Effects of endogenous GLP-1 and GIP on glucose tolerance after Roux-en-Y gastric bypass surgery

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Svane MS, BojSEN-MÖller KN, Nielsen S, Jørgensen NB, Dirksen C, Bendtsen F, Kristiansen VB, Hartmann B, Holst JJ, Madsbad S. Effects of endogenous GLP-1 and GIP on glucose tolerance after Roux-en-Y gastric bypass surgery. Am J Physiol Endocrinol Metab 310: E505–E514, 2016. First published January 19, 2016; doi:10.1152/ajpendo.00471.2015.—Exaggerated secretion of glucagon-like peptide 1 (GLP-1) is important for postprandial glucose tolerance after Roux-en-Y gastric bypass (RYGB), whereas the role of glucose-dependent insulinotropic polypeptide (GIP) remains to be resolved. We aimed to explore the relative importance of endogenously secreted GLP-1 and GIP on glucose tolerance and β-cell function after RYGB. We used DPP-4 inhibition to enhance concentrations of intact GIP and GLP-1 and the GLP-1 receptor antagonist exendin-(9–39) (Ex-9) for specific blockade of GLP-1 actions. Twelve glucose-tolerant patients were studied after RYGB in a randomized, placebo-controlled, 4-day crossover study with standard mixed-meal tests and concurrent administration of placebo, oral sitagliptin, Ex-9 infusion, or combined Ex-9-sitagliptin. GLP-1 receptor antagonist increased glucose excursions, clearly attenuated β-cell function, and aggravated postprandial hyperglucagonemia compared with placebo, whereas sitagliptin had no effect despite two- to threefold increased concentrations of intact GLP-1 and GIP. Similarly, sitagliptin did not affect glucose tolerance or β-cell function during GLP-1R blockage. This study confirms the importance of GLP-1 for glucose tolerance after RYGB via increased insulin and attenuated glucagon secretion in the postprandial state, whereas amplification of the GIP signal (or other DPP-4-sensitive glucose-lowering mechanisms) did not appear to contribute to the improved glucose tolerance seen after RYGB.

Roux-en-Y gastric bypass; glucagon-like peptide-1; glucose-dependent insulinotropic polypeptide; exendin-(9–39); dipeptidyl peptidase-4 inhibition

ROUX-EN-Y GASTRIC BYPASS (RYGB) is an effective treatment of severe obesity and induces large and sustainable weight loss, which is superior to diet, intensive lifestyle, or pharmaceutical interventions (47, 51). Moreover, RYGB improves glycemic control within days after surgery, before major weight loss (24), and induces remission of type 2 diabetes in the majority of patients (5, 47). Patients with preoperative normal glucose tolerance (NGT) also display changed postprandial glycemic profile after RYGB with increased peak plasma glucose, followed by a lower nadir (24, 45). A combination of improved hepatic insulin sensitivity induced by hypocaloric postoperative diet (18), rapid glucose absorption (19), and exaggerated postprandial insulin secretion seems to be responsible for the early changes in glucose metabolism after RYGB in patients with NGT and type 2 diabetes (3).

The postprandial gastrointestinal induction of insulin secretion, the incretin effect, is due to the insulinotropic effects of the two hormones glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) (38, 56). In glucose-tolerant subjects, GLP-1 and GIP contribute nearly equally to the incretin effect, which explains up to 70% of the postprandial insulin secretion (36, 38, 56). The incretin effect is severely impaired in type 2 diabetes (35) but is also reduced in obese people with NGT (28), which is possibly due to a subtle β-cell defect and attenuated effects of GLP-1 and GIP (14, 28, 37). However, after RYGB, a markedly enhanced insulin secretion is seen in response to various oral challenges, as opposed to intravenous stimuli (3, 11, 25, 32, 41, 46), and a restoration of the preoperatively blunted incretin effect has been demonstrated (30). A seven-to 10-fold increased postprandial GLP-1 secretion is a consistent finding after RYGB (3, 24), which may be explained by the rapid transit of nutrients to distal parts of the small intestine with a high density of L cells (9).

The role of glucagon-like peptide 1 (GLP-1) for improved β-cell function and remission of diabetes after RYGB and gastric sleeve surgery has nevertheless been questioned in previous studies using pharmacological blockade of the GLP-1 receptor (GLP-1R) by the antagonist exendin-(9–39) (Ex-9) (22, 54) supported by rodent GLP-1R knockout models (58). In other studies, however, Ex-9 clearly attenuated insulin secretion (21, 23, 45, 50) and impaired glucose tolerance (21, 23), affirming the importance of increased postprandial GLP-1 levels for enhanced β-cell function post-RYGB. This inconsistency necessitates more studies to clarify the role of GLP-1 for insulin secretion after RYGB surgery, which was one of the aims of the present study.

