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Blockade of Glucagon-like Peptide 1 Receptor Corrects Postprandial Hypoglycemia After Gastric Bypass

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BACKGROUND & AIMS: Postprandial glycemia excursions increase after gastric bypass surgery; this effect is even greater among patients with recurrent hypoglycemia. These patients also have increased postprandial levels of insulin and glucagon-like peptide 1 (GLP-1). We performed a clinical trial to determine the role of GLP-1 in postprandial glycemia in patients with hyperinsulinemic hypoglycemia syndrome after gastric bypass. **METHODS:** Nine patients with recurrent hypoglycemia after gastric bypass (H-GB), 7 patients who were asymptomatic after gastric bypass (A-GB), and 8 healthy control subjects underwent a mixed-meal tolerance test (350 kcal) using a dual glucose tracer method on 2 separate days. On 1 day they received continuous infusion of the GLP-1 receptor antagonist exendin (9-39) (Ex-9), and on the other day they received a saline control. Glucose kinetics and islet and gut hormone responses were measured before and after the meal. **RESULTS:** Infusion of Ex-9 corrected hypoglycemia in all patients with H-GB. The reduction in postprandial insulin secretion by Ex-9 was greater in the H-GB group than in the other groups (H-GB, 50% ± 8%; A-GB, 13% ± 10%; controls, 14% ± 10%) ($P < .05$). The meal-derived glucose appearance was significantly greater in subjects who had undergone gastric bypass compared to the controls and in the H-GB group compared to the A-GB group. Ex-9 shortened the time to reach peak meal-derived glucose appearance in all groups without a significant effect on overall glucose flux. Postprandial glucagon levels were higher among patients who had undergone gastric bypass than controls and increased with administration of Ex-9. **CONCLUSIONS:** Hypoglycemia after gastric bypass can be corrected by administration of a GLP-1 receptor antagonist, which might be used to treat this disorder. These findings are consistent with reports that increased GLP-1 activity contributes to hypoglycemia after gastric bypass. ClinicalTrials.gov, Number: NCT01803451.

Keywords: Roux-en-Y Gastric Bypass Surgery; Hyperinsulinemic Hypoglycemia Syndrome; Glucagon-like Peptide 1; Islet Function.

Roux-en-Y gastric bypass surgery (GB), which is now widely used for treatment of obesity, alters glucose fluxes and metabolism.[1,2] GB leads to an earlier and higher peak level of glucose and lower nadir glucose levels after food intake as well as secretion of insulin and glucagon-like peptide 1 (GLP-1) that is accentuated and occurs earlier during the postprandial period.[3] This pattern is in part due to more rapid transit of nutrients from the small gastric remnant into the small intestine, resulting in large fluxes of splanchnic glucose.[1] In healthy humans, more rapid passage of nutrients into the intestine is associated with higher plasma GLP-1 concentrations,[4,5] and postprandial hyperinsulinemia after GB is typically attributed to the combined effects of elevated glucose and GLP-1 levels. In fact, blockade of the GLP-1 receptor (GLP-1R) has a disproportionately greater effect on meal-induced insulin release in subjects who have undergone GB.[6]

Perhaps the most dramatic effect of GB on glucose metabolism is a syndrome of postprandial hyperinsulinemic hypoglycemia that emerges in a minority of patients several years after this procedure is performed.[7,8] Affected patients have greater insulin and GLP-1 responses to meal ingestion compared with subjects who have undergone GB without symptomatic hypoglycemia.[9] Examination of surgical specimens from patients with the hypoglycemia syndrome who were treated with partial pancreatectomy suggested islet cell hypertrophy,[8] but this has been disputed.[10] Despite the potential association of elevated GLP-1 levels with the post-GB hypoglycemia syndrome, there is not yet conclusive evidence that they are directly linked. In a previous study of the GLP-1R antagonist exendin (9-39) (Ex-9), we noted a trend toward a larger contribution of endogenous GLP-1 to postprandial insulin response in a group of subjects with postprandial hypoglycemia who had undergone GB compared with an asymptomatic GB group.[6] However, in this study, which focused on the effects of GLP-1–stimulated

Abbreviations used in this paper: **A-GB, asymptomatic after gastric bypass; AUC, area under the curve; CON, control subjects; EGP, endogenous glucose production; Ex-9, exendin (9-39); GB, Roux-en-Y gastric bypass surgery; GI, gastrointestinal; GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide 1; GLP-1R, glucagon-like peptide 1 receptor; H-GB, recurrent hypoglycemia after gastric bypass; HOMA-IR, homeostatic model assessment of insulin resistance; ISR, insulin secretion rate; MTT, meal tolerance test; OGIS, oral glucose insulin sensitivity index; Ra_{TOT}, glucose appearance; Ra_{Oral}, meal-derived glucose appearance; Rd, glucose disappearance.**

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insulin secretion, blood glucose was clamped and the effects of GLP-1R blockade on glycemia could not be determined.

In the present study, Ex-9 was used during dual-tracer meal tolerance studies to investigate the effect of endogenous GLP-1 on postprandial glucose kinetics in subjects with and without symptomatic hypoglycemia who had undergone GB as well as a group of nonsurgical controls. We hypothesized that GLP-1 has a greater effect on blood glucose levels in subjects with hypoglycemia who have undergone GB compared with asymptomatic subjects.

Subjects and Methods

Subjects

Nine patients with recurrent hypoglycemia after GB (H-GB), 7 subjects who were asymptomatic after GB (A-GB), and 8 healthy control subjects (CON) with normal glucose tolerance and no prior history of gastrointestinal (GI) surgery were recruited. The subjects in the H-GB group had recurrent episodes of neuroglycopenic symptoms (cognitive dysfunction, loss of consciousness, and/or seizure) within 5 hours of meal ingestion that were associated with blood glucose levels <50 mg/dL and resolved immediately with carbohydrate intake (Whipple triad).[11] The subjects in the A-GB group denied hypoglycemic symptoms and had no documented episodes of low blood glucose levels. Seven subjects in the H-GB group and 3 subjects in the A-GB group had symptoms consistent with dumping syndrome (nausea, diarrhea, weakness, sleepiness, palpitation, dizziness, headaches, feeling warmth, and abdominal fullness[12]). Dumping symptoms started soon after surgery, occurred after intake of specific foods, and were not relieved by carbohydrate ingestion, in contrast to autonomic hypoglycemic symptoms. None of the subjects had GI obstruction, renal dysfunction, or liver disorders, and none were taking any medications that interfere with glucose metabolism for at least 1 week before the studies. Two subjects in the H-GB group and 2 subjects in the A-GB group had a history of type 2 diabetes that was controlled with diet or oral medications before surgery and resolved completely after surgery.

