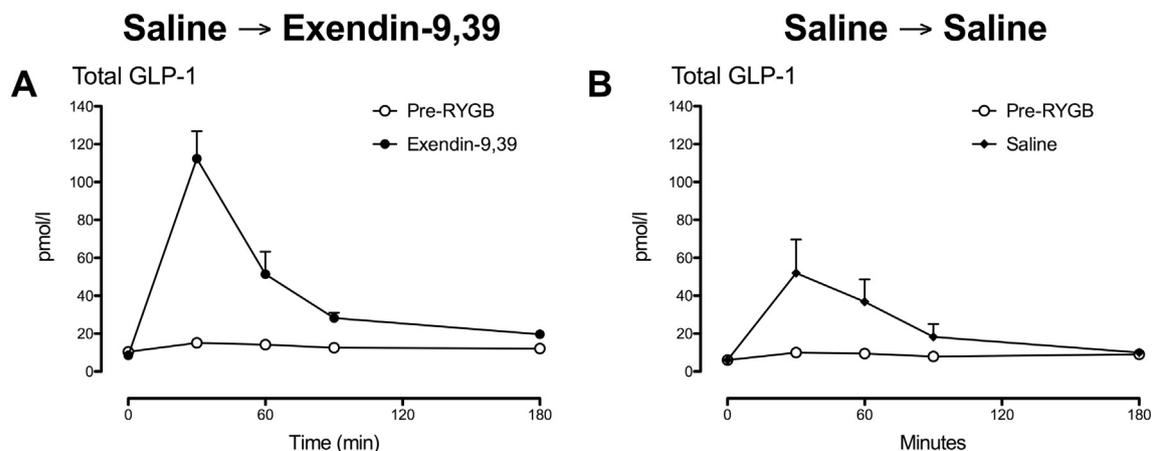
Fig. 1. Total GLP-1 concentrations in the Saline → Exendin-9,39 (left panels) and the Saline → Saline (right panels) group pre-RYGB + Saline (○) and post-RYGB + Exendin-9,39 (●) or post-RYGB + Saline (♦).