The postprandial secretion of GIP after RYGB has been reported to be unchanged (20, 24, 25), increased (13, 30), or decreased (29, 43). We have demonstrated unchanged insulinotropic effect of GIP postoperatively in glucose-tolerant subjects after intravenous infusion during a hyperglycemic clamp (8), but the relative importance of GIP compared with GLP-1 for glucose tolerance after RYGB remains unclear in the literature. An antagonist for the GIP receptor for human use is not available; consequently, the separate effect of GIP on...
insulin secretion must be evaluated indirectly by other methods. Active forms of GLP-1 and GIP have very short half-lives due to rapid degradation by the ubiquitous enzyme dipeptidyl peptidase-4 (DPP-4), and inhibition of the enzyme increases concentrations of both intact (i)GLP-1 and iGIP (59). Hence, by blocking the GLP-1 receptor with Ex-9, it is possible to evaluate the effect of endogenous GLP-1 on glucose tolerance during a mixed meal, and by administration of a DPP-4 inhibitor, the effect of enhanced concentrations of both GLP-1 and GIP can be assessed. Finally, the combined administration of a DPP-4 inhibitor and infusion of Ex-9 should reveal the role of enhanced GIP without contributions from GLP-1 compared with Ex-9 alone. Therefore, we investigated the effect of DPP-4 inhibition on meal-induced insulin secretion and glucose metabolism both alone and with Ex-9 infusion.

METHODS

Participants. Twelve obese patients [4 men and 8 women; age (mean ± SE) 36.0 ± 7.0 yr, BMI 33.5 ± 1.7 kg/m² with NGT, fasting plasma glucose < 7.0 mM, and Hb A₁c < 6.5% (48 mmol/mol) without glucose-lowering medicine] were recruited from the bariatric surgery program at Hvidovre Hospital (Hvidovre, Denmark). Two patients had type 2 diabetes prior to surgery but experienced normalization of glycemic control without glucose-lowering medication postoperatively. All patients had undergone uncomplicated RYGB 5.4 ± 1.0 mo prior to inclusion. Exclusion criteria were symptoms of severe dumping or established postprandial hypoglycemia. The study was approved by the Municipal Ethics Committee of Copenhagen (registration no. H-1-2013-131) in accordance with the Declaration of Helsinki and by the Danish Data Protection Agency and was registered at www.clinicaltrials.gov (NCT02336659). Written, informed consent was obtained from all patients before entering the study.

Experimental design. The study was a randomized, placebo-controlled, single-blinded crossover study. Patients were examined with a standard mixed-meal test on 4 different experimental days performed in randomized order. The study days were separated by ≥ 48 h and included the following interventions: 1) placebo (saline infusion and oral placebo), 2) Ex-9 (Ex-9 infusion and oral placebo), 3) sitagliptin (sita; saline infusion and oral sita), and 4) Ex-9/sita (Ex-9 infusion and oral sita).

Tablets with 100 mg sita or placebo were administered at 2200 the evening before and at 0700 on study days. On each study day, patients met at 0800 after a 10-h overnight fast. Patients were weighed (Tanita, Tokyo, Japan) and seated in a reclined position in a hospital bed. Strenuous activity was not allowed. Catheters were inserted into antecubital veins of both arms (one for blood sampling and one for infusion), and three fasting blood samples were drawn (−40 to −30 min). At t = −30 min, a primed (43.3 pmol/kg) continuous infusion (900 pmol·kg⁻¹·min⁻¹) of Ex-9 was initiated or an equivalent volume of saline was infused using a precision infusion pump (P2000; IVAC Medical Systems, Hampshire, UK). Patients continued fasting for 30 min to allow the drug to reach target tissues and concentrations to stabilize before the meal. Three further blood samples were drawn just before ingestion of the meal (−10, −5, and 0 min), and these were used to define basal concentrations. At t = 0 min, a standard mixed meal consisting of half a slice of whole meal toast with one slice of cheese, margarine spread and marmalade, 2 dl of yogurt with glucose syrup, 20 g of oatmeal, 16 raisins, and five almonds (comprising a total of 364 kcal, 53% carbohydrate, 33% fat, and 14% protein) was provided and ingested evenly over a 20-min period. To estimate gastric emptying, 1 g of paracetamol (Pamol; Nycomed, Roskilde, Denmark) was added to the portion of the meal ingested within the first 5 min. Blood was sampled frequently before and following the meal for a total of 4 h (fasting: −40, −35, and −30 min; basal: −10, −5, and 0 min; postprandial: 5, 10, 15, 20, 30, 45, 60, 90, 120, 180, and 240 min).