The protocol was approved by the institutional review board of the University of Cincinnati, and all participants provided written informed consent before the studies. All authors had access to the study data and reviewed and approved the final manuscript.

Peptides

Synthetic Ex-9 (C S Bio Co, Menlo Park, CA) was $>95\%$ pure, sterile, and free of pyrogens. Lyophilized Ex-9 was dissolved in 0.25% human serum albumin and dispensed by the research pharmacy at Cincinnati Children's Hospital. The use of synthetic Ex-9 is approved under US Food and Drug Administration Investigational New Drug (IND) 65,837.

Experimental Protocols

The subjects were instructed to maintain normal carbohydrate ingestion and not engage in excessive physical activity for 3 days before each visit. Participants were admitted to the General Clinical Research Center at Cincinnati Children's Hospital after an overnight fast on 2 separate days separated by 1 to 2 weeks. Body composition was assessed using dual-energy x-ray absorptiometry, and waist circumference was measured. Intravenous catheters were placed in each forearm for the withdrawal of blood and the infusion of Ex-9 or saline; the arm used for blood sampling was continuously warmed with a heating pad.

After withdrawal of fasting blood samples at -120 minutes, a primed continuous infusion of [6,6-$^2H_2$]glucose (22 μmol/kg prime and 0.22 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ constant) was initiated and continued for the duration of the study.[13] At -60 minutes, subjects received either a primed continuous infusion of Ex-9 (7500 pmol/kg prime and 750 $pmol \cdot kg^{-1} \cdot min^{-1}$ constant) for the remainder of the study or saline as a control[6,14,15]; the order of the Ex-9 infusions was varied so that 10 of the subjects received Ex-9 on their first day of study and 14 received saline first. At 0 minutes, a 237-mL liquid test meal containing 350 kcal and a calorie distribution of 57% carbohydrate, 15% protein, and 28% fat (Ensure Plus; Abbott Laboratories, Abbott Park, IL) mixed with 1 g of universally labeled glucose ([U-^{13}C]glucose) was consumed within 10 minutes. Blood samples were drawn from 0 to 300 minutes (Figure 1), stored on ice, and plasma separated within 60 minutes for storage at -80°C until assay.

Assays

Blood samples were collected as previously described.[6] Blood glucose concentrations were determined using an automated glucose analyzer. Insulin concentrations were determined with a previously described radioimmunoassay.[14] C-peptide and glucagon levels were measured by commercial radioimmunoassays (Millipore, Billerica, MA), and total GLP-1 (Meso Scale Diagnostics, LLC, Gaithersburg, MD) and total glucose-dependent insulinotropic peptide (GIP) (Millipore) levels were measured using a commercial enzyme-linked immunosorbent assay according to the manufacturers' specifications. Plasma enrichment of isotopes was determined using gas chromatography–mass spectrometry.

Calculations and Analysis

Fasting values of blood glucose and hormones were computed as the average of the 4 samples drawn from -130 to -60 minutes and the premeal values as the average of the 5 samples drawn from -10 to 0 minutes. Insulin secretion rates (ISRs) were derived from plasma C-peptide concentrations using deconvolution with population estimates of C-peptide.[16] Glucose, insulin, ISR, glucagon, and GLP-1 values from 0 to 180 minutes and GIP levels from 0 to 150 minutes after meal ingestion were used to compute incremental area under the curve (AUC) using the trapezoidal rule.

Rates of glucose appearance (Ra_{TOT}), glucose disappearance (Rd), meal-derived glucose appearance (Ra_{Oral}), and endogenous glucose production (EGP) were derived from plasma [6,6-$^2H_2$]glucose and [U-^{13}C]glucose enrichments as previously described[17] using an approach based on Steele's equations[18,19] (Supplementary Methods). AUC values for rates of glucose appearance, Rd, Ra_{Oral}, and EGP were calculated for 0 to 120 minutes. AUC values for all parameters were also calculated for 0 to 30 minutes and 0 to 60 minutes to evaluate the early response to meal ingestion because previous work indicates that this is when many of the changes associated with GB occur.

Insulin clearance was calculated for both fasting and fed states by dividing fasting ISR by fasting insulin and the $AUC_{ISR(0,180min)}$ by the $AUC_{Insulin(0,180min)}$.[20,21] Beta-cell function during the

