The Importance of Endogenously Secreted GLP-1 and GIP for Postprandial Glucose Tolerance and β-Cell Function After Roux-en-Y Gastric Bypass and Sleeve Gastrectomy Surgery

Morten Hindsø,[1] Nora Hedbäck,[1] Maria S. Svane,[1,2] Andreas Møller,[1] Christoffer Martinussen,[1] Nils B. Jørgensen,[1] Carsten Dirksen,[1] Lærke S. Gasbjerg,[3] Viggo B. Kristiansen,[2] Bolette Hartmann,[3] Mette M. Rosenkilde,[3] Jens J. Holst,[3,4] Sten Madsbad,[1] and Kirstine N. Bojsen-Møller[1]

Diabetes 2023;72:336–347 | https://doi.org/10.2337/db22-0568

Enhanced secretion of glucagon-like peptide 1 (GLP-1) seems to be essential for improved postprandial β-cell function after Roux-en-Y gastric bypass (RYGB) but is less studied after sleeve gastrectomy (SG). Moreover, the role of the other major incretin hormone, glucose-dependent insulinotropic polypeptide (GIP), is relatively unexplored after bariatric surgery. We studied the effects of separate and combined GLP-1 receptor (GLP-1R) and GIP receptor (GIPR) blockade during mixed-meal tests in unoperated (CON), SG-operated, and RYGB-operated people with no history of diabetes. Postprandial GLP-1 concentrations were highest after RYGB but also higher after SG compared with CON. In contrast, postprandial GIP concentrations were lowest after RYGB. The effect of GLP-1R versus GIPR blockade differed between groups. GLP-1R blockade reduced β-cell glucose sensitivity and increased or tended to increase postprandial glucose responses in the surgical groups but had no effect in CON. GIPR blockade reduced β-cell glucose sensitivity and increased or tended to increase postprandial glucose responses in the CON and SG groups but had no effect in the RYGB group. Our results support that GIP is the most important incretin hormone in unoperated people, whereas GLP-1 and GIP are equally important after SG, and GLP-1 is the most important incretin hormone after RYGB.

Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (SG) surgery improve insulin sensitivity and enhance postprandial β-cell function (1–5), resulting in high rates of type 2 diabetes remission after both procedures (6,7). The improvement in the postprandial β-cell function is best characterized after RYGB and seems to be independent of weight loss, linked to the modified gastrointestinal anatomy, and associated with an altered incretin hormone response (1,2,4,8–11).

The incretin hormones, glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), are secreted from the intestine upon food ingestion and regulate insulin secretion from pancreatic β-cells in a glucose-dependent manner (12,13). Moreover, GLP-1 inhibits glucagon secretion and gastric emptying, whereas GIP stimulates glucagon secretion during low glucose concentrations (12,13). The density of GIP-producing K cells is highest in the proximal small intestine, whereas GLP-1–producing L cells are more dominant distally (14). The rate of intestinal nutrient exposure after meal intake, and thereby the stimulation of K and L cells, is increased after both SG and RYGB (15,16). Furthermore, the proximal part of the small intestine is bypassed after RYGB. Consequently, the postprandial GLP-1 response increases after both procedures but is most

[1]Department of Endocrinology, Copenhagen University Hospital, Hvidovre, Denmark
[2]Department of Surgical Gastroenterology, Copenhagen University Hospital, Hvidovre, Denmark
[3]Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[4]Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

Corresponding authors: Morten Hindsø, morten.hindsoe@regionh.dk, and Kirstine N. Bojsen-Møller, kirstine.nyvold.bojsen-moeller@regionh.dk

Received 22 June 2022 and accepted 4 December 2022

Clinical trial reg. no. NCT03950245, clinicaltrials.gov

This article contains supplementary material online at https://doi.org/10.2337/figshare.21675275.

pronounced after RYGB (2,16,17). Postoperative changes in the postprandial GIP response are modest and less consistent. Hence, increased postprandial systemic GIP concentrations are sometimes, but not always, reported after SG; whereas, moderately reduced, unchanged, or increased concentrations are reported after RYGB (2,17).

Several studies using the GLP-1 receptor (GLP-1R) antagonist exendin(9-39)NH_2 (18–23) collectively indicate that GLP-1 has a more prominent role in the regulation of postprandial β-cell function after compared with before RYGB (24), but only one study has used this approach after SG (25). Recent studies using the novel GIP receptor (GIPR) antagonist GIP(3-30)NH_2 (26,27) in combination with exendin(9-39)NH_2 indicate that GIP is the main incretin hormone in healthy unoperated people (28,29). However, the role of endogenous GIP in the regulation of postprandial glycemia and β-cell function after bariatric surgery is unexplored.

Therefore, we studied separate and combined effects of GLP-1R and GIPR blockade in RYGB-operated, SG-operated, and unoperated (CON) people with no history of diabetes. We hypothesized that GLP-1 would be the quantitatively most important incretin hormone after RYGB and GIP the most important incretin hormone after SG.

RESEARCH DESIGN AND METHODS

Ethics

The study was registered at ClinicalTrials.gov (NCT03950245), approved by the Capital Regional Ethical Committee (Hillerød, Denmark) and the Danish Data Protection Agency, and conducted in accordance with the standards set by the Declaration of Helsinki. Written informed consent was obtained from all participants before inclusion.

Participants

Weight stable (±3 kg within the last month) RYGB-operated, SG-operated, and CON participants with no history of diabetes and a current HbA_{1c} <48 mmol/mol were recruited (n = 12 per group). Participants were matched on age, sex, and BMI (surgical groups also on preoperative BMI and time from surgery). Standard laparoscopic RYGB and SG procedures were performed at least 1 year before inclusion at the Department of Surgical Gastroenterology, Copenhagen University Hospital, Hvidovre, Denmark, as previously described (16). Exclusion criteria were hyperthyroidism, inadequately treated hypothyroidism, hemoglobin <6.5 mmol/L, pregnancy, breastfeeding, glucose-lowering medications, and systemic use of steroids.

Experimental Design

In a crossover design, each participant underwent four liquid mixed-meal tests performed in random order (separated by a minimum of 48 h) during participant-blinded continuous infusions of placebo (saline), exendin(9-39)NH_2 (bolus: 43,000 pmol/kg, infusion rate: 900 pmol/kg/min), GIP(3-30)NH_2 (infusion rate: 800 pmol/kg/min), or combined exendin(9-39)NH_2 and GIP(3-30)NH_2. To ensure that the GLP-1R was sufficiently blocked during conditions of high postprandial GLP-1 concentrations, three RYGB participants underwent an additional meal test with a 33% increased bolus (57,000 pmol/kg) and infusion rate (1,200 pmol/kg/min) of exendin(9-39)NH_2.

On experimental days, the participants met after an overnight fast (10–12 h) and were placed in a reclined position. Intravenous catheters were inserted into antecubital veins on each arm for blood sampling and continuous infusions, respectively. The infusions were started 30 min prior to meal intake and were continued throughout the experimental day. After 30 min of basal infusions, the participants ingested a liquid mixed meal (Fresubin 2 kcal DRINK: 200 mL, 400 kcal; carbohydrate, 45% total energy [E]; protein, 20%E; fat, 35%E) evenly over 20 min. Crushed paracetamol (acetaminophen; 1 g) was dissolved into the first 20 mL of the test meal to estimate paracetamol absorption rate as a measure of intestinal nutrient exposure rate (16). Blood was sampled at −40,−30,−10, 0, 5, 10, 15, 20, 30, 45, 60, 90, 120, 180, and 240 min relative to the intake of the test meal.

Peptides and Infusions

High-purity (>99%) GIP(3-30)NH_2 and exendin(9-39)NH_2 (Caslo, Lyngby, Denmark) were dissolved in 0.5% human albumin (CSL Behring, Marburg, Germany) with and without 10 mmol/L sodium hydrogen carbonate, respectively, under sterile conditions at the Capital Region Pharmacy (Herlev, Denmark). After sterile filtration and testing for pyrogens and sterility, vials were stored at −20°C. On experimental days, the peptide solutions were thawed and diluted in saline with 0.5% human albumin to a total volume of 250 mL. Placebo infusions were 250 mL isotonic saline (Fresenius Kabi, Uppsala, Sweden) with 0.5% human albumin. Hence, on each experimental day, 2 × 250 mL were infused (saline+saline, saline+exendin[9-39]NH_2, saline+GIP[3-30]NH_2, or exendin[9-39]NH_2+GIP[3-30]NH_2).

Biochemistry

Plasma glucose concentrations were measured bedside using the glucose oxidase method (YSI 2300 Stat Plus; YSI, Yellow Springs, OH) after centrifugation of blood samples in EDTA-coated tubes for 45 s at 7,500g at room temperature. Blood samples for serum C-peptide and insulin (fasting samples only) analyses were collected into clot activator tubes and left to coagulate at room temperature for 30 min before centrifugation at 2,000g for 10 min at 4°C. Blood samples for plasma glucagon, total GLP-1, total GIP, paracetamol, exendin(9-39)NH_2, and GIP(3-30)NH_2 analyses were collected into EDTA-coated tubes and immediately centrifuged at 2,000g for 10 min at 4°C. After centrifugation, serum and plasma samples were stored at −20°C (GLP-1, GIP, exendin[9-39]NH_2, and GIP[3-30]NH_2) or −80°C (C-peptide, insulin, glucagon, and paracetamol) until analyses.