Study drugs/peptides. Ex-9 was purchased from Bachem (Switzerland), and the same lot was used for all experiments. The peptide was dissolved in sterilized water containing 1% (wt/vol) human albumin, sterilized, dispensed in appropriate volumes, and stored frozen (−20°C) by the Capital Region of Denmark Central Pharmacy (Herlev, Denmark). The peptide had >99.5% purity by high-performance liquid chromatography. The Ex-9 infusion rate of 900 pmol·kg⁻¹·min⁻¹ was chosen since this dosage has previously been reported to block the GLP-1R by 95% (33, 48). Sita (Januvia; 100 mg) was purchased from MSD (Ballerup, Denmark) and was dispensed in capsules similar to placebo capsules by the Capital Region of Denmark Central Pharmacy (Herlev, Denmark).

Assays. Blood was collected into clot activator tubes for C-peptide analysis, prechilled EDTA tubes containing aproptinin (500 KIU/ml blood, Trasylo; Bayer, Leverkusen, Germany), a specific DPP-4 inhibitor (valine-pyrrolidone, final concentration of 0.01 mM; a gift from Novo Nordisk, Bagsværd, Denmark) for analysis of GLP-1, GIP, and glucagon, and EDTA tubes for analysis of paracetamol, Ex-9, and DPP-4 activity. Clot activator tubes were left to coagulate for 30 min, whereas EDTA tubes were placed on ice until they were centrifuged at 4°C. Plasma glucose was measured bedside using the glucose oxidase technique (YSI model 2300 STAT Plus; YSI, Yellow Springs, OH). Serum for measurements of C-peptide was stored at −80°C and plasma at −20°C until batch analysis.

Serum C-peptide and plasma paracetamol concentrations were analyzed by Cobas immunoassay (Roche). Plasma samples were assayed for total (t)GLP-1 immunoreactivity, using antisem 89390 as described previously (40). Intact GLP-1 was measured using a previously described and validated in-house two-site sandwich ELISA (57). Sensitivity for both assays was below 2 pmol/l, and intra-assay coefficient of variation (CV) was <6%. Total GIP was measured using antibody 80867 directed against the COOH-terminal region (31, and intact GIP [GIP (1–42)] was measured using antibody 98171 specific for the intact NH₂ terminus, as described previously (6). Sensitivity was <5 pmol/l, and intra-assay CV was <10%. Glucagon was measured using the commercial available Linco assay (Millipore, Billerica, MA), which has previously been shown not to cross-react with Ex-9 (26). Ex-9 was measured using antibody 3145 obtained from rabbits after immunization with exendin-4. The antibody shows 100% cross-reactivity with Ex-9 but <0.01% cross-reactivity with GLP-1, glucagon, or GIP (26). DPP-4 enzymatic activity was determined at t = −30 min using a modified method (34). In short, 40 μl of EDTA-plasma was incubated at room temperature with 140 μl of substrate (50 mM Tris-HCl, pH 7.5, containing 1 mM H-Gly-Pro-p-nitroaniline; Bachem cat. no. L1880). The absorbance was determined at 405 nm with an ELISA reader (BioTek EL800) at 0 min and again at 60 min. The results are calculated using absorbance units and expressed as percent of activity in basal levels (days without sita administration).

Statistical analysis and calculations. Total area under the curve (Tauc) was calculated using the trapezoid rules, and the subtraction of basal values yielded incremental AUC (iAUC).

Preheparin insulin secretion rates (ISR) for each individual from each experimental day were derived by deconvolution of peripheral C-peptide concentrations, utilizing a two-compartment model of C-peptide kinetics and population-based C-peptide parameters (52) as implemented in the ISEC software program (16). ISR are expressed in pmol·kg⁻¹·min⁻¹.

β-Cell function was evaluated by two indexes. To estimate the important initial β-cell response during a meal, which has a major impact on glucose tolerance, the β-cell glucose sensitivity (β-GS) was calculated as the dose-response relationship between glucose concentration and ISR during the meal. Time to glucose peak (tpeak glucose) was identified for each individual on each experimental day; then, ISR from t = 0 to tpeak glucose were plotted against corresponding plasma
glucose concentrations. The slopes of these linear relations were evaluated by cross-correlation analysis, as described previously (27), and reflect the change in ISR per millimolar increase in plasma glucose expressed as pmol·kg\(^{-1}\)·min\(^{-1}\)·mM\(^{-1}\). Second, an index of \(\beta\)-cell function during the whole postprandial state was calculated as \(\frac{tAUC_{ISR}}{tAUC_{glucose}}\) throughout the 240-min period of the mixed-meal test.