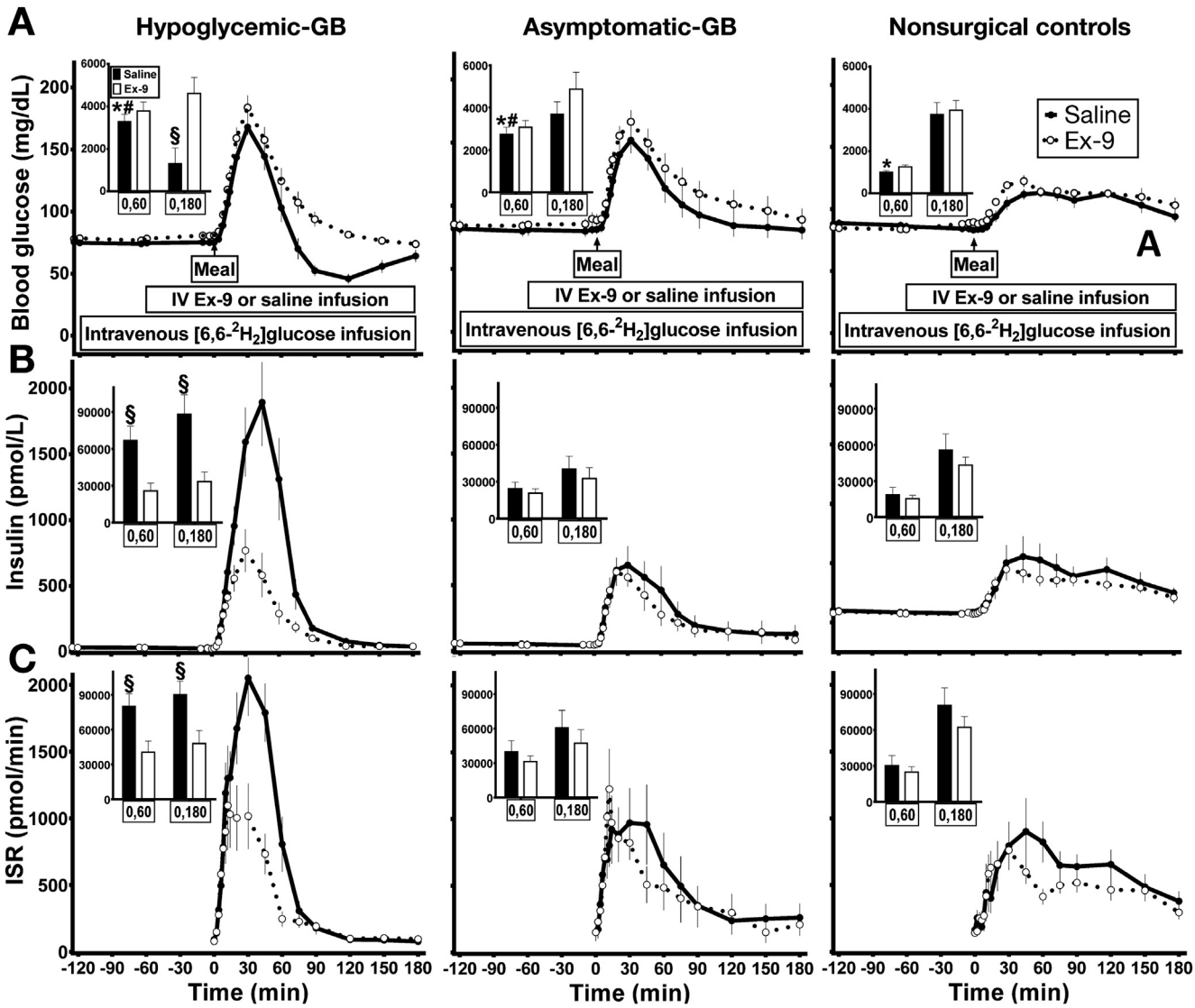

Figure 1. (*A*) Blood glucose, (*B*) plasma insulin, and (*C*) insulin secretion responses to meal ingestion in subjects who underwent GB, with (H-GB, *left panels*) and without (A-GB, *middle panels*) recurrent hypoglycemia, and nonsurgical controls (*right panels*) during studies with (*dashed line, white bar*) and without (*solid line, black bar*) infusion of Ex-9. The corresponding AUCs for 0 to 60 minutes and 0 to 180 minutes are shown (*insets*). $^{*}P < .05$ compared with Ex-9 study, $^{\#}P < .05$ compared with the CON group, $^{\S}P < .05$ for the interaction of group and treatment.

meal studies was also compared using a previously validated model[22,23] that expresses ISR as the sum of 2 components: (1) glucose sensitivity, the effect of glucose concentration over time on ISR as a dose-response function, and (2) rate sensitivity, the effect of the rate of change in glucose concentrations on ISR, which represents principally early insulin release. Beta-cell glucose sensitivity as the slope of ISR and blood glucose concentration was computed separately for the first part of the meal tolerance test (MTT), as glucose levels increased to peak values, and the latter part, as glucose levels declined toward fasting levels.

Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as fasting glucose (mg/dL) × fasting insulin (pmol/L)/8.66.[24] MTT-derived insulin sensitivity ($OGIS_{120min}$) was measured as previously described.[25,26] A semiquantitative symptom questionnaire (adapted with modification from Sigstad[12]; Supplementary Methods) was administered every 15 minutes starting from meal ingestion. Subjects scored 0 when they had none of the symptoms and 1 if they had any symptoms at each time point. The sum of these symptoms during MTT studies constituted the total symptom score. These values were also calculated for the periods from the start of meal ingestion to the peak glucose level for each study as the early symptom score.

Statistical Analysis

Data are presented as mean ± SEM. Baseline characteristics were compared using analysis of variance or χ^2 test. The parameters obtained from each subject in studies with and without Ex-9 were compared among the H-GB, A-GB, and CON groups using 2-way repeated-measures analysis of variance and post hoc comparisons when indicated. Associations between nadir glucose concentrations and postprandial insulin responses with other parameters were performed using

Spearman correlation. Statistical analyses were performed using SPSS 20 (SPSS Inc, Chicago, IL).

Results

Characteristics of Subjects

The subjects in the 3 groups had similar body mass index, waist circumference, fat and lean mass, and glycated hemoglobin A_{1c} values (Table 1). The H-GB and CON groups were almost all women, and the A-GB group included 4 men. The control subjects were slightly younger than surgical subjects, and the surgical subjects had comparable age and rates of diabetes before GB (Table 1). Total weight loss and time since GB were similar in the surgical groups, although subjects in the H-GB group had a trend towards greater weight regain from their postoperative nadir after surgery compared with subjects in the A-GB group (Table 1). More subjects in the H-GB group had a history of dumping symptoms.

Effects of GLP-1R Blockade on Glucose Fluxes Before and After the Meal

Subjects who underwent GB had lower fasting glucose levels compared with controls as well as a higher and earlier peak glucose level in response to meal ingestion (Figure 1 and Table 2). During the MTT, blood glucose levels decreased to <50 mg/dL in 8 of the subjects in the H-GB group; all became symptomatic within 60 to 120 minutes. In contrast, none of the subjects in the A-GB group had postprandial glucose levels <50 mg/dL or symptoms of hypoglycemia. Although the early glucose response to meal ingestion (glucose peak and $AUC_{Glucose(0,60min)}$) did not differ significantly between the 2 surgical groups, the average nadir glucose level was significantly lower in the H-GB group compared with the other groups, with no differences between the A-GB and CON groups (Table 2). Blockade of the GLP-1R with Ex-9 increased both fasting and postprandial glucose levels in all 3 groups (Figure 1 and Table 2). The postprandial glycemic effect of Ex-9 infusion was similar among the 3 groups for the first 60 minutes, with increases in the $AUC_{Glucose(0,60min)}$ of 18% ± 10% in the H-GB group, 14% ± 6% in the A-GB group, and 30% ± 14% in the CON group. However, over the entire course of the MTT, the H-GB group had a significantly larger glycemic response to Ex-9, with an increase in the $AUC_{Glucose(0,180min)}$ of 200% ± 72% compared with 37% ± 12% in the A-GB group and 14% ± 12% in the CON group ($P < .001$; Figure 1). None of the patients in the H-GB group had a blood glucose level <50 mg/dL or symptoms of hypoglycemia during administration of Ex-9. In all 3 groups, the time to reach peak glucose level became shorter and the time to reach nadir glucose level became longer as a result of GLP-1R blockade (Table 2).