inserted retrogradely into a vein on the dorsum of the contra-lateral hand. This was placed in a heated Plexiglas box maintained at 55 °C to allow sampling of arterialized venous blood. A primed (12 mg/kg) continuous (0.12 mg/kg/min) infusion of $[6,6-^2H_2]$ glucose was initiated. At time 0 (0930), while sitting in a recliner, subjects consumed a meal (220 kcal, 56% carbohydrate, 25% fat, 19% protein) consisting of 1 scrambled egg, 15 g of Canadian bacon, 100 ml of water and Jell-O containing 35 g of glucose labeled with $[1-^{13}C]$ glucose – (4% enrichment). Consumption of the test meal is standardized as previously described [12]. Simultaneously, Exendin-9,39 or saline was infused. An infusion of $[6-^3H]$ glucose was also started, and the infusion rate varied to mimic the anticipated appearance of meal $[1-^{13}C]$ glucose. The rate of infusion of $[6,6-^2H_2]$ glucose was altered to approximate the anticipated fall in EGP thereby minimizing changes in specific activity [17,18].
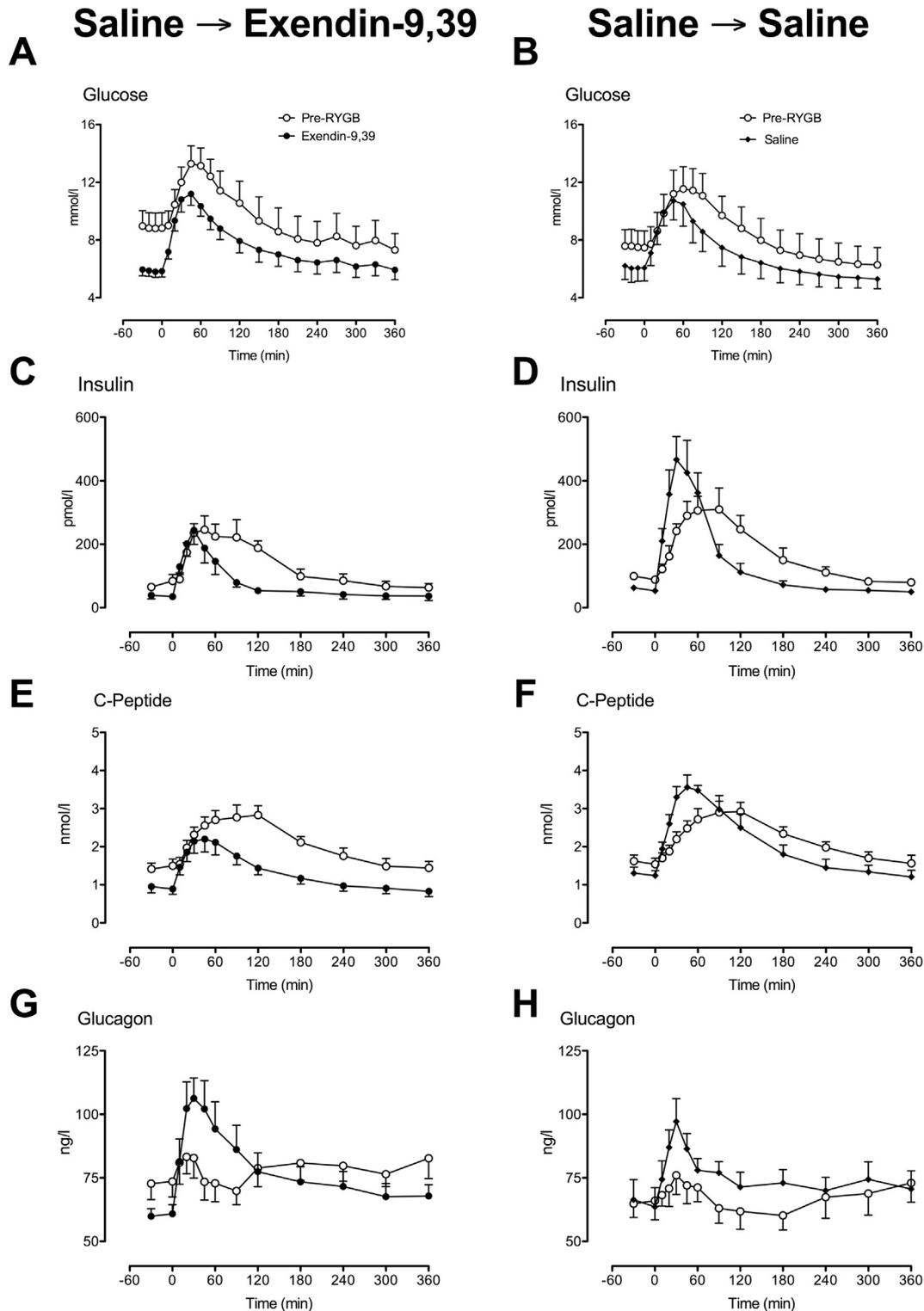
Fig. 2. Glucose, insulin, C-peptide and glucagon concentrations in the Saline → Exendin-9,39 (left panels) and the Saline → Saline (right panels) group pre-RYGB + Saline (○) and post-RYGB + Exendin-9,39 (●) or post-RYGB + Saline (♦).

2.3. Analytical Techniques

Plasma samples were placed on ice, centrifuged at 4 °C, separated, and stored at −20 °C until assayed. Glucose concentrations were measured using a glucose oxidase method (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin was measured using a chemiluminescence assay (Access Assay; Beckman, Chaska, MN). Plasma glucagon and C-peptide were measured by Radio-Immunoassay (EMD Millipore, Billerica, MA). Collection tubes for GLP-1 had 100 μM of DPP-4 inhibitor added. Total GLP-1 concentrations were measured using a C-terminal assay (EMD Millipore, Billerica, MA). Plasma $[6,6-^2H_2]$ glucose and $[1-^{13}C]$ glucose enrichments were measured using gas chromatographic mass spectrometry (Thermoquest, San Jose, CA) to simultaneously monitor the C-1 and C-2 and C-3 to C-6 fragments, as described previously [19]. In addition, $[6-^3H]$ glucose specific activity was measured by liquid scintillation counting following deproteinization and passage over anion and cation exchange columns [18].

2.4. Calculations

The systemic rates of meal appearance (Meal Ra), EGP and glucose disappearance (Rd) were calculated using Steele's model [20]. Meal Ra was calculated by multiplying rate of appearance of $[1-^{13}C]$ glucose (obtained from the infusion rate of $[6-^3H]$ glucose and the clamped plasma ratio of $[6-^3H]$ glucose and $[1-^{13}C]$ glucose) by the meal enrichment. EGP was calculated from the infusion rate of $[6,6^2H_2]$ glucose and the ratio of $[6,6^2H_2]$ glucose to endogenous glucose concentrations. Rd was calculated by subtracting the change in glucose mass from the overall rate of glucose appearance as previously described [21,22]. Values from −30 to 0 min were averaged and considered as basal. Area above basal (AAB) was calculated using the trapezoidal rule to calculate Area Under the Curve (AUC) and then subtracting basal AUC [23].