Values are presented as means ± SE unless stated otherwise. Differences between the 4 experimental days were analyzed by ANOVA in a linear mixed-effects model, with experimental day (placebo, Ex-9, sita, or Ex-9/sita) as fixed effect and individual subjects as random effect. Logarithmic transformation was used if distribution was skewed. Post hoc comparisons between the experimental days were performed using post hoc estimates from linear mixed-effects model comparisons. Statistical analysis was performed in R version 3.1.2 (www.R-project.org) with the package “nlme” for the linear mixed-effects models. \(P\) values <0.05 were considered statistically significant.

**RESULTS**

Fasting and basal glucose and hormone concentrations. Fasting plasma glucose was slightly lower after sita administration, albeit only significantly so when the Ex-9/sita and Ex-9 days were compared (Table 1). Ex-9 infusion for 30 min increased plasma glucose (basal concentration) when compared with saline regardless of sita administration (\(P<0.01\) for both Ex-9 vs. placebo and Ex-9/sita vs. sita). Fasting and basal C-peptide concentrations did not differ between the 4 experimental days. Both intact (i) GLP-1 and iGIP were higher in the fasting state on sita days, whereas fasting values of tGLP-1 were similar. Fasting tGIP varied slightly between the 2 sita days. Ex-9 infusion increased basal concentrations of both iGLP-1 and tGIP-1 while not influencing GIP or glucagon (ANOVA \(P=0.10\)) concentrations significantly. Fasting glucagon did not differ between study days.

Ex-9 infusion and DPP-4 inhibition. Ex-9 concentrations were similar on the 2 days with Ex-9 administration, with mean values throughout the 4-h meal test of 410 ± 22 (Ex-9) and 434 ± 24 nmol/l (Ex-9/sita), respectively (\(P=0.3\)). The Ex-9 concentration reached target concentration before meal ingestion and increased slightly during the meal test (Fig. 1).

Inhibition of DPP-4 plasma activity was successfully achieved on both sita days with full DPP-4 inhibition accomplished (data not shown).

**Postprandial glucose and C-peptide concentrations.** During the meal test, Ex-9 infusion resulted in increased peak glucose (\(P=0.01\)), 2-h glucose (\(P<0.001\)), and also iAUC glucose (\(P<0.001\)) compared with the placebo day (Table 2 and Fig. 2A). Sita administration alone decreased peak glucose concentration (\(P=0.01\)) but had no effect on 2-h glucose (\(P=1.00\)) or iAUC glucose (\(P=0.47\)) compared with placebo. The glucose response to the combination Ex-9/sita was similar to

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**Table 1. Glucose and glucoregulatory hormones at fasting and effect of a basal saline/Ex-9 infusion on the 4 experimental days with oral placebo and saline infusion (placebo), oral placebo and Ex-9 infusion (Ex-9), oral sita and saline infusion (sita), or oral sita and Ex-9 infusion (Ex-9/sita)**

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>Placebo</th>
<th>Ex-9</th>
<th>Sit</th>
<th>Ex-9/Sita</th>
<th>Mixed-Effect ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/l</td>
<td>5.2 ± 0.2</td>
<td>5.3 ± 0.2</td>
<td>5.0 ± 0.1</td>
<td>5.0 ± 0.1</td>
<td>0.04 &lt;0.01</td>
</tr>
<tr>
<td>C-peptide, pmol/l</td>
<td>936 ± 67</td>
<td>944 ± 67</td>
<td>965 ± 76</td>
<td>950 ± 95</td>
<td>0.92 0.22</td>
</tr>
<tr>
<td>ISR, pmol kg(^{-1})·min(^{-1})</td>
<td>2.7 ± 0.2</td>
<td>2.7 ± 0.2</td>
<td>2.7 ± 0.2</td>
<td>2.7 ± 0.2</td>
<td>0.95 0.23</td>
</tr>
<tr>
<td>Total GLP-1, pmol/l</td>
<td>5.5 ± 0.7</td>
<td>5.2 ± 0.7</td>
<td>6.5 ± 0.6</td>
<td>6.5 ± 0.9</td>
<td>0.21 &lt;0.01</td>
</tr>
<tr>
<td>Intact GLP-1, pmol/l</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>1.1 ± 0.4</td>
<td>1.3 ± 0.6</td>
<td>0.02 &lt;0.01</td>
</tr>
<tr>
<td>Total GIP, pmol/l</td>
<td>8.6 ± 1.0</td>
<td>8.7 ± 0.6</td>
<td>9.8 ± 0.8</td>
<td>7.5 ± 0.5</td>
<td>&lt;0.01 &lt;0.01</td>
</tr>
<tr>
<td>Intact GIP, pmol/l</td>
<td>8.6 ± 0.6</td>
<td>7.2 ± 0.6</td>
<td>12 ± 0.6</td>
<td>9.4 ± 0.7</td>
<td>&lt;0.01 &lt;0.01</td>
</tr>
<tr>
<td>Glucagon, pg/ml</td>
<td>73 ± 7</td>
<td>73 ± 7</td>
<td>71 ± 8</td>
<td>72 ± 9</td>
<td>0.94 0.10</td>
</tr>
</tbody>
</table>