The appearance of $U^{13}C$-labeled glucose in the circulation paralleled that of blood glucose concentrations after the test meal. However, the rates of early Ra_{Oral} were significantly greater in the H-GB group compared with the A-GB group and greater in the A-GB group compared with the CON group ($AUC_{RaOral(0,60min)}$:1548 ± 213, 1137 ± 161, and 544 ± 35 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in the H-GB, A-GB, and CON groups; $P < .01$; Figure 2). Blocking the GLP-1R did not affect early or overall Ra_{Oral} rates except for shortening the time to reach peak Ra_{Oral} values, suggesting faster nutrient transit during the Ex-9 studies. Basal levels of EGP were similar among the 3 groups, declined after the ingestion of glucose, and increased again within 30 minutes, with no significant differences among the groups (Figure 2). Infusion of Ex-9 showed a tendency to increase premeal EGP values ($P = .1$) but had no significant effect on $AUC_{EGP(0,120min)}$ in any groups. The early ($AUC_{Rd(0,60min)}$) and overall ($AUC_{Rd(0,120min)}$) rates of glucose disposal were significantly larger in subjects who underwent GB compared with the nonsurgical controls ($P < .05$). There was no difference in the rates of Rd between the saline and Ex-9 studies (Figure 2).

Table 1. Baseline Characteristics of Study Subjects

	H-GB group (n = 9)	A-GB group (n = 7)	CON group (n = 8)	*P* value
Age (*y*)	44.6 ± 4.5	47.6 ± 3.0	35.1 ± 3.3	.08
Body mass index (kg/m^2)	30.9 ± 2.5	33.8 ± 3.4	32.8 ± 1.1	.69
Sex (female/male)	9/0	3/4	7/1	.02
Diabetes mellitus (yes/no)	2/7	2/5	0/8	.70
Dumping symptoms (yes/no)	7/2	3/4	NA	.15
Waist circumference (*cm*)[a]	95.5 ± 4.8	105.0 ± 8.6	108.9 ± 2.7	.30
Total fat mass (*kg*)[a]	32.9 ± 5.8	34.6 ± 8.3	35.0 ± 1.6	.90
Total lean mass (*kg*)[a]	49.7 ± 2.5	60.9 ± 6.8	52.7 ± 2.1	.20
Glycated hemoglobin A_{1c} (%)	5.2 ± 0.2	5.2 ± 0.1	5.1 ± 0.1	.80
Time since surgery (*y*)	3.9 ± 0.5	3.6 ± 0.7		.72
Preoperative body mass index (kg/m^2)	48.0 ± 2.6	55.0 ± 2.6		.09
Total weight loss (*kg*)	45.4 ± 4.4	60.6 ± 10.0		.15
Weight regain (*kg*)	17.3 ± 6.0	−4.5 ± 10.0		.07

NOTE. Data are presented as mean ± SEM unless otherwise specified. Statistical *P* values for analysis of variance or χ^2 analysis are provided in the last column.
NA, not applicable.
[a]Measured in 7 subjects in each group.

Table 2. Effects of Meal Ingestion on Glucose and Beta Cell Response in the H-GB, A-GB, and CON Groups in Studies With and Without Intravenous Infusion of Ex-9

	H-GB group (n = 9)		A-GB group (n = 7)		CON group (n = 8)		Statistical tests (*P* values)		
	Saline	Ex-9	Saline	Ex-9	Saline	Ex-9	Ex-9 vs saline	Group status	Interaction
Fasting glucose (*mg/dL*)[a]	74.9 ± 2.4	80.5 ± 2.2	81.0 ± 3.8	88.8 ± 4.9	83.7 ± 3.1	88.6 ± 2.1	.00	.10	.62
Time to reach peak glucose (*min*)	33.3 ± 2.2	30.6 ± 2.1	39.3 ± 8.9	30.7 ± 2.8	62.5 ± 12.2	41.3 ± 3.7	.02	.02	.21
Time to reach nadir glucose (*min*)	98 ± 8	155 ± 14	141 ± 18	165 ± 15	154 ± 14	158 ± 12	.01	.12	.08
Nadir glucose (*mg/dL*)	42.3 ± 3.7	70.8 ± 4.1	77.4 ± 8.3	88.5 ± 8.9	86.6 ± 3.3	95.1 ± 3.6	.00	.00	.00
Peak glucose (*mg/dL*)	170.9 ± 7.4	185.0 ± 9.3	157.7 ± 9.1	169.9 ± 8.6	119.0 ± 4.3	128.4 ± 4.0	.00	.00	.87
$AUC_{Glucose(0,60min)}$ ($mg \cdot dL^{-1} \cdot min^{-1}$)	3289 ± 339	3785 ± 426	2747 ± 332	3075 ± 321	1004 ± 73	1255 ± 95	.00	.00	.64
$AUC_{Glucose(0,180min)}$ ($mg \cdot dL^{-1} \cdot min^{-1}$)	1317 ± 721	4615 ± 757	3693 ± 579	4879 ± 790	3739 ± 564	3940 ± 456	.00	.33	.00
Fasting insulin (*pmol/L*)[a]	27.2 ± 3.7	23.9 ± 3.5	47.1 ± 14.4	44.8 ± 11.6	77.1 ± 14.7	78.0 ± 16.1	.83	.00	.97
$AUC_{Insulin(0,30min)}$ ($pmol \cdot L^{-1} \cdot min^{-1}$)	18.6 ± 3.4	10.7 ± 2.1	10.2 ± 1.9	10.1 ± 1.6	4.9 ± 1.9	4.7 ± 0.9	.02	.01	.01
$AUC_{Insulin(0,60min)}$ ($pmol \cdot L^{-1} \cdot min^{-1}$)	67.0 ± 11.6	25.8 ± 6.3	24.3 ± 5.2	20.7 ± 3.6	18.6 ± 6.1	15.3 ± 2.7	.00	.01	.00
$AUC_{Insulin(0,180min)}$ ($pmol \cdot L^{-1} \cdot min^{-1}$)	88.3 ± 15.6	33.3 ± 8.0	40.2 ± 10.5	32.5 ± 8.9	55.7 ± 13.5	43.3 ± 6.5	.00	.25	.01
Fasting ISR (*pmol/min*)[a]	91.8 ± 13.3	80.0 ± 9.1	150.7 ± 65.4	151.8 ± 54.7	166.0 ± 24.5	141.5 ± 10.8	.19	.24	.51
$AUC_{ISR(0,30min)}$ (*pmol*)	34.6 ± 5.6	22.5 ± 5.7	17.9 ± 4.0	18.4 ± 2.6	9.5 ± 3.2	11.3 ± 2.5	.06	.02	.00
$AUC_{ISR(0,60min)}$ (*pmol*)	80.1 ± 10.9	40.5 ± 9.7	39.9 ± 9.6	31.3 ± 5.0	30.1 ± 8.6	24.9 ± 4.3	.00	.02	.00
$AUC_{ISR(0,180min)}$ (*pmol*)	90.3 ± 11.4	48.0 ± 11.3	60.8 ± 15.0	47.2 ± 11.9	80.4 ± 14.8	62.2 ± 9.1	.00	.55	.05
Beta cell rate sensitivity ($pmol \cdot m^{-2} \cdot mM^{-1}$)	1692 ± 605	1656 ± 522	1615 ± 555	1633 ± 465	3381 ± 1020	3122 ± 666	.80	.10	.96
Beta cell glucose sensitivity ($pmol \cdot min^{-1} \cdot m^{-2} \cdot mM^{-1}$)	367 ± 43	155 ± 34	186 ± 30	160 ± 28	394 ± 95	279 ± 64	.00	.10	.07
Fasting insulin clearance	2.9 ± 0.2	3.0 ± 0.4	2.7 ± 0.5	2.8 ± 0.3	1.9 ± 0.2	1.9 ± 0.2	.70	.03	.96
Postprandial insulin clearance	1.4 ± 0.3	1.4 ± 0.3	1.6 ± 0.2	1.8 ± 0.3	1.6 ± 0.1	1.7 ± 0.1	.89	.81	.88
HOMA-IR	0.7 ± 0.1	0.7 ± 0.1	1.4 ± 0.5	1.5 ± 0.5	2.3 ± 0.5	2.5 ± 0.6	.83	.00	.95
$OGIS_{120min}$ ($mL \cdot min^{-1} \cdot m^{-2}$)	537 ± 17	486 ± 16	472 ± 30	439 ± 29	406 ± 19	393 ± 12	.00	.00	.14