Insulin action (S_i) was measured using the oral minimal model. β-Cell responsivity was estimated using the oral C-peptide minimal model, incorporating age-associated changes in C-peptide kinetics [24]. The model assumes that insulin secretion comprises static (ϕ_s) and dynamic (ϕ_d) components with total β-cell responsivity to glucose (Φ) derived from these two components [15]. Disposition indices (DI) were subsequently calculated by multiplying Φ by S_i. We also used the Oral GLP-1 model [25], which assumes that GLP-1 stimulates above-basal but not basal insulin secretion [26], to quantify the potentiation of insulin secretion by endogenous GLP-1 after RYGB. The model also assumes that above-basal insulin secretion is linearly modulated by GLP-1 through an index π, which quantifies the increase of above-basal insulin secretion due to a 1 pmol/l increase in circulating GLP-1 concentrations.

2.5. Statistical Analysis

All data are presented as Mean ± SEM unless otherwise noted. To quantify between-group differences for the change from baseline over time, we used (symmetric) percent change [27] calculated as $100*Log_e$ (Present value / Baseline value). Between-group differences were tested using an unpaired, two-tailed *t*-test. Within-group differences (pre-RYGB vs. post-RYGB) were compared using a paired, two-tailed t-test. Statistical analyses used Primer 5 (GraphPad Software, San Diego, CA). A *p*-value <0.05 was considered statistically significant.

3. Results

3.1. Volunteer Characteristics (Table 1)

Of the twelve subjects who completed the study, 7 received Exendin-9,39 during the second study (Saline → Exendin-9,39) and 5 received saline (Saline → Saline). Subject characteristics are summarized in Table 1. Gender, age, HbA$_{1c}$, BMI and weight did not differ

between groups before surgery and subjects lost a comparable amount of weight after surgery.

3.2. Total GLP-1 Concentrations Before and After RYGB (Fig. 1)

Fasting total GLP-1 concentrations were unchanged post-surgery. However, RYGB, increased postprandial peak and integrated total GLP-1 concentrations. As previously observed [12], Exendin-9,39 infusion increased peak concentrations compared to the group receiving saline (112 ± 14 vs. 52 ± 17 pmol/l, $p = 0.03$).

3.3. Glucose, Insulin, C-Peptide and Glucagon Before and After RYGB (Fig. 2, Table 1)

RYGB (upper panels) lowered fasting glucose concentrations in both groups (Table 1). The postprandial peak incremental increase in glucose concentrations also did not differ between groups. The symmetrical percent change in peak postprandial glucose concentrations (pre-RYGB vs. post-RYGB) did not differ between groups ($p > 0.05$). Integrated glucose concentrations calculated as Area Above Basal (AAB) were increased by RYGB in the presence of Exendin-9,39 (181 ± 154 vs. 582 ± 129 mmol per 6 h, $p = 0.02$) but unchanged with saline (275 ± 88 vs. 315 ± 66 mmol per 6 h, $p = 0.62$). However, the symmetrical percent change in AAB glucose (pre-RYGB vs. post-RYGB) did not differ between groups ($p = 0.08$).

Fasting insulin concentrations (upper middle panels) were significantly lowered by RYGB in both groups (Table 1). Peak insulin concentrations after RYGB were unchanged in the Saline → Exendin-9,39 (281 ± 45 vs. 255 ± 49 pmol/l, $p = 0.59$) but increased in the Saline → Saline group (213 ± 82 vs. 522 ± 81 pmol/l, $p = 0.007$). The symmetrical percent change in peak postprandial insulin concentrations (pre-RYGB vs. post-RYGB) also differed between groups ($p < 0.05$).

Fasting and postprandial C-peptide concentrations (lower middle panels) reflected the changes in insulin concentrations observed in both groups although there was a proportionately greater decrease in fasting C-peptide in the Saline→Exendin-9,39 group (Table 1). The symmetrical percent change in peak postprandial C-peptide concentrations also differed between groups ($p = 0.05$).

Fasting glucagon concentrations (lower panels) decreased from baseline after RYGB in the Saline → Exendin-9,39 but not in the Saline → Saline group (Table 1). On the other hand, the symmetrical percent change in peak ($p = 0.85$) and incremental ($p = 0.88$) postprandial glucagon concentrations did not differ between groups.