Data are means ± SE. Sita, sitagliptin; ISR, insulin secretion rate; GLP-1, glucagon-like peptide-1; GIP, glucose-dependent insulinotropic polypeptide. Fasting, mean of concentrations at \(t=-40\) to \(t=-30\) min; basal, mean of concentrations at \(t=10\) to \(t=0\); \(\Delta\text{basal}\) = basal – fasting values. *\(P<0.05\) placebo vs. Ex-9, †\(P<0.05\) placebo vs. sita; ‡\(P<0.05\) for the comparison of Ex-9 vs. sita/Ex-9.
that of Ex-9 alone. Time to peak of plasma concentration did not differ between study days, although Ex-9 infusion tended to shorten time to peak (placebo vs. Ex-9, \( P = 0.08 \) and sita vs. Ex-9/sita, \( P = 0.06 \)).

C-peptide concentrations and ISR (peak as well as iAUC) were significantly decreased during Ex-9 infusions compared with placebo (Table 2 and Fig. 2, B and C). Administration of sita alone had no effect on C-peptide or ISR compared with placebo. Incremental AUC of C-peptide and ISR on days with Ex-9 and combined Ex-9/sita were comparable (\( P = 0.78 \) and \( P = 0.51 \), respectively).

\( \beta \)-Cell glucose sensitivity. \( \beta \)-Cell responsiveness to changes in glucose concentrations (\( \beta \)-GS) on the up slope of glucose excursions was \( \sim 20\% \) decreased during Ex-9 compared with placebo (\( P = 0.02 \)), whereas \( \beta \)-GS with sita alone did not differ from the placebo day (\( P = 0.41 \)) (Fig. 3). \( \beta \)-GS deteriorated equally during Ex-9 infusion with and without the presence of sita (Ex-9 vs. Ex-9/sita, \( P = 0.21 \)).

The integrated index of \( \beta \)-cell function throughout the postprandial period (tAUCISR/tAUCglucose) was also significantly lower on Ex-9 compared with placebo. Sita alone had no effect on the integrated index compared with placebo, and there were no differences between days with administration of Ex-9 and Ex-9/sita (placebo: \( 1.2 \pm 0.1 \); Ex-9: \( 0.9 \pm 0.1 \); sita: \( 1.2 \pm 0.1 \); Ex-9/sita: \( 0.9 \pm 0.1 \); \( P < 0.001 \) for placebo vs. Ex-9, \( P = 0.36 \) for placebo vs. sita, and \( P = 0.52 \) for Ex-9 vs. Ex-9/sita).

Postprandial GLP-1, GIP, and glucagon. Incremental AUC iGIP-1 was enhanced 2.5-fold by Ex-9 (\( P < 0.001 \)) and approximately threefold by sita compared with placebo (\( P < 0.001 \)); on the Ex-9/sita day, iAUC iGIP-1 was elevated even further (~7-fold compared with placebo, \( P < 0.001 \); Table 3 and Fig. 4B). Ex-9 infusion enhanced postprandial iAUC tGIP-1 secretion approximately twofold (\( P < 0.001 \)), whereas sita tended to decrease iAUC tGIP-1 compared with placebo (\( P = 0.06 \)) (Fig. 4A). The increases in iGLP-1 were comparable on Ex-9 and Ex-9/sita days (peak iGLP-1: \( P = 0.66 \); iAUC tGLP-1: \( P = 0.74 \)). Moreover, sita increased the iGLP-1/tGLP-1 ratio significantly (tAUC iGLP-1/tAUC tGLP-1: placebo 0.1 ± 0.02, Ex-9 0.2 ± 0.02, sita 0.6 ± 0.1, Ex-9/sita 0.6 ± 0.04; \( P = 0.09 \) for placebo vs. Ex-9, \( P < 0.001 \) for sita vs. Ex-9/sita, and \( P < 0.001 \) for Ex-9 vs. Ex-9/sita).