NOTE. Data are presented as mean ± SEM unless otherwise specified. Statistical effects (treatment [saline/Ex-9], group status [H-GB/A-GB/CON], and their interaction) are provided in the last 3 columns.
[a]Fasting values from 110 to 120 minutes of study (immediately before meal ingestion).

Contribution of GLP-1 to Beta Cell Function, Insulin Sensitivity, and Insulin Clearance

Fasting insulin levels were lower in patients who underwent GB compared with controls, which is consistent with their greater insulin sensitivity and fasting insulin clearance (Table 2). The H-GB group had an earlier and more robust beta cell response after the test meal, although the overall AUC_{ISR} for the 3-hour postmeal period did not differ significantly among the groups (Figure 1 and Table 2). GLP-1R blockade diminished both early and total insulin secretion in all groups, with the largest effect seen in the H-GB group and no difference between the A-GB and CON groups (relative reduction in $AUC_{ISR(0,180min)}$: 50% ± 8% in the H-GB group, 13% ± 10% in the A-GB group, and 14% ± 10% in the CON group; $P < .05$; Table 2).

Beta cell glucose sensitivity during the first part of the MTT tended to be higher in the H-GB and CON groups compared with the A-GB group (Figure 3 and Table 2), and there was a trend toward lower beta cell rate sensitivity in the GB group compared with the CON group (Table 2). Infusion of Ex-9 lowered beta cell glucose sensitivity in all 3 groups, with the maximum effect observed in the H-GB group (relative reduction in glucose sensitivity: 60% ± 8% in the H-GB group, 15% ± 44% in the A-GB group, and 20% ± 18% in the CON group; $P = .07$) (Figure 3 and Table 2) but had no effect on beta cell rate sensitivity. The H-GB group also had higher rates of glucose-stimulated insulin

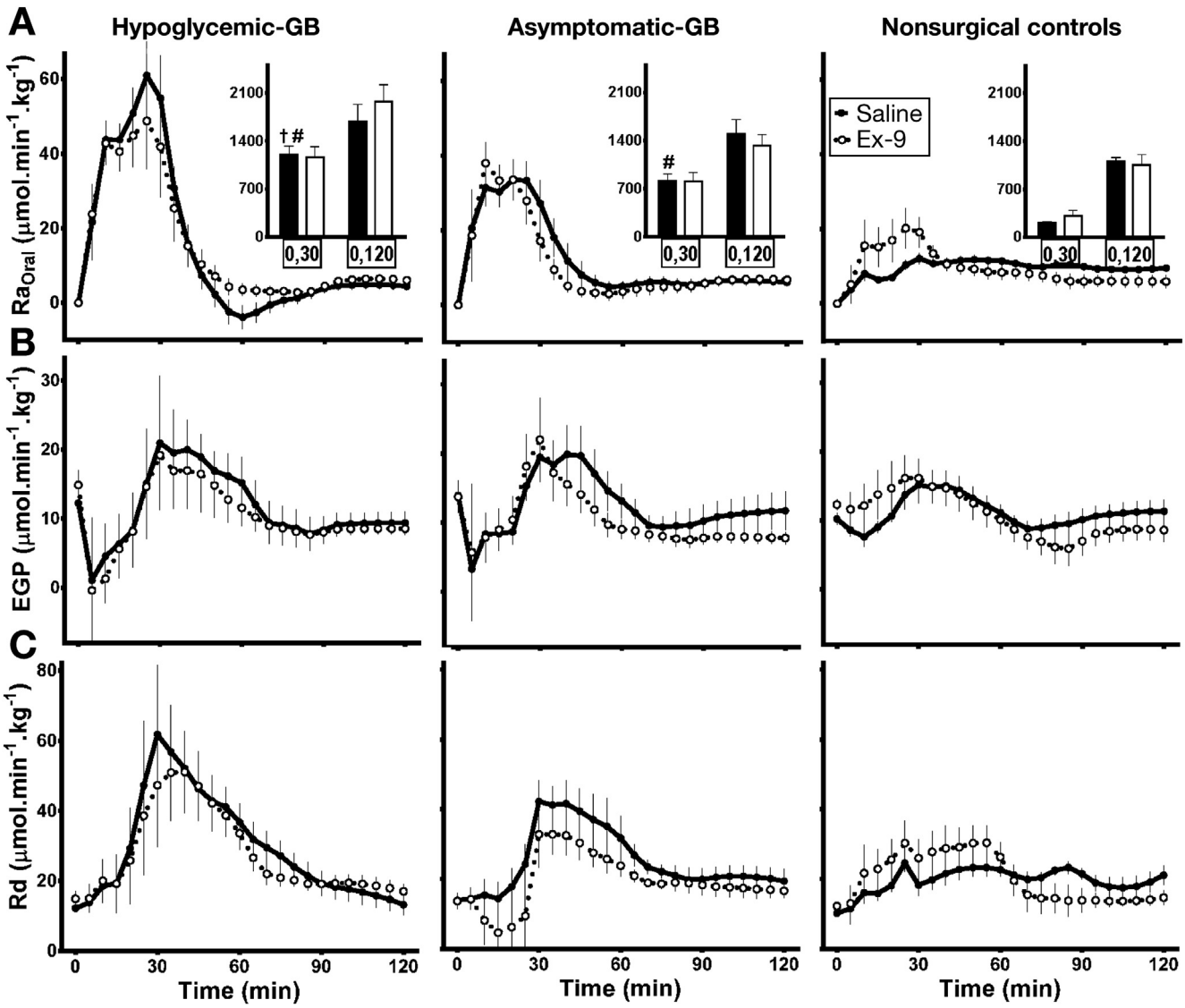

Figure 2. The rates of (*A*) Ra_{Oral} (A), (*B*) EGP, and (*C*) Rd in subjects who underwent GB, with (H-GB, *left panels*) and without (A-GB, *middle panels*) recurrent hypoglycemia, and nonsurgical controls (*right panel*) during studies with (*dashed lines*) and without (*solid lines*) infusion of Ex-9. AUC_{RaOral} for 0 to 30 minutes and 0 to 120 minutes are shown (*insets*). $^{\#}P < .05$ compared with the CON group, $^{\dagger}P < .05$ compared with the A-GB group.