3.4. Insulin Action (S_i), Dynamic β-Cell Responsivity (ϕ_d), Static β-Cell Responsivity (ϕ_s), Total β-Cell Responsivity (Φ) and Disposition Index (DI) Before and After RYGB (Fig. 3, Table 2)

Insulin action (upper panels) was unchanged by RYGB in both groups. The dynamic component of β-cell responsivity (ϕ_d – upper middle panels) was significantly increased ($p = 0.02$) by RYGB in the Saline → Saline group (Table 2). The symmetrical percent change in ϕ_d did not differ between groups. On the other hand, while the static component of β-cell responsivity (ϕ_s – middle panels) was not significantly changed by Exendin-9,39 infusion after RYGB, there was a tendency for this to increase after RYGB in the Saline → Saline group so that the symmetric percent change differed significantly between the two groups (Table 2).

Total β-cell responsivity (Φ – lower middle panels) was unchanged by RYGB in the Saline → Exendin-9,39 and slightly, but not significantly, increased after RYGB in the Saline → Saline group. This was borne out by the symmetric percent change which differed ($p = 0.04$) between groups (Table 2).

However, DI (lower panels) which expresses Φ as a function of insulin action was unchanged by RYGB in the Saline → Exendin-9,39. Although there was a tendency for DI to increase in the Saline → Saline
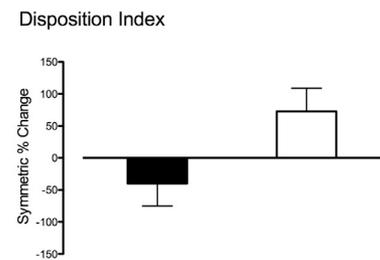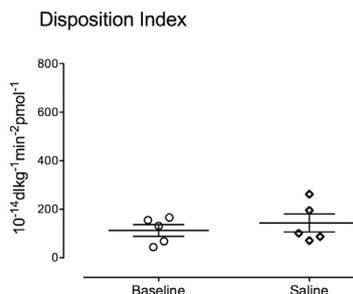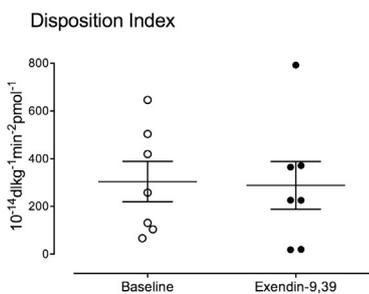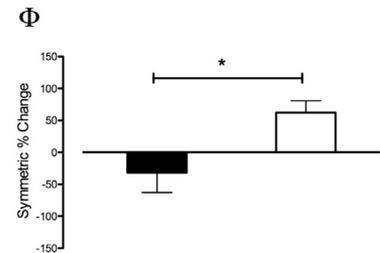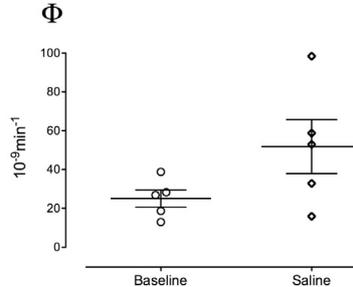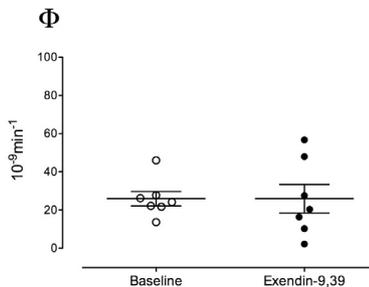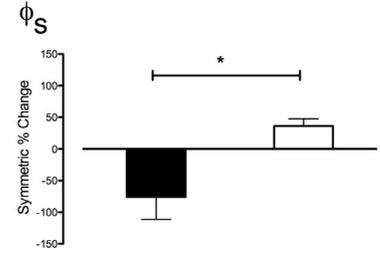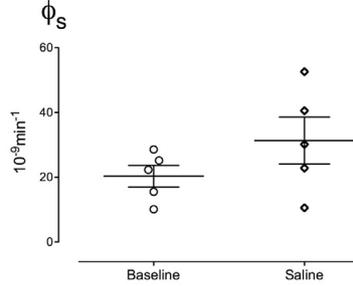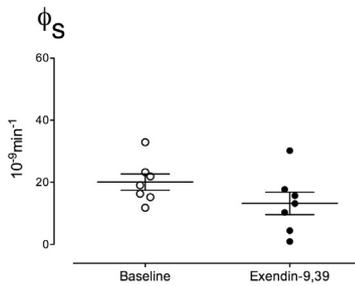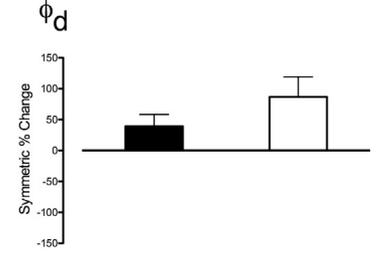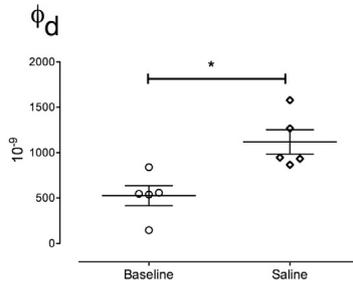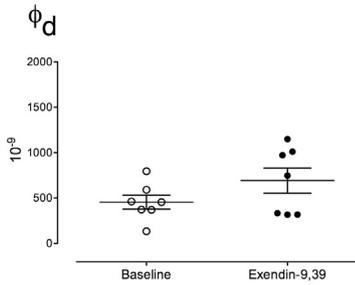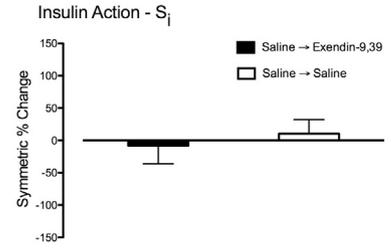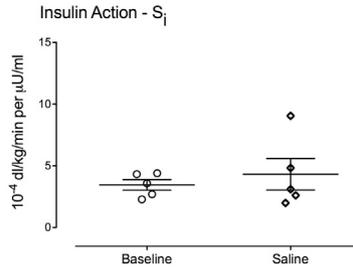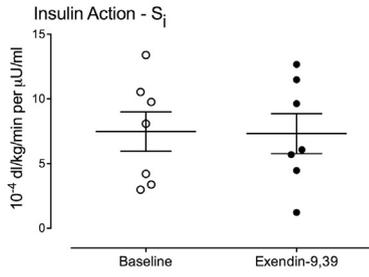
Fig. 3. Insulin action (S_i), dynamic (ϕ_d), static (ϕ_s) and total (Φ) β-cell responsivity to glucose as well as Disposition Index, in the Saline → Exendin-9,39 group and the Saline → Saline group pre-RYGB + Saline (○) and post-RYGB + Exendin-9,39 (●) or post-RYGB + Saline (♦). The third column shows Symmetrical percent change for each study arm. * denotes $p < 0.05$.