Intact GIP secretion (iAUC) was not affected by Ex-9 alone but increased more than twofold on both sita days compared with placebo and Ex-9 alone (\( P < 0.001 \) for both placebo vs. sita and Ex-9 vs. Ex-9/sita; Table 3 and Fig. 4D). Postprandial tGIP varied slightly between study days. The iGIP/tGIP ratio was increased on sita days (tAUC iGIP/tAUC tGIP: placebo 0.6 ± 0.04, Ex-9 0.5 ± 0.04, sita 1.0 ± 0.08, Ex-9/sita 1.0 ± 0.06; \( P = 0.22 \) for placebo vs. Ex-9, \( P < 0.001 \) for placebo vs. sita, and \( P < 0.001 \) for Ex-9 vs. Ex-9/sita).

Both peak concentrations and iAUC glucagon increased significantly on Ex-9 days (\( P < 0.001 \) and \( P = 0.006 \) for peak and iAUC, respectively), whereas sita attenuated both peak (\( P = 0.01 \)) and iAUC (\( P = 0.03 \)) compared with placebo (Fig. 2D). Combined Ex-9/sita decreased peak glucagon compared with Ex-9 (\( P < 0.001 \)), whereas iAUCs were comparable (\( P = 0.35 \)).

Gastric emptying. Time to peak of plasma paracetamol concentrations did not differ between the 4 study days (ANOVA, \( P = 0.7 \); Fig. 1).

**DISCUSSION**

We aimed to investigate the combined and separate effects of endogenously secreted GLP-1 and GIP on glucose tolerance during a mixed-meal test after RYGB in glucose-tolerant subjects. This was done by combining the use of the specific GLP-1R antagonist Ex-9, which blocks the action of endogenous GLP-1, with administration of the DPP-4 inhibitor sitagliptin, which elevates the concentrations of intact GLP-1 and GIP. Indeed, we achieved ~2.5-fold increased levels of both intact GLP-1 and GIP after administration of sitagliptin. We obtained high and stable concentrations of Ex-9 before meal ingestion, which were sustained throughout the whole postprandial period. Ex-9 infusion increased levels of total and intact GLP-1, which were most pronounced after DPP-4 inhibition, probably as a result of diminished negative feedback on the L cell, as suggested previously (12, 53).

Blocking GLP-1R pathways by Ex-9 resulted in marked impairment of \( \beta \)-cell function and increased plasma glucose concentrations, in agreement with previous RYGB studies showing attenuated insulin secretion both in people with preoperative type 2 diabetes (21, 23, 54) and with NGT (50) as well as deterioration of glucose tolerance after Ex-9 compared with saline infusion (21, 23, 50). Nevertheless, the role of GLP-1 for improved glucose metabolism after RYGB was
Fig. 2. Concentrations (means ± SE; left) and incremental area under the curve (iAUC; right) of plasma glucose (A), C-peptide (B), insulin secretion rates (ISR; C), and plasma glucagon (D) in response to a mixed meal. Placebo: solid black line; Ex-9: dashed black line; sita: solid gray line; Ex-9/sita: dashed gray line.
questioned by Vetter et al. (54), who examined the effect of Ex-9 in 8 patients after RYGB compared with 8 controls with an equivalent 10% weight loss obtained by intensive lifestyle modification (ILM); they found larger inhibition of insulin secretion post-RYGB compared with ILM, supporting the importance of GLP-1 for postprandial insulin secretion after RYGB, but equal deterioration in glycemia in the two groups with Ex-9 after the interventions. However, this is consistent with the larger improvements in hepatic insulin sensitivity post-RYGB compared with post-ILM, which makes the comparison of glucose tolerance between the groups difficult. Studies in rodent GLP-1R knockout models have also questioned the importance of GLP-1 for glucose metabolism after bariatric surgery (58), therefore suggesting other mechanisms, including changed intestinal glucose metabolism (44). It is puzzling to translate results to human physiology, and species differences definitely seem to exist in this field. Accordingly, the present experiments are an important addition to the evidence from human studies supporting that endogenous GLP-1 is of importance for glucose tolerance after RYGB. Additional factors are undoubtedly contributing, including caloric restriction, marked improvement in hepatic and peripheral insulin sensitivity (3), relief of glucotoxicity (32), and accelerated nutrient passage (4). In nonoperated individuals, Ex-9 may accelerate gastric emptying (7, 49) and subsequently glucose absorption, although this is not demonstrated consistently (39), but in RYGB-operated patients where the stomach is bypassed, the effect is negligible as expected (23, 45, 50). The present paracetamol absorption data confirm previous findings of rapid intestinal meal delivery in RYGB-operated patients (10) uninfluenced by Ex-9 (23).