secretion as blood glucose levels declined in the latter part of the MTT compared with the A-GB and CON groups, but blockade of GLP-1R signaling reduced the insulin-glucose dose response in the latter part of the MTT in all groups. C-peptide levels were modestly higher in the H-GB group as glucose levels increased after meal consumption but were dramatically higher as glucose levels decreased in the latter part of the test. Blockade of GLP-1R signaling almost completely eliminated this disparity in C-peptide levels (Figure 3).

Fasting insulin sensitivity, computed as 1/HOMA-IR, and total glucose clearance during the MTT ($OGIS_{120min}$) were significantly greater in the H-GB and A-GB groups compared with the CON group. Infusion of Ex-9 reduced $OGIS_{120min}$ values in all 3 groups but had no effect on 1/HOMA-IR (Table 2). Although fasting insulin clearance was greater in surgical subjects compared with controls, insulin clearance after eating was not different among the groups and was not significantly affected by Ex-9 in any group (Table 2).

GI Hormone and Alpha Cell Responses After Meal Ingestion and Postprandial Symptoms With and Without GLP-1R Blockade

Plasma concentrations of glucagon, GLP-1, and GIP before and after the test meal are shown in Figure 4 and Table 3. Fasting plasma glucagon levels were similar among the 3 groups; however, postprandial glucagon levels followed significantly different courses in the GB and CON groups. Meal ingestion suppressed glucagon slightly in the CON group but increased both early and overall glucagon responses in the H-GB and A-GB groups. Infusion of Ex-9

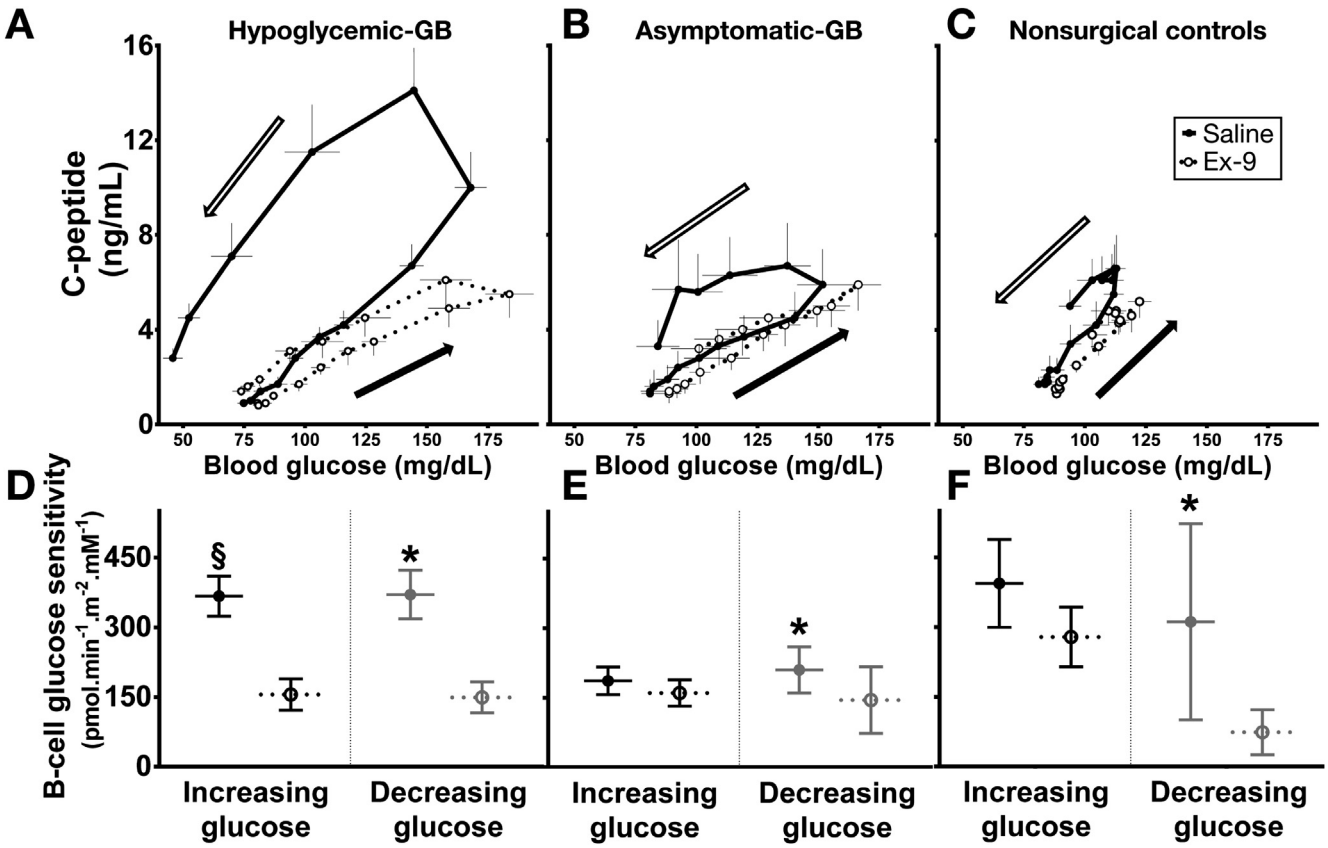

Figure 3. Circulatory C-peptide levels across blood glucose values and beta cell glucose sensitivity in subjects who underwent GB with (*A* and *D*) and without (*B* and *E*) hypoglycemia syndrome and nonsurgical controls (*C* and *F*) during MTT studies with (*open circles, dashed line*) and without (*closed circles, solid line*) infusion of Ex-9 (0–120 minutes for subjects who underwent GB and 0–180 minutes for healthy controls). *Black arrows* show the initial phase of the MTT (increasing glucose level), and the *white arrows* show the latter phase of the MTT (decreasing glucose level). *$P < .05$ compared with the Ex-9 study, §$P = .07$ for the interaction of group and treatment.

had no influence on fasting glucagon levels, but postprandial glucagon levels were increased in all 3 groups ($P < .01$).