Table 2
Indices of insulin secretion and action of the participants in each group, before and after RYGB.

	Saline → Exendin-9,39		Saline → Saline		$^*p$-Value
	Baseline	Exendin-9,39	Baseline	Saline	
S_i (10^{-4} dl/kg/min per µU/ml)	7.5 ± 1.5 $^{**}p = 0.93$	7.3 ± 1.5	3.5 ± 0.4 $^{**}p = 0.46$	4.3 ± 1.3	0.64
$\phi_{dynamic}$ (10^{-9})	455 ± 77 $^{**}p = 0.09$	692 ± 138	526 ± 111 $^{**}p = 0.02$	1118 ± 134	0.21
ϕ_{static} (10^{-9} min^{-1})	20 ± 3 $^{**}p = 0.7$	13 ± 4	20 ± 3 $^{**}p = 0.07$	31 ± 7	0.03
Φ (10^{-9} min^{-1})	26 ± 0.04 $^{**}p = 1.0$	26 ± 8	25 ± 4 $^{**}p = 0.09$	52 ± 14	0.04
DI (10^{-14} dl/kg/min per pmol/l)	304 ± 84 $^{**}p = 0.81$	288 ± 100	132 ± 31 $^{**}p = 0.28$	402 ± 225	0.06

* Represents the value of an unpaired, two-tailed t-test for between-group differences in symmetrical percent change for a given parameter.

** Represents the value of a paired, two-tailed t-test for within-group differences for a given parameter.

group, this difference was not significant. Similarly, the symmetric percent change in DI did not differ between groups (Table 2).