Serum C-peptide and insulin concentrations were measured on an IMMULITE 2000 analyzer (Siemens Healthcare Diagnostics, Tarrytown, NY). Plasma glucagon concentrations were measured using a sandwich ELISA kit (cat no. 10-1271-01; Mercodia, Uppsala, Sweden) and the modified "sequential protocol" to eliminate potential cross-reactivity with gut-derived proglucagon products (30). Paracetamol concentrations were measured with a spectrophotometric method (cat no. 506-30, Acetaminophen L3K kit; Sekisui Diagnostics, Abbott, Copenhagen, Denmark). Plasma concentrations of total GLP-1, total GIP, exendin(9-39)NH_2, and GIP(3-30)NH_2 were measured with radioimmunoassays, as previously described (28).

Calculations

Fasting concentrations were calculated as the mean of preinfusion samples (t = −40 and −30 min), and basal concentrations were values obtained after 30 min of premeal infusions just before meal intake (t = 0 min). The HOMA2 model was used to estimate insulin sensitivity (1/HOMA2-insulin resistance [IR]) (https://www.dtu.ox.ac.uk/homacalculator/). Insulin clearance was calculated as the fasting C-peptide–to–insulin ratio. The incremental area under the curve (iAUC) and total area under the curve (tAUC) were calculated using the trapezoidal rule, with and without subtraction of basal concentrations, respectively. Insulin secretion rates (ISR) were derived from deconvoluted C-peptide data using ISEC software (31). β-Cell glucose sensitivity (β-GS) was calculated as the slope of the linear relation between ISR and corresponding glucose concentrations from basal levels (t = 0 min) to the time of the peak plasma glucose concentration (32). The disposition index was calculated as the product of β-GS and insulin sensitivity.

To compare the effects of single and combined GLP-1R and GIPR blockade between groups, we calculated placebo-subtracted effects as absolute changes from placebo (ΔGLP1-R, ΔGIPR, and ΔGLP1-R/GIPR blockade, respectively). The prespecified primary and secondary outcomes were between-group differences in the placebo-subtracted effect of GIPR versus GLP-1R blockade (expressed as ΔGIPR blockade − ΔGLP-1R blockade) on the iAUC of glucose and β-GS, respectively.

Missing plasma/serum concentrations (<1% of all analyses) were imputed as a weighted average from adjacent values.

Statistical Analyses

Between-group differences (including the primary and secondary outcomes) were analyzed by one-way ANOVA, followed by the post hoc Tukey honestly significant difference test. For outcomes with variance inhomogeneity, data were logarithmically transformed or analyzed by the Welch heteroscedastic F test, followed by the post hoc Games-Howell test. When residuals were not normally distributed, data were logarithmically transformed or analyzed by the Kruskal-Wallis test, followed by post hoc Bonferroni-adjusted pairwise exact Wilcoxon rank sum tests.

Within-group differences between the four experimental days (placebo, GLP-1R blockade, GIPR blockade, and combined GLP-1R/GIPR blockade) were analyzed by ANOVA in a linear mixed-effects model (with the experimental day as a categorical fixed effect and individual participants as a random effect) with reporting of post hoc comparisons of single and combined hormone receptor blockades versus placebo as well as GLP-1R blockade versus GIPR blockade. Logarithmic transformation was used if needed for optimal model fit.

To test for potential within-group synergistic effects of GLP-1R and GIPR blockade, the placebo-subtracted effect of combined GLP-1R/GIPR blockade was compared against the sum of the placebo-subtracted effect of single GLP-1R and single GIPR blockade using a two-tailed paired t test.

We based our sample size calculation on data from a meal study in healthy unoperated people demonstrating an ∼40% greater increase in the iAUC of glucose during GIPR versus GLP-1R blockade (absolute mean difference 55 mmol/L; SD 64) (33). Assuming that the importance of GIP versus GLP-1 would be largely unaltered after SG but markedly reduced after RYGB (with greater importance of GLP-1), we powered the study (n = 12 per group) to be able to detect an absolute between-group difference in the effect of GIPR blockade versus GLP-1R blockade on the iAUC of glucose of 80 mmol/L × min (with 80% power and a two-sided α-error of 0.05).

$P < 0.05$ was chosen as the level of significance. Statistical analyses were performed in R 4.1.2 software (www.Rproject.org) using the "onewaytests," "rstatix," and "nlme" packages.

Data and Resource Availability

Reasonable requests for access to the data sets should be addressed to the corresponding author. No applicable resources were generated during the study.

RESULTS

Participant Characteristics

Participant characteristics are summarized in Table 1. HbA_{1c} was slightly lower (range: CON, 33 − 44 mmol/mol; SG, 28 − 41 mmol/mol; RYGB, 29 − 38 mmol/mol), and fasting plasma glucose concentrations tended to be lower in the surgical groups compared with CON. Moreover, fasting insulin clearance (C-peptide–to–insulin ratio) was higher after RYGB than in CON. HOMA2 insulin sensitivity did not differ significantly between groups. Fasting plasma GLP-1 concentrations were higher after RYGB than after SG. Fasting plasma GIP and glucagon concentrations did not differ between groups.

Peptide Infusions

In all groups, target plasma concentrations of exendin (9-39)NH_2 (∼400 nmol/L) and GIP(3-30)NH_2 (∼75 nmol/L) were reached within the 30-min basal infusions and were maintained throughout the 4-h postprandial period (Fig. 1).

Table 1—Participant characteristics

	CON	SG	RYGB	*P* value ANOVA	CON vs. SG	CON vs. RYGB	SG vs. RYGB
Matching parameters							
Women/men, *n/n*	7/5	7/5	7/5				
Age, years	50 ± 13	50 ± 11	48 ± 8				
BMI actual, kg/m^2	33 ± 5	34 ± 4	33 ± 7				
BMI preoperative, kg/m^2	—	43 ± 5	45 ± 5				
Time from surgery, years	—	2.0 (1.2; 2.7)	1.8 (1.3; 2.4)				
Glycemic control							
HbA_{1c}, %	6.2 ± 0.5	5.7 ± 0.5	5.6 ± 0.4	<0.01	<0.05	<0.01	0.73
HbA_{1c}, mmol/mol	37 ± 3	34 ± 4	33 ± 3	<0.01	<0.05	<0.01	0.71
Fasting biochemistry							
Glucose, mmol/L	5.4 (5.3; 5.6)	4.9 (4.7; 5.4)	5.0 (4.7; 5.3)	0.09‡			
Insulin, pmol/L	47 (45; 74)	51 (47; 59)	38 (32; 41)	<0.05‡	1.0	<0.05	<0.05
C-peptide, pmol/L	754 (566; 968)	831 (667; 894)	666 (543; 779)	0.20‡			
C-peptide–to–insulin ratio	12.8 ± 3.9	15.6 ± 3.2	17.7 ± 3.4	<0.01	0.14	<0.01	0.29
ISR, pmol/kg/min	2.1 (1.7; 2.6)	2.3 (1.9; 2.4)	1.9 (1.6; 2.2)	0.20‡			
HOMA2-S	0.61 ± 0.23	0.58 ± 0.12	0.73 ± 0.20	0.15			
GLP-1, pmol/L	7.8 ± 2.9	6.8 ± 2.2	10.4 ± 4.0	<0.05	0.70	0.13	<0.05
GIP, pmol/L	13.6 ± 2.6	11.9 ± 2.0	12.7 ± 2.9	0.29			
Glucagon, pmol/L	5.3 (4.1; 8.0)	5.0 (4.2; 6.7)	6.0 (3.9; 9.0)	0.76‡			

Data are mean ± SD or median (IQR), unless indicated otherwise. Fasting biochemistry outcomes are the means of fasting samples obtained prior to initiation of the continuous infusions from all four experimental days. HOMA2-S, HOMA2 insulin sensitivity (based on fasting glucose and C-peptide concentrations). ‡Kruskal-Wallis test.

GLP-1 and GIP Concentrations

Placebo Infusions

Postprandial profiles and the iAUCs of plasma GLP-1 and GIP concentrations during placebo infusions are shown in Fig. 2. Postprandial GLP-1 concentrations were highest after RYGB but were also higher after SG compared with CON. In contrast, postprandial GIP concentrations were lowest in the RYGB group.

GLP-1R and GIPR Blockade

GLP-1R blockade and combined GLP-1R/GIPR blockade increased postprandial GLP-1 concentrations (albeit only significantly in the surgical groups) without affecting GIP concentrations, whereas GIPR blockade affected neither GIP nor GLP-1 concentrations (Supplementary Fig. 1 and Supplementary Table 1).

Glucose Concentrations

Placebo Infusions

During placebo infusions, the overall postprandial (iAUC) glucose response was similar between groups (Fig. 3*D*), but the profile differed (Fig. 3*A–C*) with highest peaks in the RYGB group (CON: 7.0 ± 0.3 mmol/L [mean ± SEM], SG: 8.1 ± 0.6, RYGB: 9.4 ± 0.5; ANOVA $P < 0.01$).