Studies of DPP-4 inhibition after RYGB have not been reported previously. Administration of sitagliptin increased the concentrations of endogenously secreted intact GLP-1 and GIP ~2.5-fold, which is in line with previous findings (2), but surprisingly, this did not affect postprandial glucose excursions, insulin secretion, or indices of β-cell function. In other studies with glucose-tolerant subjects, acute DPP-4 inhibition has also failed to affect postprandial glucose excursions or enhance the incretin effect (12, 42). An explanation might be that responses corresponding to the maximal β-cell secretory capacity were achieved on the placebo day in the present study. This explanation seems particularly relevant for RYGB subjects, given the markedly enhanced postprandial GLP-1 concentrations. However, this cannot explain the failure of DPP-4 inhibition to improve glucose concentrations or β-cell function during GLP-1R antagonism. Indeed, comparing glucose concentrations and β-cell function between study days with Ex-9 alone and the combined Ex-9/sita administration allows us to explore the effect on glucose tolerance of enhanced, intact GIP

### Table 3. Postprandial gut-derived glucoregulatory hormone responses on the 4 experimental days with placebo, Ex-9, sita, or Ex-9/sita

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Ex-9</th>
<th>Sita</th>
<th>Ex-9/Sita</th>
<th>Mixed-Effect ANOVA</th>
<th>Placebo vs. Ex-9</th>
<th>Placebo vs. Sita</th>
<th>Ex-9 vs. Ex-9/Sita</th>
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<tbody>
<tr>
<td><strong>Total GLP-1</strong></td>
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<td></td>
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<tr>
<td>Peak, pmol/l iAUC, pmol·1−1·min</td>
<td>44 ± 4</td>
<td>87 ± 10</td>
<td>32 ± 6</td>
<td>90 ± 8</td>
<td>P &lt; 0.001</td>
<td>&lt;0.001</td>
<td>0.16</td>
<td>0.66</td>
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<tr>
<td><strong>Intact GLP-1</strong></td>
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<tr>
<td>Peak, pmol/l iAUC, pmol·1−1·min</td>
<td>2,838 ± 278</td>
<td>4,918 ± 647</td>
<td>1,758 ± 394</td>
<td>4,732 ± 538</td>
<td>P &lt; 0.001</td>
<td>&lt;0.001</td>
<td>0.06</td>
<td>0.74</td>
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<tr>
<td><strong>Total GIP</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Peak, pmol/l iAUC, pmol·1−1·min</td>
<td>8.6 (7–10)</td>
<td>19 (14–21)</td>
<td>16 (14–33)</td>
<td>63 (46–78)</td>
<td>P &lt; 0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<td><strong>Intact GIP</strong></td>
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<tr>
<td>Peak, pmol/l iAUC, pmol·1−1·min</td>
<td>533 (292–669)</td>
<td>1,143 (721–1,361)</td>
<td>1,352 (665–1,926)</td>
<td>3,149 (1,824–4,537)</td>
<td>P &lt; 0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td>Glucagon</td>
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<tr>
<td>Peak, pg/ml iAUC, pg·ml−1·min</td>
<td>3,493 ± 334</td>
<td>3,571 ± 378</td>
<td>3,264 ± 390</td>
<td>2,628 ± 299</td>
<td>P = 0.03</td>
<td>0.81</td>
<td>0.49</td>
<td>0.03</td>
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</table>

Data are means ± SE. iAUC, incremental area under the curve; iGLP-1, intact GLP-1. Peak iGLP-1, iAUCGLP-1, and peak glucagon are presented as median (interquartile range) because of skewed distribution.