3.5. GLP-1-Induced Potentiation of Insulin Secretion (Fig. 4)

The oral GLP-1 model demonstrated that after RYGB, infusion of Exendin-9,39 inhibited the stimulatory effects of endogenous GLP-1 on insulin secretion as compared to saline (0.7 ± 0.3 vs. $11.0 \pm 2.9\%$ per pmol/l, $p = 0.02$).

3.6. Endogenous Glucose Production, Meal Appearance and Glucose Disappearance Before and After RYGB (Fig. 5)

Post-RYGB, fasting EGP decreased ($p < 0.05$) in both the Saline → Exendin-9,39 (18.4 ± 1.1 vs. 13.9 ± 1.3 µmol/kg/min) and the Saline → Saline (17.8 ± 2.4 vs. 14.1 ± 1.7 µmol/kg/min) groups (upper panels). Nadir postprandial suppression of EGP was unchanged ($p > 0.05$) by RYGB in both groups. The symmetric percent change in fasting and nadir EGP did not differ between groups.

As expected given the alteration in upper gastrointestinal anatomy, peak Meal Ra increased post-RYGB in both groups (middle panels). The timing of this peak did not differ in either group. The symmetric percent change in peak Meal Ra did not differ between groups. AAB meal appearance (Meal Ra) was not altered by RYGB in either group.

Post-RYGB, fasting glucose disappearance decreased ($p < 0.01$) in both the Saline → Exendin-9,39 (20 ± 1.1 vs. 16.2 ± 1.3 µmol/kg/min) and the Saline → Saline (20.0 ± 2.3 vs. 15.4 ± 1.9 µmol/kg/min) groups (lower panels). In contrast, peak postprandial glucose disappearance was unchanged (61.4 ± 13.0 vs. 78.1 ± 13.4 µmol/kg/min, $p = 0.41$) by RYGB in the Saline → Exendin-9,39 group but increased (47.2 ± 11.4 vs. 78.4 ± 11.4 µmol/kg/min, $p = 0.05$) in the Saline → Saline group. However,

GLP-1-induced potentiation of insulin secretion

Fig. 4. Glucagon-like peptide-1-induced potentiation of post-prandial insulin secretion (π) in the presence of Saline (open bars) and Exendin-9,39 (solid bars). * denotes $p < 0.05$.

the symmetric percent change in fasting and peak glucose disappearance did not differ significantly between groups. No within- or between-group differences in integrated (AAB) rates of glucose disappearance were observed.