GLP-1R and GIPR Blockade

GLP-1R blockade increased basal glucose concentrations in the CON and SG groups, and combined GLP-1R/GIPR blockade increased basal glucose concentrations in all groups (Table 2). However, the effect was larger in CON compared with both surgical groups (Supplementary Table 2). GIPR blockade lowered basal glucose concentrations after RYGB (Table 2), but the placebo-subtracted effect did not differ significantly between groups (Supplementary Table 2).

During the total 4-h postprandial period, GLP-1R blockade increased the iAUC of glucose after SG ($P < 0.05$) and tended to increase the iAUC of glucose after RYGB (P = 0.10), but there was no effect in CON (P = 0.48) (Table 2). However, the placebo-subtracted effect of GLP1R blockade did not differ significantly between groups (Fig. 3*E*). GLP-1R blockade increased the tAUC glucose response in all groups (Table 2), including in CON, because of the effect on basal glucose concentrations. The effect of GLP-1R blockade on postprandial plasma glucose concentrations seemed to be particularly pronounced in the early postprandial phase in the CON group ($iAUC_{0-60}$) and in the late postprandial phase in the surgical groups ($iAUC_{60-240}$) (Fig. 3*A–C* and Table 2). Moreover, GLP-1R blockade, but not GIPR blockade, increased nadir plasma glucose concentrations in all groups (Table 2). Two RYGB-operated participants experienced asymptomatic postprandial hypoglycemia (nadir glucose <3 mmol/L) during placebo infusions (2.9 and 2.7 mmol/L) but not during GLP-1R blockade (Supplementary Fig. 2).

GIPR blockade increased the iAUC of glucose in CON ($P < 0.01$) and tended to increase the iAUC of glucose after SG (P = 0.09), but there was no effect after RYGB (P = 0.52) (Table 2). The placebo-subtracted effect of GIPR blockade was significantly larger in CON compared with RYGB (Fig. 3*F*).

The placebo-subtracted effect of GIPR versus GLP-1R blockade on the iAUC of glucose (primary outcome) was larger in the CON compared with the RYGB group (Fig. 3*H*). Thus, the iAUC of glucose was higher during GIPR than GLP-1R blockade in the CON group, while the iAUC of

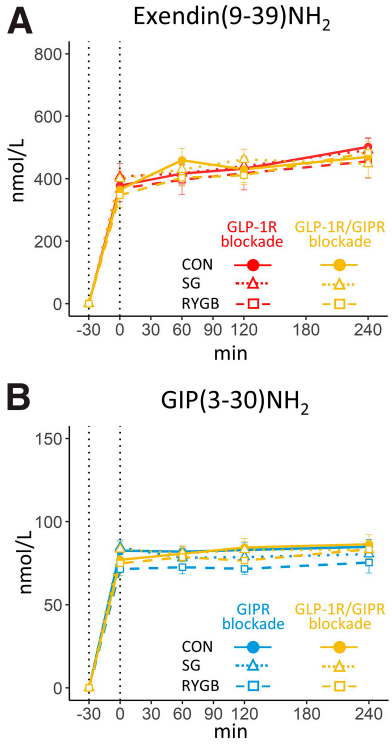

Figure 1—Plasma concentrations of exendin(9-39)NH_2 (*A*) and GIP(3-30)NH_2 (*B*) during infusions of exendin(9-39)NH_2, GIP(3-30)NH_2, and combined exendin(9-39)NH_2 and GIP(3-30)NH_2 in CON, SG, and RYGB participants. Data are mean ± SEM.

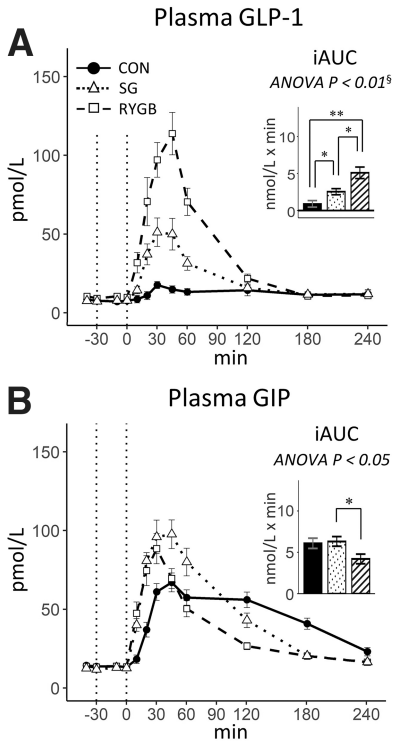

Figure 2—Postprandial profiles and iAUCs of plasma GLP-1 (*A*) and GIP (*B*) concentrations during placebo infusions in CON, SG, and RYGB participants. Data are mean ± SEM. §Welch heteroscedastic *F* test. **P* < 0.05 and ***P* < 0.01 for the difference between groups.

glucose was higher during GLP-1R than during GIPR blockade in the RYGB group (Table 2). In the SG group, the iAUC of glucose was similar during GIPR and GLP-1R blockade (Table 2).

Combined GLP-1R/GIPR blockade increased the iAUC of glucose in all groups (Table 2 and Fig. 3*G*) and was more effective than the summed effect of single GLP-1R and GIPR blockade in the CON ($P < 0.05$) and RYGB ($P < 0.01$) groups, with a similar tendency in the SG group ($P = 0.09$).

ISR

Placebo Infusions

During placebo infusions, the iAUC of ISR was similar between groups (Fig. 4*D*). However, the profile differed (Fig. 4*A*–*C*), with higher peaks in the surgical groups compared with CON (CON: 10.7 ± 0.8 pmol/kg/min [mean ± SEM], SG: 15.0 ± 1.3, RYGB: 19.8 ± 2.6; ANOVA $P < 0.01$).

GLP-1R and GIPR blockade

GLP-1R blockade had no effect on basal ISR (Table 2). GIPR blockade and combined GLP-1R/GIPR blockade lowered basal ISR in the SG group (Table 2), but the placebo-subtracted effect did not differ significantly between groups (Fig. 4*A*–*C* and Supplementary Table 2).

GLP-1R blockade and combined GLP-1R/GIPR blockade reduced the iAUC of ISR in both surgical groups, but there were no effects in CON (Table 2). The placebo-subtracted effect of GLP-1R and combined GLP-1R/GIPR blockade was greater after RYGB compared with CON (Fig. 4*E* and *G*).

GIPR blockade reduced the iAUC of ISR in the CON group only (Table 2), but without significant differences between groups (Fig. 4*F*).

The placebo-subtracted effect of GIPR versus GLP-1R blockade on the iAUC of ISR was larger in CON than in both surgical groups (Fig. 4*H*). Thus, the iAUC of ISR was lower during GIPR blockade than during GLP-1R blockade in the CON group, while the iAUC of ISR was lower during GLP-1R blockade than during GIPR blockade in both surgical groups (Table 2).

β-Cell Function

Placebo Infusions

Neither β-GS (Fig. 5*D*) nor disposition index estimates (Supplementary Fig. 3) differed between groups during placebo infusions.

GLP-1R and GIPR Blockade

GLP-1R blockade reduced β-GS in the surgical groups only (Table 2), but without significant differences between groups (Fig. 5*E*). GIPR blockade reduced β-GS in the CON and SG groups only (Table 2), resulting in a greater effect of GIPR blockade in the CON than in the RYGB group (Fig. 5*F*).