Postprandial plasma GLP-1 levels were substantially higher in the surgical subjects compared with controls, with a trend toward larger responses in the H-GB group compared with the A-GB group. Blocking the GLP-1R increased premeal levels of GLP-1 in the A-GB and H-GB groups and postmeal levels in the A-GB and CON groups with no effect in the H-GB group ($P < .05$ for interaction between treatment and groups). Premeal GIP levels were similar in the 3 groups and meal ingestion increased the early response of GIP to meal ingestion in the A-GB and H-GB groups compared with the CON group, although the overall postprandial GIP levels were similar in the 3 groups. GLP-1R blockade caused small but significant increases in the early and overall GIP responses to meal ingestion in the 3 groups.

Consistent with a higher frequency of dumping symptoms in the past, the H-GB group had higher scores for both GI and non-GI symptoms compared with the A-GB group during the MTT studies (GI and non-GI symptoms: 10 ± 0 and 17 ± 5 in the H-GB group vs 3.9 ± 3 and 3 ± 0 in the A-GB group; $P < .05$), with the most pronounced differences occurring in the early part of the test meal. Of note, GLP-1R blockade diminished the early non-GI symptoms during MTT in the H-GB group ($P < .05$ for the interaction of group and treatment).

Association of Nadir Glucose Level With Hormonal Responses

Among the surgical subjects, nadir glucose concentrations during the control MTT studies were inversely correlated with the early GLP-1 response to meal ingestion ($AUC_{GLP\text{-}1(0,60min)}$; $r = -0.554$, $P = .032$) and Ra_{Oral} values ($r = -0.524$, $P = .037$), while there were no significant relationships between Ra_{Oral} and the GLP-1 and GIP responses to the meal. The nadir glucose level was also inversely correlated with the early insulin response ($AUC_{ISR(0,60min)}$: $r = -0.48$, $P = .06$) and beta cell glucose sensitivity ($r = -0.60$, $P = .014$), but there was no association with the overall insulin response ($AUC_{ISR(0,180min)}$) or beta cell rate sensitivity among the surgical patients. The magnitude of the GLP-1 effect on ISR (the difference in ISR with and without Ex-9) also correlated inversely with nadir glucose values ($r = 0.60$, $P = .013$), but there was no

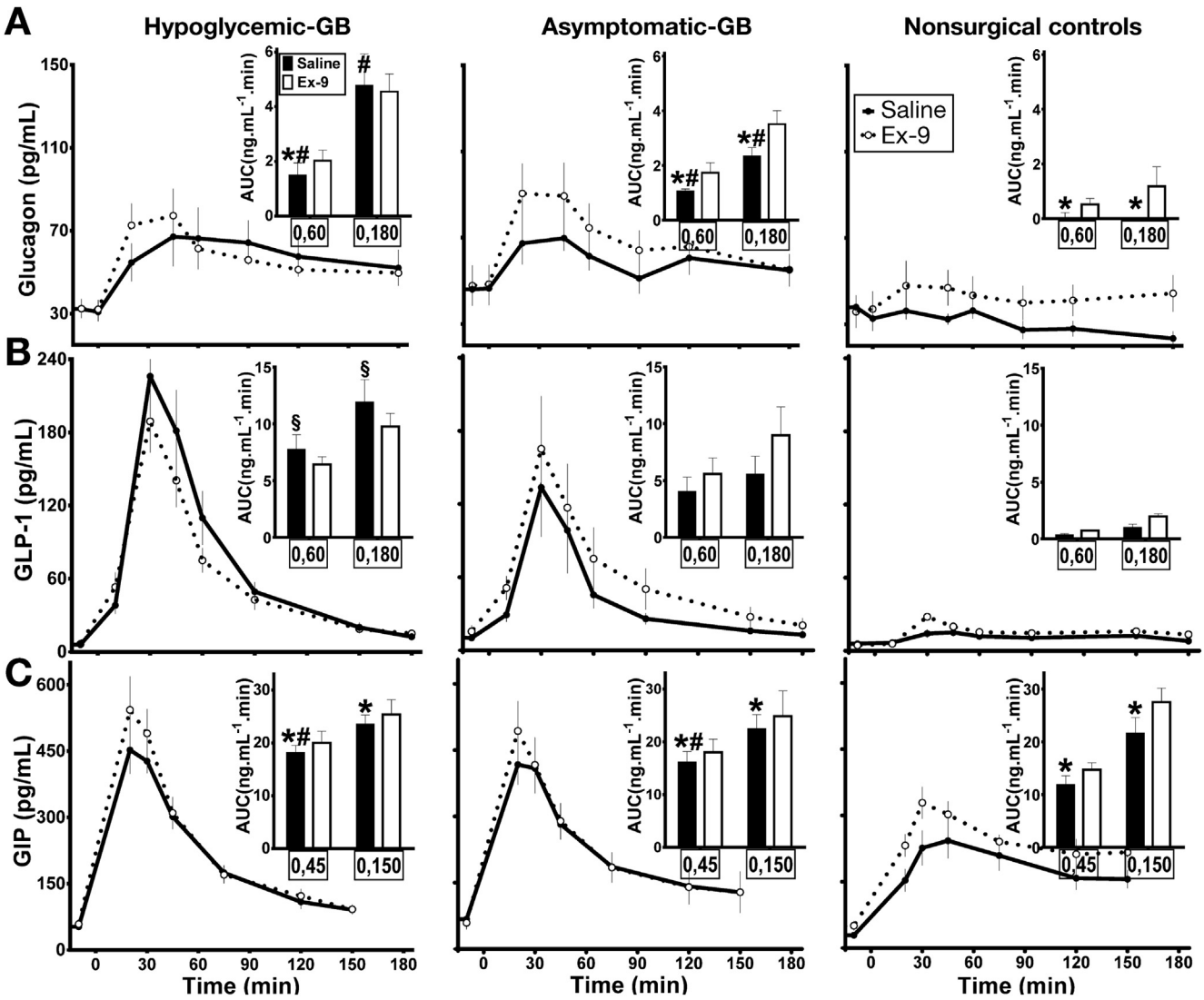

Figure 4. (*A*) Plasma glucagon, (*B*) GLP-1, and (*C*) GIP responses to meal ingestion in subjects who underwent GB, with (H-GB, *left panels*) and without (A-GB, *middle panels*) recurrent hypoglycemia, and nonsurgical controls (*right panels*) during studies with (*dashed lines, white bars*) and without (*solid lines, black bars*) infusion of Ex-9. Corresponding AUCs are shown (*insets*). *$P < .05$ compared with the Ex-9 study, #$P < .05$ compared with nonsurgical controls, §$P < .05$ for the interaction of group and treatment.

correlation between the size of the GLP-1 effect and circulating levels of GLP-1 or GIP during the saline studies.