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concentrations without the influence of GLP-1. Surprisingly, the glucose tolerance and β-cell function did not differ between these 2 experimental days. Again, it might be assumed that a maximal effect of endogenously secreted GIP on β-cell function was already obtained on the control days. Accordingly, Asmar et al. (1) reported no further effect on insulin secretion when additional GIP was infused during a meal that by itself caused a large endogenous GIP response. However, the peaks of total and intact GIP in the present study amounted to only about one-third of the responses during meal tests with DPP-4 inhibition in nonoperated individuals, which raises the possibility that an attenuated GIP response after RYGB surgery explains the present findings (17, 59). Nevertheless it was unexpected that more than twofold increased levels of active GIP could not compensate for the absence of activation of GLP-1R pathways, particularly given our previous demonstration of a preserved insulinotropic action of GIP after RYGB (8). DPP-4 is not a selective enzyme for degradation of GLP-1 and GIP, and other substrates are affected by sitagliptin administration. Nonetheless, the glucoregulatory actions of DPP-4 inhibitors are mediated predominantly through GLP-1 and GIP pathways (15), and changes in other DPP-4 substances may be of minor importance for glucose metabolism. However, the changed gut hormonal profile after RYGB, interplay between the hormones, and potential interactions with DPP-4 substrates are complex and not fully clarified.

Notably, the method applied in this study has been used previously by Aulinger et al (2) to address the role of endogenous incretin hormones; oral glucose tolerance tests were performed in nonoperated patients with type 2 diabetes on 4 days of placebo, sitagliptin, Ex-9, or Ex-9/sitagliptin administration. They found attenuated glucose excursions and increased insulin secretion during DPP-4 inhibition compared with placebo. Furthermore, during the combined DPP-4 inhibition and Ex-9 infusion, they found a restoration of ~50% of the insulinotropic effect blocked by GLP-1R antagonism alone, supporting GIP as an important factor. Obviously, comparison of glucose metabolism between the studies is difficult due to different glucose absorption rates in RYGB operated vs. nonoperated. Moreover, hepatic and peripheral insulin sensitivity and sensitivity to incretins, particularly GIP, are likely to differ considerably between RYGB-operated patients with NGT and patients with type 2 diabetes (55).

Several studies have consistently reported paradoxically elevated glucagon secretion in the postprandial state after RYGB (13, 20, 24). This paradoxical hyperglucagonemia is seen despite enhanced GLP-1 and insulin response (3, 24). In our study, Ex-9 greatly increased postprandial glucagon concentrations compared with placebo, in line with previous findings (2, 23). This glucagon hypersecretion during Ex-9 possibly contributes to the worsened glucose profiles seen during these experimental days. In contrast, DPP-4 inhibition reduced the glucagon response. Thus, glucose metabolism after RYGB seems to be regulated through GLP-1 secretion by dual effects via increased insulin and inhibition of glucagon.

Fig. 4. Concentrations (means ± SE) of total glucagon-like peptide-1 (GLP-1; A), intact GLP-1 (B), total glucose-dependent insulino-}

tropic polypeptide (GIP; C), and intact GIP (D). Placebo: solid black line; Ex-9: dashed black line; sita: solid gray line; Ex-9/sita: dashed gray line.
A theoretical limitation of our study is the premise that the GLP-IR is actually maximally blocked by Ex-9 infusion. This assumption is based on previously published results, and the Ex-9 dosage used in this study has been shown to result in a 95% inhibition of the insulinotropic effect of pharmacological concentrations of GLP-1 (33, 48). In our study, however, peak values of intact GLP-1 on the Ex-9/sitagliptin day reached even higher concentrations than reported previously, where the effectiveness of Ex-9 infusion has not been evaluated. Yet we found a significantly lower β-cell function comparing Ex-9/sitagliptin and sitagliptin days. Therefore, a minor remaining GLP-1 activity on the Ex-9/sitagliptin day would not change our conclusion. Another limitation in the present study is that all patients had normal glucose tolerance at the time of inclusion (i.e., no patients with persisting postoperative diabetes). However, patients with persisting postoperative diabetes early after RYGB may represent a very selected population that does not reflect the majority of patients, and furthermore, by limiting our study to patients with NGT, we avoid the confounding of glucotoxicity on insulin secretion and glucose metabolism (32). Sensitivity to GIP is dependent on glucose tolerance, and patients with NGT would be expected to respond to increased concentrations of GIP, as opposed to patients with hyperglycemia, where GIP action is blunted (55).

In summary, we report the action of GLP-1 receptor blockade with and without DPP-4 inhibition on glucose tolerance after RYGB and thereby explore the separate and combined effects of endogenously secreted GLP-1 and GIP. Our findings support the exaggerated endogenous GLP-1-response as a contributing explanation for improved postprandial glucose tolerance after RYGB, whereas we were unable to identify additional major DPP-4-sensitive glucose-lowering mechanisms besides GLP-1. The present study highlights the importance of GLP-1 for regulation of postprandial insulin and glucagon secretion after RYGB surgery.

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DISCLOSURES

No conflicts of interests relevant to this article, financial or otherwise, are reported by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