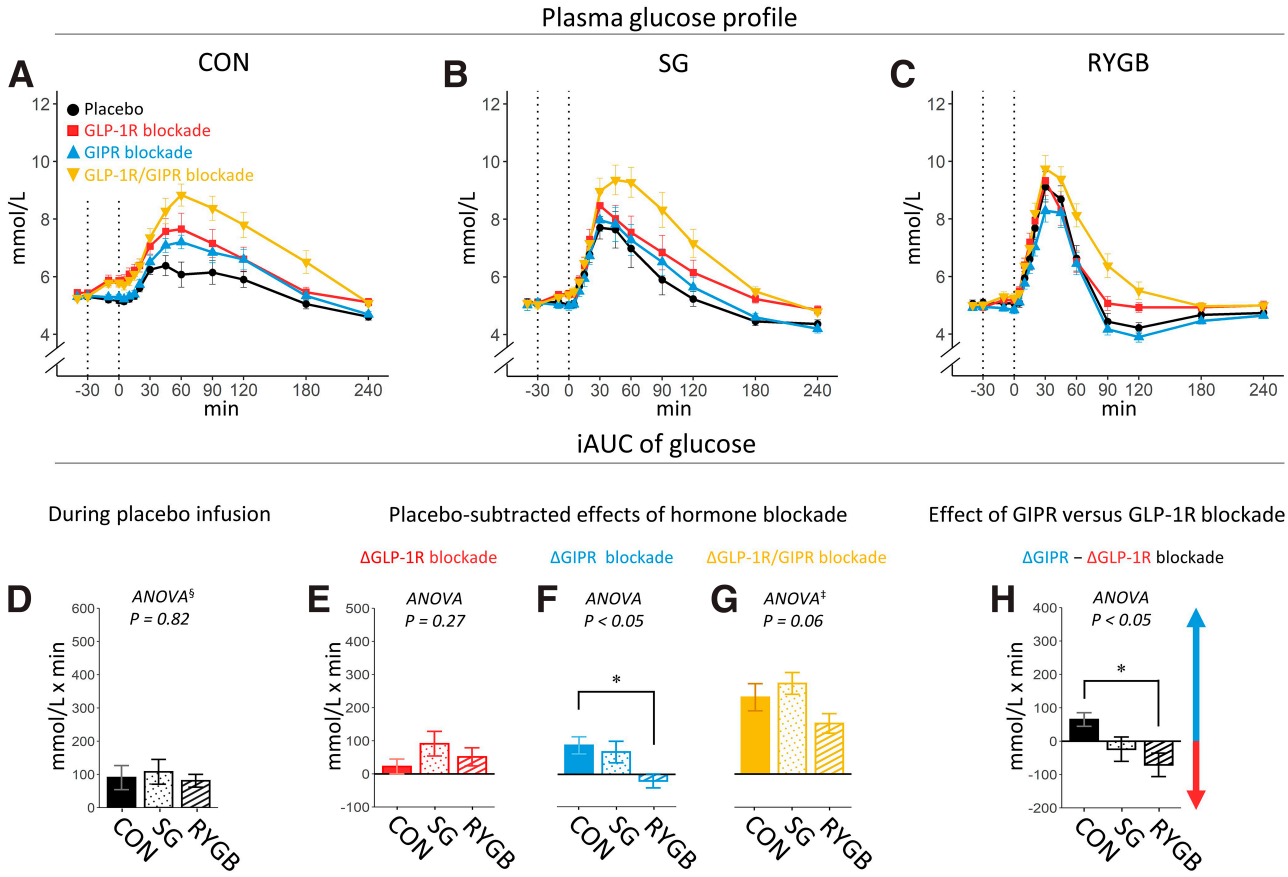

Figure 3—Postprandial profiles of plasma glucose concentrations during placebo infusion, GLP-1R blockade, GIPR blockade, and combined GLP-1R/GIPR blockade in CON (*A*), SG (*B*), and RYGB (*C*) participants. Corresponding iAUCs are presented as absolute outcomes during placebo infusion (*D*) and as placebo-subtracted effects (absolute changes from placebo) of GLP-1R (*E*), GIPR (*F*), and combined GLP-1R/GIPR blockade (*G*). *H*: In addition, the effect of GLP-1R blockade was subtracted from the effect of GIPR blockade to evaluate between-group differences in the importance of GIP vs. GLP-1. ‡Kruskal-Wallis test. §Welch heteroscedastic *F* test. Data are mean ± SEM. **P* < 0.05 and ***P* < 0.01 for the difference between groups.

The placebo-subtracted effect of GIPR blockade versus GLP-1R blockade on β-GS (secondary outcome) differed significantly between all of the groups (Fig. 5*H*). Hence, β-GS was lower during GIPR blockade than during GLP-1R blockade in CON, while β-GS was similar during GIPR and GLP-1R blockade after SG and was lower during GLP-1R blockade than during GIPR blockade after RYGB (Table 2).

Combined GLP-1R/GIPR blockade increased β-GS in all groups (Table 2 and Fig. 5*G*) and was more effective than the summed effect of single GLP-1R and GIPR blockade in the RYGB group ($P < 0.05$) but not in the CON ($P = 0.56$) and SG ($P = 0.27$) groups.

Glucagon Concentrations

During placebo infusions, there were no significant between-group differences in the iAUC of glucagon (ANOVA $P = 0.14$).

GLP-1R blockade and combined GLP-1R/GIPR blockade had no significant effects on basal plasma glucagon concentrations (Table 2). GIPR blockade reduced basal glucagon concentrations in the CON group (Table 2), but the placebo-subtracted effect did not differ between groups (Supplementary Table 2). GLP-1R and GIPR blockade had no effects on the iAUC of glucagon in any group, but GLP-1R blockade increased the tAUC of glucagon in the CON group (Fig. 6*A–C* and Table 2).

Paracetamol Absorption Rates

During placebo infusions, time to the peak of paracetamol concentrations was shortest after RYGB ($P < 0.01$ compared against both CON and SG) but did not differ between CON and SG ($P = 0.30$) (Table 2). GLP-1R and GIPR blockade had no significant effects in any group (Fig. 6*D–F* and Table 2).

Increased Exendin(9-39)NH_2 Infusion Rate

In the three RYGB participants who underwent an extra meal test, no additional impact was observed on the iAUC of glucose, the iAUC of ISR, or β-GS when the exendin (9-39)NH_2 bolus and infusion rate were increased by 33% (Supplementary Fig. 2).

Table 2—Within-group comparisons

	Placebo	GLP-1R blockade	GIPR blockade	GLP-1R/GIPR blockade	ANOVA *P*
CON					
Basal glucose (*t* = 0), mmol/L	5.2 ± 0.1	5.9 ± 0.2**	5.3 ± 0.1††	5.8 ± 0.2**	<0.01
iAUC glucose, mmol/L × min	90 ± 36	113 ± 38	177 ± 33**†	322 ± 42**	<0.01
$iAUC_{0-60}$ glucose, mmol/L × min	42 ± 8	61 ± 11*	60 ± 6*	86 ± 9**	<0.01
$iAUC_{60-240}$ glucose, mmol/L × min	49 ± 31	52 ± 30	118 ± 32*†	236 ± 39**	<0.01
tAUC glucose, mol/L × min	1.34 ± 0.04	1.52 ± 0.06**	1.45 ± 0.04*	1.71 ± 0.07**	<0.01
Nadir glucose, mmol/L	4.4 ± 0.1	5.0 ± 0.1**	4.7 ± 0.1†	5.1 ± 0.1**	<0.01
Basal ISR (*t* = 0), pmol/kg/min‖	2.0 ± 0.2	2.2 ± 0.2	2.0 ± 0.2	1.9 ± 0.2	0.10
iAUC ISR, nmol/kg‖	0.86 ± 0.10	0.86 ± 0.10	0.72 ± 0.07*†	0.89 ± 0.10	<0.05
β-GS, (pmol/kg/min)/(mmol/L)‖	4.7 ± 0.5	4.1 ± 0.6	2.5 ± 0.2**††	1.7 ± 0.1**	<0.01
Basal glucagon (*t* = 0), pmol/L‖	6.9 ± 0.9	8.3 ± 2.0	5.3 ± 1.3*†	8.1 ± 1.8	<0.05
iAUC glucagon, pmol/L × min	−151 ± 168	67 ± 343	132 ± 151	−165 ± 214	0.73
tAUC glucagon, nmol/L × min	1.5 ± 0.2	2.1 ± 0.3*	1.4 ± 0.2††	1.8 ± 0.3	<0.01
Time to peak paracetamol, min‖	62 ± 9	109 ± 23	93 ± 20	99 ± 18	0.35
SG					
Basal glucose (*t* = 0), mmol/L	5.1 ± 0.1	5.4 ± 0.1**	5.0 ± 0.1††	5.3 ± 0.1*	<0.01
iAUC glucose, mmol/L × min	108 ± 37	199 ± 55*	175 ± 31	381 ± 59**	<0.01
$iAUC_{0-60}$ glucose, mmol/L × min	106 ± 19	116 ± 16	116 ± 14	158 ± 15**	<0.01
$iAUC_{60-240}$ glucose, mmol/L × min	2 ± 21	83 ± 41*	58 ± 22	223 ± 46**	<0.01
tAUC glucose, mol/L × min	1.33 ± 0.05	1.49 ± 0.07**	1.38 ± 0.06†	1.66 ± 0.07**	<0.01
Nadir glucose, mmol/L	4.1 ± 0.1	4.8 ± 0.2**	4.2 ± 0.2††	4.8 ± 0.1**	<0.01
Basal ISR (*t* = 0), pmol/kg/min	2.1 ± 0.1	1.9 ± 0.1	1.7 ± 0.1**	1.8 ± 0.1*	<0.05
iAUC ISR, nmol/kg‖	0.89 ± 0.10	0.75 ± 0.07*	0.88 ± 0.08†	0.73 ± 0.06*	<0.01
β-GS, (pmol/kg/min)/(mmol/L)	4.5 ± 0.6	3.4 ± 0.4**	3.5 ± 0.3*	1.9 ± 0.3**	<0.01
Basal glucagon (*t* = 0), pmol/L	5.5 ± 0.9	5.3 ± 0.8	4.1 ± 0.7	4.9 ± 1.0	0.22
iAUC glucagon, pmol/L × min	156 ± 135	300 ± 508	310 ± 112	227 ± 110	0.81
tAUC glucagon, nmol/L × min	1.5 ± 0.2	1.6 ± 0.2	1.3 ± 0.1	1.4 ± 0.2	0.10
Time to peak paracetamol, min	51 ± 8	48 ± 10	47 ± 10	57 ± 9	0.84
RYGB					
Basal glucose (*t* = 0), mmol/L	5.1 ± 0.1	5.2 ± 0.1	4.9 ± 0.1*††	5.3 ± 0.2**	<0.01
iAUC glucose, mmol/L × min	81 ± 19	132 ± 26	61 ± 23†	233 ± 29**	<0.01
$iAUC_{0-60}$ glucose, mmol/L × min	148 ± 15	147 ± 17	134 ± 14	175 ± 13*	<0.01
$iAUC_{60-240}$ glucose, mmol/L × min	−68 ± 14	−15 ± 14*	−73 ± 19†	58 ± 23**	<0.01
tAUC glucose, mol/L × min	1.30 ± 0.04	1.38 ± 0.05*	1.23 ± 0.03††	1.50 ± 0.06**	<0.01
Nadir glucose, mmol/L	4.0 ± 0.2	4.7 ± 0.2**	3.7 ± 0.2††	4.8 ± 0.2**	<0.01
Basal ISR (*t* = 0), pmol/kg/min	1.7 ± 0.2	1.7 ± 0.1	1.4 ± 0.1	1.6 ± 0.1	0.05
iAUC ISR, nmol/kg‖	0.93 ± 0.11	0.61 ± 0.08**	0.86 ± 0.11††	0.56 ± 0.05**	<0.01
β-GS, (pmol/kg/min)/(mmol/L)‖	3.7 ± 0.4	2.6 ± 0.2**	4.3 ± 0.5††	1.5 ± 0.4**	<0.01
Basal glucagon (*t* = 0), pmol/L	5.0 ± 1.0	6.1 ± 1.0	4.4 ± 0.8	5.1 ± 0.9	0.11
iAUC glucagon, pmol/L × min	241 ± 118	114 ± 143	198 ± 137	298 ± 148	0.74
tAUC glucagon, nmol/L × min	1.4 ± 0.2	1.6 ± 0.2	1.2 ± 0.2	1.5 ± 0.2	0.08
Time to peak paracetamol, min‖	23 ± 4	22 ± 4	20 ± 3	23 ± 7	0.89