4. Discussion

4 weeks after RYGB, subjects with type 2 diabetes have lower fasting glucose concentrations, explained by decreased rates of fasting endogenous glucose production. The use of a competitive antagonist for GLP-1 at its cognate receptor, Exendin-9,39, results in a small, but significant increase in postprandial glucose excursion. This is explained by a decrease in both peak and integrated postprandial insulin (and C-peptide) concentrations as well as by net β-cell responsivity to glucose (quantified using Φ). Use of the Oral GLP-1 model [25], confirmed the inhibition of GLP-1-induced potentiation of insulin secretion (quantified using π) when Exendin-9,39 is infused at 300 pmol/kg/min in keeping with prior data [12].
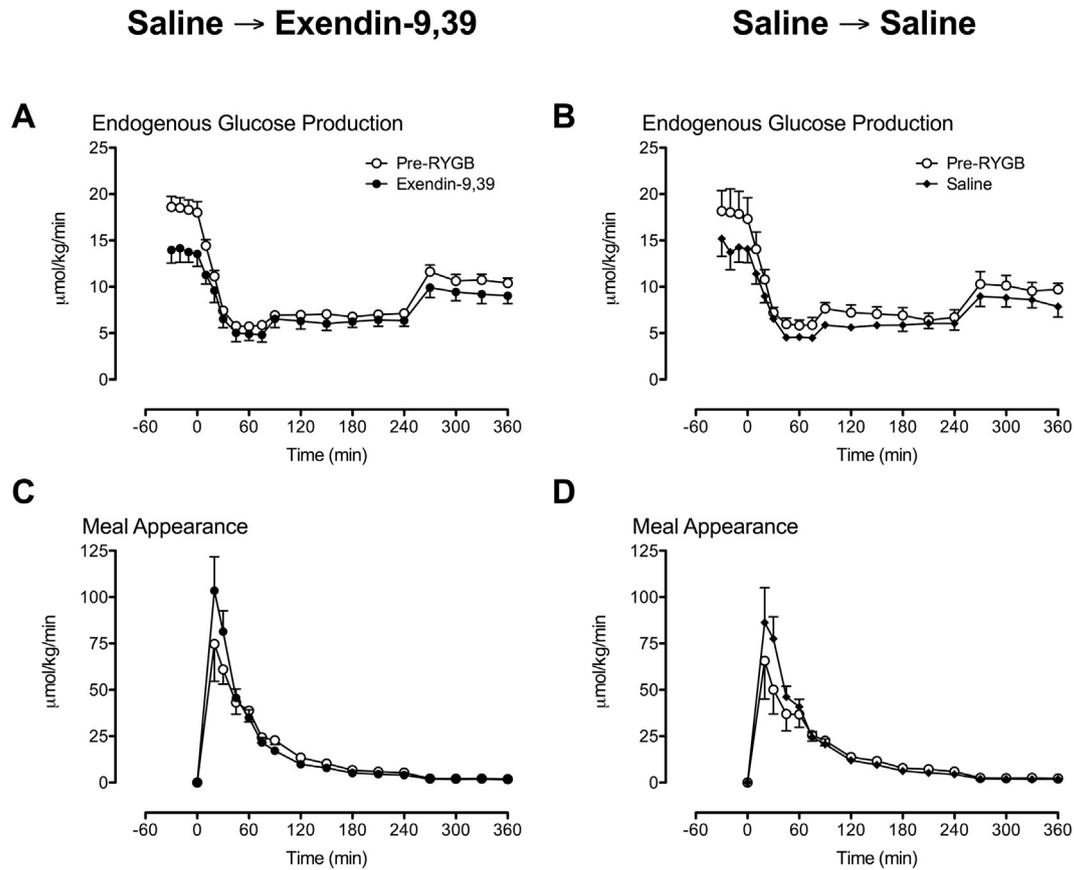
RYGB results in a substantial reduction in fasting glucose within 4 weeks of surgery despite minimal weight loss. This is due to a decrease in fasting EGP and is also observed when people with type 2 diabetes subjected to equivalent caloric restriction [7]. Since this occurred in the presence of decreased fasting insulin concentrations, it implies an improvement in hepatic insulin action (at least in the fasting state) or a change in the ability of factors other than insulin to regulate glucose production. On the other hand, net postprandial insulin action (quantified by S_i) was not altered by RYGB regardless of whether or not GLP-1 action was inhibited by Exendin-9,39. This implies that, as before [12], the effect of Exendin-9,39 to increase incremental glucose concentrations is primarily explained by decreased insulin secretion.

Impaired GLP-1-induced potentiation of insulin secretion resulted in a decrease in β-cell response to sustained hyperglycemia (ϕ_s), consistent with prior reports of the mechanism by which GLP-1 and GLP-1-based therapy alter insulin secretion [12,18]. This implies that the elevated GLP-1 concentrations observed after RYGB increases static β-cell responsivity (ϕ_s) thought to be equivalent to the 2nd phase of insulin secretion [15]. Consistent with prior observations, Exendin-9,39 did not alter dynamic β-cell responsivity (ϕ_d) thought to be equivalent to the 1st phase of insulin secretion [15]. This implies that GLP-1 concentrations post-RYGB do not measurably alter the size of the immediately releasable pool of insulin [12]. Since this accumulates during fasting it is also possible that a lack of effect of Exendin-9,39 on ϕ_d may be an artefact of the experimental design where GLP-1 receptor blockade is initiated at the start of the meal.

Meal appearance represents the net result of intestinal absorption and splanchnic extraction of the ingested meal [28]. Given that integrated meal appearance did not differ from pre-RYGB values, this implies that postprandial GLP-1 concentrations do not affect these parameters in the post-RYGB setting and that in the presence of permissive insulin
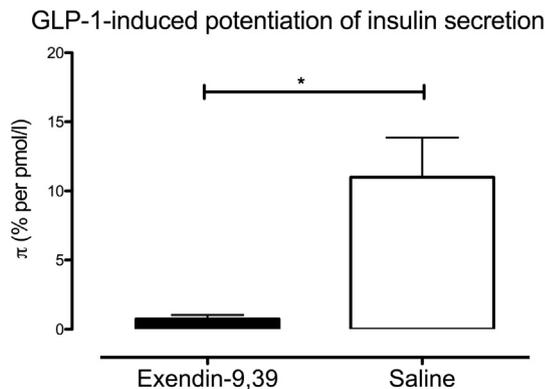
Saline → Exendin-9,39

A Endogenous Glucose Production

Saline → Saline

B Endogenous Glucose Production

C Meal Appearance

D Meal Appearance

Fig. 5. Endogenous glucose production, rate of systemic meal appearance and glucose disappearance in the Saline → Exendin-9,39 and the Saline → Saline group pre-RYGB + Saline (○) and post-RYGB + Exendin-9,39 (●) or post-RYGB + Saline (♦).

concentrations splanchnic extraction of ingested carbohydrate is primarily driven by postprandial glucose concentrations [29].