Data are mean ± SEM. ‖Model on logarithmically transformed data. *$P < 0.05$ and **$P < 0.01$ for within-group comparison against placebo. †$P < 0.05$ and ††$P < 0.01$ for within-group comparison of GIPR vs. GLP-1R blockade.

DISCUSSION

We studied the effects of separate and combined GLP-1R and GIPR blockade during meal tests in matched groups of unoperated, SG-operated, and RYGB-operated people without diabetes. Our main finding was an altered importance of endogenously secreted GIP versus GLP-1 for the postprandial iAUC of glucose and β-GS after bariatric surgery. In unoperated people, GIP was more important than GLP-1, as also previously reported (28,29). In contrast, GLP-1 and GIP were equally important after SG, and GLP-1 was more important than GIP after RYGB.

This is the first study to directly compare the importance of endogenously secreted incretin hormones for postprandial glucose metabolism after RYGB versus SG. Moreover, our study design offers advantages over previous RYGB and SG studies in which only the GLP-1R was blocked. Hence, we could demonstrate effects during combined GLP-1R/GIPR blockade that were not evident during single hormone receptor blockade. Thus, despite no effect of single GLP-1R blockade in the CON group and no effect of single GIPR blockade in the RYGB group, there were synergistic effects of GLP-1R and GIPR blockade on the iAUC of glucose in both groups. Similarly, despite no effect of single GIPR blockade on β-GS in the RYGB group, there were synergistic effects of GLPR and GIPR blockade. Therefore, our results indicate that in healthy unoperated people, GIP is more important than GLP-1, but GLP-1 still plays an important role. After RYGB, the

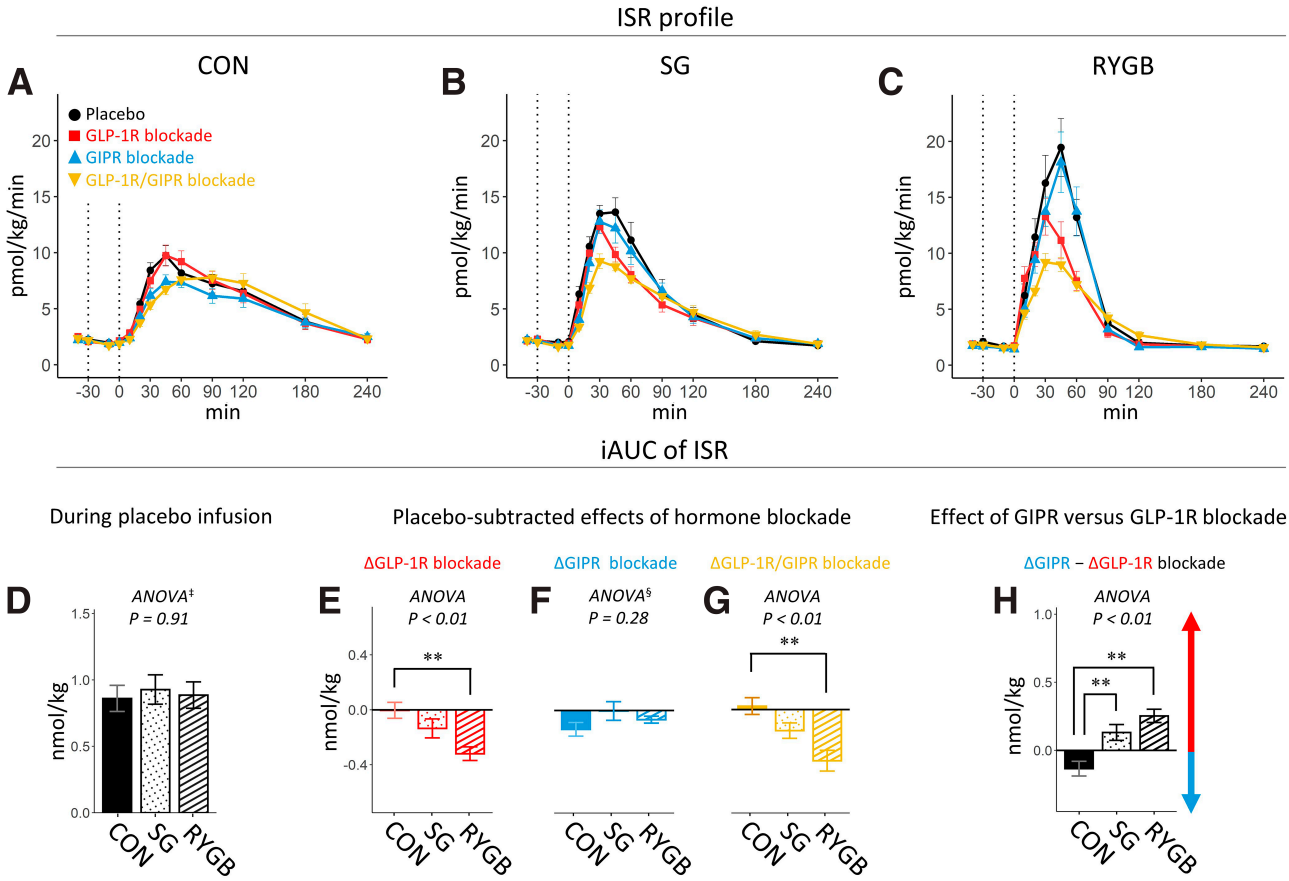

Figure 4—Postprandial profiles of ISRs during placebo infusion, GLP-1R blockade, GIPR blockade, and combined GLP-1R/GIPR blockade in CON (*A*), SG (*B*), and RYGB (*C*) participants. Corresponding iAUCs are presented as absolute outcomes during placebo infusion (*D*) and as placebo-subtracted effects (absolute changes from placebo) of GLP-1R (*E*), GIPR (*F*), and combined GLP-1R/GIPR blockade (*G*). *H*: In addition, the effect of GLP-1R blockade was subtracted from the effect of GIPR blockade to evaluate between-group differences in the importance of GIP vs. GLP-1. Data are mean ± SEM. ‡Kruskal-Wallis test. §Welch heteroscedastic *F* test. $*P < 0.05$ and $**P < 0.01$ for the difference between groups.

importance of GIP is reduced but not completely lost. In a previous more indirect study of endogenous GIP function after RYGB, an approximately twofold elevation of postprandial systemic concentrations of intact (= active) endogenous GIP (via inhibition of dipeptidyl peptidase 4 [DPP-4]) had no impact on postprandial glucose concentrations or β-cell function during GLP-1R blockade (32). However, synergistic effects of GIP and GLP-1 were not addressed in that design.

Our results are consistent with several previous post-RYGB studies (18–23) and one previous post-SG study (25) showing numerically greater reductions in the postprandial β-cell function during GLP-1R blockade after RYGB and SG versus unoperated people, although the small cohorts often limit the power to detect significant between-group differences (24). The impact of GLP-1R blockade on postprandial glucose tolerance is less clear (24). However, GLP-1R blockade clearly raises nadir glucose, as also observed in the current study, and prevents hypoglycemia in RYGB-operated patients with symptomatic postprandial hypoglycemia (21,34,35). Several factors limit the direct comparison of postprandial effects of GLP-1R blockade in bariatric versus unoperated individuals. The postprandial glucose profiles are markedly different, with higher peaks especially after RYGB compared with CON. Moreover, in our study, GLP-1R blockade increased premeal glucose concentrations more in the CON group than in both surgical groups. Previous studies indicate that the effect of exendin(9-39)NH_2 in the fasting state is particularly associated with increased glucagon concentrations (36–38). However, the current study was designed to address postprandial effects, and we were not able to detect between-group differences in basal glucagon concentrations.

Another aspect to consider is the inhibitory effect of GLP-1 on the gastric emptying rate in unoperated people (12,28,39). We found a markedly faster paracetamol absorption rate after RYGB compared with CON but surprisingly, no difference between SG and CON. The latter contrasts previous findings (15,16) and could reflect the meal stimuli in our study (a liquid mixed meal with a modest fat content ingested evenly over 20 min). Moreover,

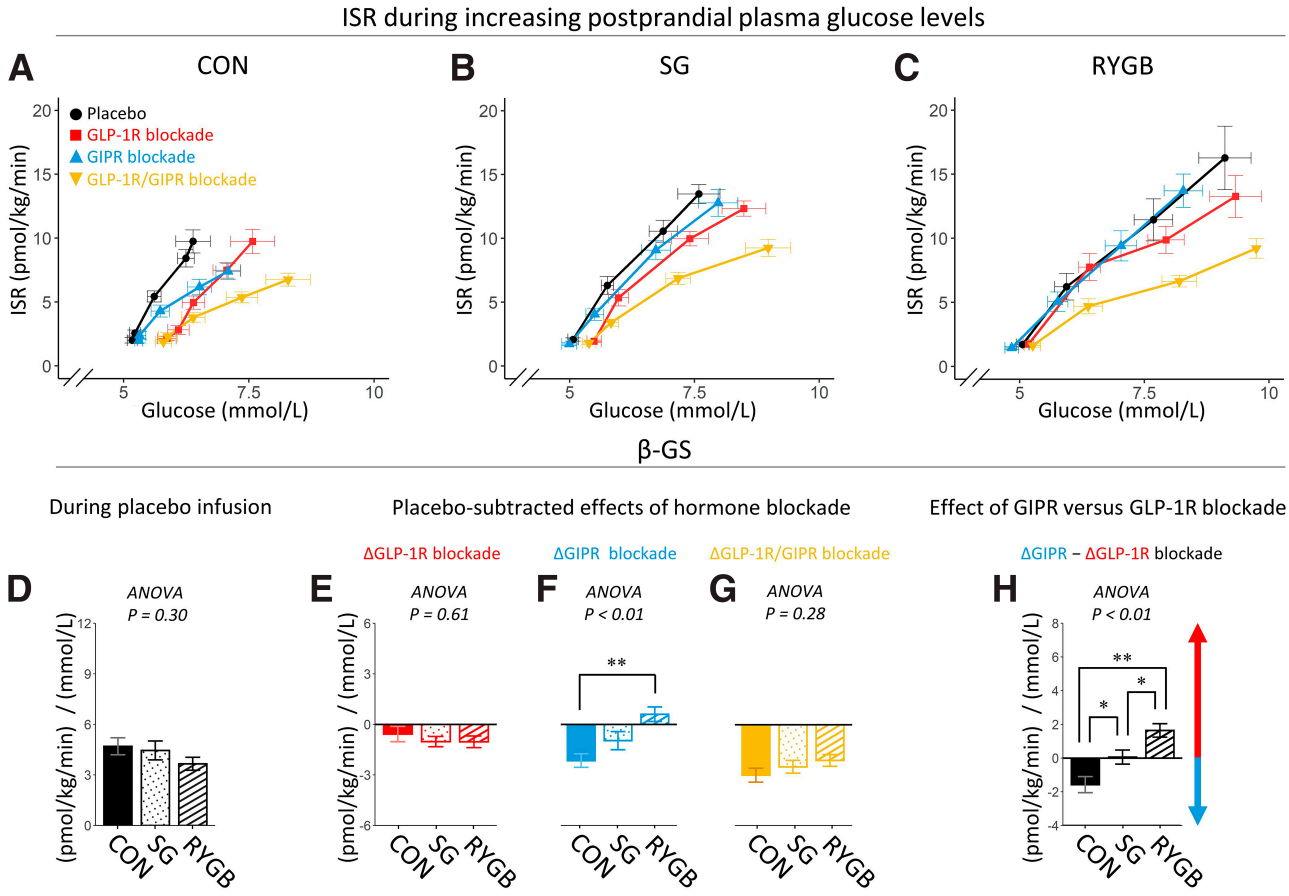

Figure 5—ISRs related to increasing plasma glucose concentrations during placebo infusion, GLP-1R blockade, GIPR blockade, and combined GLP-1R/GIPR blockade in CON (*A*), SG (*B*), and RYGB (*C*) participants. Estimates of β-GS are presented as absolute outcomes during placebo infusion (*D*) and as placebo-subtracted effects (absolute changes from placebo) of GLP-1R (*E*), GIPR (*F*), and combined GLP-1R/GIPR blockade (*G*). *H*: In addition, the effect of GLP-1R blockade was subtracted from the effect of GIPR blockade to evaluate between-group differences in the importance of GIP vs. GLP-1. Data are mean ± SEM. *$P < 0.05$ and **$P < 0.01$ for the difference between groups.

GLP-1R blockade neither affected time to the peak of paracetamol concentrations in the surgical groups (as expected) nor in CON.

Reports of RYGB- and SG-induced changes in the postprandial GIP response are conflicting (2,17), probably particularly reflecting differences in surgical technique, the type of meal stimulus, sample size, and the choice of control group. Using a commercially available liquid mixed meal with a macronutrient composition resembling that of a regular meal resulted in a reduced postprandial GIP response after RYGB compared with SG and a similar tendency compared with CON. While this is a standardized and easily reproducible test meal, a solid meal may be more real-life relevant. Nonetheless, whereas the macronutrient composition of a meal undoubtedly affects postprandial glucose excursions and gut and pancreatic hormone responses (40), the meal texture (solid vs. liquid) seems to have surprisingly little influence on postprandial glucose tolerance and insulin secretion in both unoperated people as well as in individuals who have undergone RYGB and SG (41).

Exendin(9-39)NH_2 is an antagonist or inverse agonist on the GLP-1R (24,37,38,42–44). Infused at a rate of 300 pmol/kg/min, exendin(9-39)NH_2 blocks ∼90% of GLP-1 mediated insulin secretion during coinfusion of GLP-1 to mimic physiological postprandial GLP-1 concentrations in unoperated people (24,37,38,42–44). However, postprandial GLP-1 concentrations are greatly elevated after RYGB. Therefore, we used a higher exendin(9-39)NH_2 infusion rate (bolus: 43,000 pmol/kg, continuous infusion: 900 pmol/kg/min) than in most other post-RYGB studies (24). Furthermore, we tested the effect of a 33% increased exendin(9-39)NH_2 infusion rate in three of the RYGB-operated participants without any additional effects. It is also unclear to what extent exendin(9-39)NH_2 blocks local effects of endogenous GLP-1 in the intestinal wall and splanchnic vessels (e.g., activation of sensory vagal afferents [12]), where the concentration of intact (= active) GLP-1 is much higher than in the systemic circulation due to rapid degradation by DPP-4 (45). Finally, animal and ex vivo human studies indicate that not only