RYGB is associated with a paradoxical increase in postprandial glucagon concentrations – as observed in this experiment, in both the presence or absence of Exendin-9,39. Inhibition of GLP-1 actions by Exendin-9,39 resulted in a non-significant trend to higher postprandial concentrations, suggesting a weak inhibitory effect of endogenous GLP-1 on glucagon secretion after RYGB as observed previously [11,12]. The role and contribution of postprandial glucagon concentrations to EGP and overall glucose metabolism in RYGB will require further study.

In the current experiment, RYGB did not alter net postprandial insulin action (quantified by S_i), despite effects on fasting EGP. This contrasts with a recent experiment where a small improvement in insulin action, measured with an intravenous glucose tolerance test, twenty-one days after RYGB was noted. However, the effect of RYGB was smaller than that produced by caloric restriction alone [30]. In addition, using the same methodology we used in this experiment, we have reported that insulin action post-RYGB did not differ from that observed in age- and weight-matched controls [12]. Nevertheless, it is possible that our study was underpowered to detect a small effect of RYGB on insulin action in the early postoperative phase.

The dose required to completely inhibit GLP-1 action in humans is unknown. We infused Exendin-9,39 at 300 pmol/kg/min. Based on prior experiments [31–33] and the estimated portal concentrations of GLP-1 [34], this rate should result in near complete inhibition of GLP-1 actions at its cognate receptor [35]. Nevertheless, it is possible that the inhibition of GLP-1 was incomplete in the current experiment and therefore the effect of endogenous GLP-1 on insulin secretion underestimated. Indeed, π calculated using the Oral GLP-1 model ≠ 0. However, we have previously demonstrated that the effect of this

infusion rate on glucose concentrations [12] was similar to those observed with much higher infusion rates of Exendin-9,39 [11,13].

The increase in GLP-1 concentrations after GLP-1 receptor blockade by exendin-9,39 has been observed previously [12]. Conversely, elevated concentrations of intact GLP-1 in the presence of dipeptidyl peptidase-4 inhibition decreases total GLP-1 concentrations [17,36,37]. These observations imply that the L-cell are responsive to circulating concentrations of active GLP-1 [36] but the mechanism by which this occurs is unclear.

Despite the negative effect of Exendin-9,39 on postprandial insulin secretion and on β-cell responsivity to glucose, the net effect on postprandial glucose metabolism was small. Indeed EGP suppressed to the same nadir regardless of the presence or absence of Exendin-9,39. Similarly, meal appearance was not significantly affected. The relative lack of effect of a decrease in β-cell function on postprandial glucose concentrations emphasizes the importance of glucose effectiveness in the maintenance of postprandial glucose homeostasis. Since glucose effectiveness is impaired in people with type 2 diabetes; [38] this may perhaps explain why we observed a slightly greater effect of Exendin-9,39 on net glucose concentrations in this experiment compared to our prior observations in non-diabetic subjects [12]. Our study suffers from two limitations. The first is that the blood volume requirements precluded studying all subjects twice in the post-operative period (once with saline infusion and once with Exendin-9,39). The second is the relatively small numbers of subjects studied making the results vulnerable to differences in baseline characteristics and the inherent variability of the individual parameters measured. Nevertheless, our results are compatible with previous findings [12] and are supported by the oral GLP-1 model [25] which confirms the inhibitory action of Exendin-9,39 on GLP-1-induced insulin secretion.

In summary, like caloric restriction, RYGB results in a decrease in fasting glucose concentrations in subjects with type 2 diabetes. This is primarily due to decreased fasting rates of endogenous glucose production. Exendin-9,39, four weeks after surgery, inhibits endogenous GLP-1 action and results in a small, but significant, increase in incremental glucose concentrations due to decreased β-cell responsivity to glucose. Increased postprandial GLP-1 secretion cannot explain all of the metabolic changes observed after RYGB but contributes to postprandial β-cell function after RYGB in people with type 2 diabetes.

Acknowledgments

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