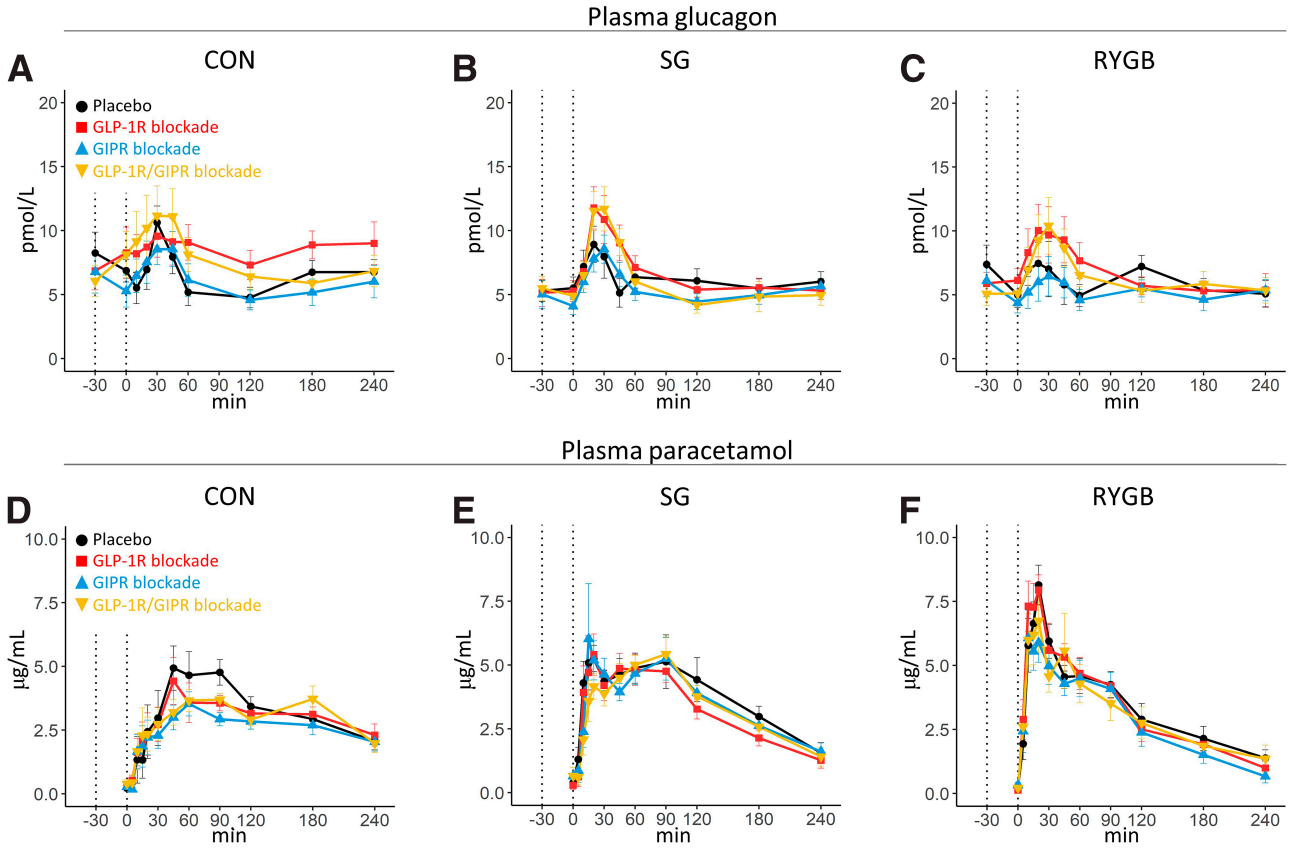

Figure 6—Postprandial profiles of plasma glucagon (*A*–*C*) and paracetamol (*D*–*F*) concentrations during placebo infusion, GLP-1R blockade, GIPR blockade, and combined GLP-1R/GIPR blockade in CON (*A* and *D*), SG (*B* and *E*), and RYGB (*C* and *F*) participants. Data are mean ± SEM.

GLP-1 but also glucagon regulate insulin secretion via action on the GLP-1R (46,47). Although the physiological importance in vivo in humans is unclear, it is possible that some of the effects of exendin(9-39)NH_2 on insulin secretion could be attributed to the effect of blocked glucagon action.

GIP(3-30)NH_2 is a relatively new experimental tool but pharmacologically well described as a competitive antagonist on the GIPR without cross-reactivity on closely related receptors (26,27,48,49). We only expected modest changes in postprandial GIP concentrations after bariatric surgery. Therefore, we used a GIP(3-30)NH_2 infusion rate (800 pmol/kg/min) previously demonstrated to block >80% of GIP-mediated insulin secretion in unoperated people during coinfusion of GIP to mimic physiological postprandial GIP concentrations (48). The uncertainty of the degree of inhibition of the endogenous hormone function seems less than for exendin(9-39)NH_2, as GIP is not thought to act through sensory vagal afferents and reaches the systemic circulation in its intact (= active) form to a greater extent than GLP-1 (slower DPP-4–mediated degradation) (12).

We used a cross-sectional design and matched the groups on sex, age, and BMI, allowing us to explore weight-loss–independent effects on glucose tolerance and β-cell function, which is a strength of the study. Nevertheless, our study participants may represent a selective group as a slightly greater weight loss is often reported after RYGB compared with SG (2,7). Moreover, HbA_{1c} was slightly lower in the surgical groups than in the CON group, but since all participants had an HbA_{1c} <48 mmol/mol, this should not affect conclusions with respect to the observed effects of incretin hormone blockade.

Conclusion

In people without diabetes, the importance of endogenously secreted GIP versus GLP-1 is altered after bariatric surgery, reflecting the postoperative changes in the postprandial secretion of the hormones. Hence, GIP is the most important incretin hormone in unoperated people, while GIP and GLP-1 are equally important after SG, and GLP-1 is the most important incretin hormone after RYGB. Future studies should explore the separate and combined effects of endogenously secreted GLP-1 and GIP in other bariatric cohorts (e.g., in patients with type 2 diabetes remission or postbariatric symptomatic postprandial hypoglycemia).

Acknowledgments. The authors would like to thank Alis Sloth Andersen and Jette Nymann Andersen, both of Hvidovre University Hospital, Hvidovre, Denmark, and Lene Brus Albæk and Tabatha Emilia de A Constantini, both of the Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark, for laboratory assistance.

Funding. The study was conducted at the Department of Endocrinology, Copenhagen University Hospital Hvidovre (Hvidovre, Denmark) and was supported by grants from the Novo Nordisk Foundation Excellence Project (NNF18 OC0032330), the Hvidovre Hospital Research Fund, the "Doctor Sofus Carl Emil Friis and Wife Olga Dorus Friis" Foundation, and the European Research Council under the European Union's Horizon 2020 research and innovation program (grant agreement no. 695069-BYPASSWITHOUTSURGERY).

Duality of Interest. M.S.S. and K.N.B.-M. have received support for attending meetings from Novo Nordisk. C.M. has received support from Novo Nordisk for organizing/attending meetings. N.B.J. has Eli Lilly and Novo Nordisk stocks and has received consulting/lecture fees and/or support for attending meetings from Novo Nordisk, Boehringer-Ingelheim, and Sanofi. C.D. has participated in advisory boards and has received lecture fees and/or support for attending meetings from Novo Nordisk, Boehringer-Ingelheim, and AstraZeneca. L.S.G. is cofounder of Antag Therapeutics, has patents relating to GIPR antagonists and dual-acting GIP–GLP-2 agonists, and has received lecture fees from Eli Lilly. B.H. is cofounder of Bainan Biotech and has patents relating to GIPR agonists. M.M.R. is cofounder of Antag Therapeutics and Bainan Biotech, chairman of the board of Bainan Biotech, and has patents relating to GIPR agonists and antagonists and dual-acting GIP–GLP-2 agonists. J.J.H. is a cofounder of Bainan Biotech and Antag Therapeutics and has received consulting fees from Novo Nordisk. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. M.H., N.H., M.S.S., A.M., C.M., N.B.J., C.D., L.S.G., V.B.K., B.H., M.M.R., J.J.H., S.M., and K.N.B.-M. contributed to the data analysis and discussion. M.H., N.H., and A.M. conducted the study. M.H. and K.N.B.-M. obtained funding and wrote the primary draft of the protocol. M.H., and K.N.B-M. wrote the manuscript. N.H., M.S.S., A.M., C.M., N.B.J., C.D., L.S.G., V.B.G., B.H., M.M.R., J.J.H., and S.M. critically revised the manuscript. M.S.S., C.M., N.B.J., C.D., L.S.G., V.B.K., B.H., M.M.R., J.J.H., and S.M. contributed to the design. B.H. and J.J.H. performed hormone analysis. All authors approved the final version of the manuscript. K.N.B.-M. initiated the study. M.H. and K.N.B.-M. 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.

Prior Presentation. Parts of this study were presented in abstract form at the 57th Annual Meeting of the European Association for the Study of Diabetes, virtual meeting, 27 September–1 October 2021 (50).

References

1. Holst JJ, Madsbad S, Bojsen-Møller KN, et al. Mechanisms in bariatric surgery: gut hormones, diabetes resolution, and weight loss. Surg Obes Relat Dis 2018;14:708–714

2. Douros JD, Tong J, D'Alessio DA. The effects of bariatric surgery on islet function, insulin secretion, and glucose control. Endocr Rev 2019;40:1394–1423

3. Bojsen-Møller KN, Dirksen C, Jørgensen NB, et al. Early enhancements of hepatic and later of peripheral insulin sensitivity combined with increased postprandial insulin secretion contribute to improved glycemic control after Roux-en-Y gastric bypass. Diabetes 2014;63:1725–1737

4. 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

5. Fatima F, Hjelmesæth J, Birkeland KI, et al. Gastrointestinal hormones and β-cell function after gastric bypass and sleeve gastrectomy: a randomized controlled trial (Oseberg). J Clin Endocrinol Metab 2022;107:e756–e766

6. Borgeraas H, Hofsø D, Hertel JK, Hjelmesaeth J. Comparison of the effect of Roux-en-Y gastric bypass and sleeve gastrectomy on remission of type 2 diabetes: a systematic review and meta-analysis of randomized controlled trials. Obes Rev 2020;21:e13011

7. Hofsø D, Fatima F, Borgeraas H, et al. Gastric bypass versus sleeve gastrectomy in patients with type 2 diabetes (Oseberg): a single-centre, triple-blind, randomised controlled trial. Lancet Diabetes Endocrinol 2019;7:912–924

8. Davis DB, Khoraki J, Ziemelis M, Sirinvaravong S, Han JY, Campos GM. Roux en Y gastric bypass hypoglycemia resolves with gastric feeding or reversal: Confirming a non-pancreatic etiology. Mol Metab 2018;9:15–27

9. Svane MS, Toft-Nielsen MB, Kristiansen VB, et al. Nutrient re-routing and altered gut-islet cell crosstalk may explain early relief of severe postprandial hypoglycaemia after reversal of Roux-en-Y gastric bypass. Diabet Med 2017; 34:1783–1787

10. Pournaras DJ, Aasheim ET, Bueter M, et al. Effect of bypassing the proximal gut on gut hormones involved with glycemic control and weight loss. Surg Obes Relat Dis 2012;8:371–374

11. Dirksen C, Hansen DL, Madsbad S, et al. Postprandial diabetic glucose tolerance is normalized by gastric bypass feeding as opposed to gastric feeding and is associated with exaggerated GLP-1 secretion: a case report. Diabetes Care 2010;33:375–377

12. Holst JJ. The incretin system in healthy humans: the role of GIP and GLP-1. Metabolism 2019;96:46–55

13. Nauck MA, Meier JJ. Incretin hormones: their role in health and disease. Diabetes Obes Metab 2018;20(Suppl. 1):5–21

14. Jorsal T, Rhee NA, Pedersen J, et al. Enteroendocrine K and L cells in healthy and type 2 diabetic individuals. Diabetologia 2018;61:284–294

15. Eiken A, Fuglsang S, Eiken M, et al. Bilio-enteric flow and plasma concentrations of bile acids after gastric bypass and sleeve gastrectomy. Int J Obes 2020;44:1872–1883

16. Svane MS, Bojsen-Møller KN, Martinussen C, et al. Postprandial nutrient handling and gastrointestinal hormone secretion after Roux-en-Y gastric bypass vs sleeve gastrectomy. Gastroenterology 2019;156:1627–1641.e1

17. Moffett RC, Docherty NG, le Roux CW. The altered enteroendocrine reportoire following roux-en-Y-gastric bypass as an effector of weight loss and improved glycaemic control. Appetite 2021;156:104807

18. Jørgensen NB, Dirksen C, Bojsen-Møller KN, et al. Exaggerated glucagon-like peptide 1 response is important for improved β-cell function and glucose tolerance after Roux-en-Y gastric bypass in patients with type 2 diabetes. Diabetes 2013;62:3044–3052

19. Jiménez A, Casamitjana R, Viaplana-Masclans J, Lacy A, Vidal J. GLP-1 action and glucose tolerance in subjects with remission of type 2 diabetes after gastric bypass surgery. Diabetes Care 2013;36:2062–2069

20. Vetter ML, Wadden TA, Teff KL, et al. GLP-1 plays a limited role in improved glycemia shortly after Roux-en-Y gastric bypass: a comparison with intensive lifestyle modification. Diabetes 2015;64:434–446

21. Salehi M, Gastaldelli A, D'Alessio DA. Blockade of glucagon-like peptide 1 receptor corrects postprandial hypoglycemia after gastric bypass. Gastroenterology 2014;146:669–680.e2

22. Shah M, Law JH, Micheletto F, et al. Contribution of endogenous glucagon-like peptide 1 to glucose metabolism after Roux-en-Y gastric bypass. Diabetes 2014;63:483–493

23. 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

24. Hindsø M, Svane MS, Hedbäck N, Holst JJ, Madsbad S, Bojsen-Møller KN. The role of GLP-1 in postprandial glucose metabolism after bariatric surgery: a narrative review of human GLP-1 receptor antagonist studies. Surg Obes Relat Dis 2021;17:1383–1391

25. Jiménez A, Mari A, Casamitjana R, Lacy A, Ferrannini E, Vidal J. GLP-1 and glucose tolerance after sleeve gastrectomy in morbidly obese subjects with type 2 diabetes. Diabetes 2014;63:3372–3377

26. Lynggaard MB, Gasbjerg LS, Christensen MB, Knop FK. GIP(3-30)NH_2—a tool for the study of GIP physiology. Curr Opin Pharmacol 2020;55:31–40

27. Hansen LS, Sparre-Ulrich AH, Christensen M, et al. N-terminally and C-terminally truncated forms of glucose-dependent insulinotropic polypeptide are high-affinity

competitive antagonists of the human GIP receptor. Br J Pharmacol 2016;173:826–838
28. Gasbjerg LS, Helsted MM, Hartmann B, et al. Separate and combined glucometabolic effects of endogenous glucose-dependent insulinotropic polypeptide and glucagon-like peptide 1 in healthy individuals. Diabetes 2019;68:906–917
29. Gasbjerg LS, Helsted MM, Hartmann B, et al. GIP and GLP-1 receptor antagonism during a meal in healthy individuals. J Clin Endocrinol Metab 2020;105:dgz175
30. Wewer Albrechtsen NJ, Kjeldsen SAS, Jensen NJ, et al. On measurements of glucagon secretion in healthy, obese, and Roux-en-Y gastric bypass operated individuals using sandwich ELISA. Scand J Clin Lab Invest 2022;82:75–83
31. Hovorka R, Soons PA, Young MA. ISEC: a program to calculate insulin secretion. Comput Methods Programs Biomed 1996;50:253–264
32. Svane MS, Bojsen-Møller KN, Nielsen S, et al. Effects of endogenous GLP-1 and GIP on glucose tolerance after Roux-en-Y gastric bypass surgery. Am J Physiol Endocrinol Metab 2016;310:E505–E514
33. Gasbjerg LS, Helsted MM, Sparre-Ulrich AH, et al. Abstract 503: Postprandial effects of individual and combined GIP and GLP-1 receptor antagonism in healthy subjects. Diabetologia 2018;61(Suppl. 1):S246
34. Tan M, Lamendola C, Luong R, McLaughlin T, Craig C. Safety, efficacy and pharmacokinetics of repeat subcutaneous dosing of avexitide (exendin 9-39) for treatment of post-bariatric hypoglycaemia. Diabetes Obes Metab 2020;22:1406–1416
35. Craig CM, Liu L-F, Deacon CF, Holst JJ, McLaughlin TL. Critical role for GLP-1 in symptomatic post-bariatric hypoglycaemia. Diabetologia 2017;60:531–540
36. Holst JJ, Gasbjerg LS, Rosenkilde MM. The role of incretins on insulin function and glucose homeostasis. Endocrinology 2021;162:bqab065
37. Gasbjerg LS, Bari EJ, Christensen M, Knop FK. Exendin(9-39)NH_2: recommendations for clinical use based on a systematic literature review. Diabetes Obes Metab 2021;23:2419–2436
38. Schirra J, Sturm K, Leicht P, Arnold R, Göke B, Katschinski M. Exendin (9-39)amide is an antagonist of glucagon-like peptide-1(7-36)amide in humans. J Clin Invest 1998;101:1421–1430
39. Nauck MA, Quast DR, Wefers J, Pfeiffer AFH. The evolving story of incretins (GIP and GLP-1) in metabolic and cardiovascular disease: a pathophysiological update. Diabetes Obes Metab 2021:23(Suppl. 3):5–29
40. Jensen CZ, Bojsen-Møller KN, Svane MS, et al. Responses of gut and pancreatic hormones, bile acids, and fibroblast growth factor-21 differ to glucose, protein, and fat ingestion after gastric bypass surgery. Am J Physiol Gastrointest Liver Physiol 2020;318:G661–G672
41. Hedbäck N, Hindsø M, Bojsen-Møller KN, et al. Effect of meal texture on postprandial glucose excursions and gut hormones after Roux-en-Y gastric bypass and sleeve gastrectomy. Front Nutr 2022;9:889710
42. Morper M, Nicolaus J, Wörle B, Göke B, Schirra J. Abstract 803: The efficacy of exendin(9-39)amide as a GLP-1 receptor antagonist in human (Abstract). Diabetologia 2009;52(Suppl.1):S315
43. Schirra J, Göke B. GLP-1—a candidate humoral mediator for glucose control after Roux-en-Y gastric bypass. Diabetes 2014;63:387–389
44. Göke R, Fehmann HC, Linn T, et al. Exendin-4 is a high potency agonist and truncated exendin-(9-39)-amide an antagonist at the glucagon-like peptide 1-(7-36)-amide receptor of insulin-secreting beta-cells. J Biol Chem 1993;268:19650–19655
45. Hjøllund KR, Deacon CF, Holst JJ. Dipeptidyl peptidase-4 inhibition increases portal concentrations of intact glucagon-like peptide-1 (GLP-1) to a greater extent than peripheral concentrations in anaesthetised pigs. Diabetologia 2011;54:2206–2208
46. Capozzi ME, Svendsen B, Encisco SE, et al. β Cell tone is defined by proglucagon peptides through cAMP signaling. JCI Insight 2019;4:e126742
47. Svendsen B, Larsen O, Gabe MBN, et al. Insulin secretion depends on intra-islet glucagon signaling. Cell Rep 2018;25:1127–1134.e2
48. Gasbjerg LS, Christensen MB, Hartmann B, et al. GIP(3-30)NH_2 is an efficacious GIP receptor antagonist in humans: a randomised, double-blinded, placebo-controlled, crossover study. Diabetologia 2018;61:413–423
49. Gasbjerg LS, Bari EJ, Stensen S, et al. Dose-dependent efficacy of the glucose-dependent insulinotropic polypeptide (GIP) receptor antagonist GIP(3-30)NH_2 on GIP actions in humans. Diabetes Obes Metab 2021;23:68–74
50. Hindsø M, Hedbäck N, Møller A, et al. Importance of endogenous GLP-1 and GIP for postprandial glucose tolerance after Roux-en-Y gastric bypass and sleeve gastrectomy surgery. Diabetologia 2021;64(Suppl. 1):S200–S201