Discussion

The findings reported here show that postprandial hypoglycemia in subjects with H-GB can be corrected with GLP-1R blockade. Moreover, the disproportionate improvement in the glycemic response when Ex-9 is given to these subjects supports a pathogenic role for exaggerated GLP-1 action in the hyperinsulinemic hypoglycemic syndrome associated with GB. GLP-1 contributes to the significant increase in postprandial insulin secretion in subjects with H-GB, and administration of Ex-9 reduces the abnormally high rate of insulin secretion experienced by these subjects during the latter phase of meal absorption. Beyond these important differences in beta cell function, subjects with H-GB have an enhanced meal-derived glucose appearance, raising the possibility that alterations in GI function contribute to alterations in postprandial glycemia, and some of these may also be mediated by GLP-1. Overall, these results support the development of treatment strategies using GLP-1R blockade for patients with postsurgical hypoglycemia.

The present study is an extension of a previous assessment of GLP-1–stimulated insulin secretion in subjects with and without symptomatic hypoglycemia who had undergone GB.[6] This earlier study focused on beta cell function assessed during clamped plasma glucose levels and thus could not determine the effects of GLP-1R blockade on glycemia. In this study, we infused Ex-9 during an MTT to replicate the mealtime setting in which some subjects who

Table 3. Effects of Meal Ingestion on GI Hormone and Alpha Cell Response in the H-GB, A-GB, and CON Groups in Studies With and Without Intravenous Infusion of Ex-9

	H-GB group (n = 9)		A-GB group (n = 7)		CON group (n = 8)		Statistical tests (*P* values)		
	Saline	Ex-9	Saline	Ex-9	Saline	Ex-9	Ex-9 vs saline	Group status	Interaction
$AUC_{GIP(0,30)}$ $(ng \cdot mL^{-1} \cdot min^{-1})$	7.9 ± 0.8	9.4 ± 1.2	6.9 ± 0.9	8.3 ± 1.1	2.9 ± 0.5	4.1 ± 0.7	.01	.00	.96
$AUC_{GIP(0,45)}$ $(ng \cdot mL^{-1} \cdot min^{-1})$	12.6 ± 1.0	14.6 ± 1.7	11.1 ± 1.5	12.7 ± 1.7	5.9 ± 1.0	8.1 ± 1.0	.02	.00	.95
$AUC_{GIP(0,150)}$ $(ng \cdot mL^{-1} \cdot min^{-1})$	23.5 ± 1.8	25.4 ± 2.8	22.5 ± 2.7	24.9 ± 4.8	21.6 ± 3.0	27.6 ± 2.6	.04	.97	.54
$AUC_{GLP\text{-}1(0,30)}$ $(ng \cdot mL^{-1} \cdot min^{-1})$	2.7 ± 0.4	2.5 ± 0.3	1.5 ± 0.4	2.2 ± 0.5	0.1 ± 0.0	0.3 ± 0.0	.11	.00	.07
$AUC_{GLP\text{-}1(0,60)}$ $(ng \cdot mL^{-1} \cdot min^{-1})$	7.7 ± 1.3	6.4 ± 0.7	4.0 ± 1.3	5.6 ± 1.4	0.3 ± 0.1	0.7 ± 0.1	.77	.00	.05
$AUC_{GLP\text{-}1(0,180)}$ $(ng \cdot mL^{-1} \cdot min^{-1})$	11.9 ± 2.0	9.8 ± 1.2	5.6 ± 1.6	9.0 ± 2.5	1.0 ± 0.3	2.0 ± 0.2	.43	.00	.05
$AUC_{Glucagon(0,20)}$ $(ng \cdot mL^{-1} \cdot min^{-1})$	0.2 ± 0.1	0.4 ± 0.1	0.2 ± 0.0	0.4 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	.00	.01	.37
$AUC_{Glucagon(0,60)}$ $(ng \cdot mL^{-1} \cdot min^{-1})$	1.5 ± 0.5	2.0 ± 0.4	1.1 ± 0.0	1.7 ± 0.4	−0.1 ± 0.2	0.5 ± 0.2	.00	.01	.56
$AUC_{Glucagon(0,180)}$ $(ng \cdot mL^{-1} \cdot min^{-1})$	4.8 ± 1.2	4.6 ± 0.6	2.3 ± 0.3	3.5 ± 0.5	−0.9 ± 0.7	1.2 ± 0.7	.03	.00	.12

NOTE. Data are presented as mean ± SEM unless otherwise noted. Statistical effects (treatment [saline/Ex-9], group status [H-GB/A-GB/CON], and their interaction) are provided in the last 3 columns.

have undergone GB experience hypoglycemia and used changes in blood glucose levels as the primary outcome. The selection of subjects for this study was highly focused to recruit patients who could be characterized unequivocally as either H-GB or A-GB; we enrolled only patients with clearly documented prior postprandial hypoglycemia or those who denied any previous symptoms. Based on the glucose response to the MTT, this allocation to the 2 groups was successful and, while only representative of extremes in glucose regulation among subjects who have undergone GB, was informative about the role of GLP-1 in GB-related hypoglycemia.

We measured rates of meal glucose appearance into the circulation to evaluate the effects of GLP-1 on GI function and the potential role on plasma glucose levels. The GB group had clearly faster Ra_{Oral} compared with the CON group, as previously described,[1,27–30] but without a major effect of GLP-1R blockade. Moreover, the rate of meal glucose appearance was significantly faster in the H-GB group compared with the A-GB group, with and without Ex-9. Although our study was not designed to address the mechanisms involved in gastric emptying, passage through the intestine, or nutrient digestion, it is plausible that differences in gastrojejunostomy size, pressure gradients across this area, or intestinal glucose absorption could explain the differences in Ra_{Oral} in the H-GB and A-GB groups. The higher symptom scores in the H-GB group during the MTT is compatible with differences in GI function among the groups, and some of these improved with GLP-1R blockade. Given the greater Ra_{Oral} in the H-GB group, it seems likely that these patients had higher intraportal glucose concentrations during the MTT. Variation in portal glucose concentrations contributes to hepatic and extrahepatic glucose uptake[31] and could conceivably have a role in the differences in postprandial glucose regulation described here. These results support more in-depth study of the role of GI function in the hyperinsulinemic hypoglycemia syndrome associated with GB.

Infusion of Ex-9 unmasked a significant difference in the effect of GLP-1 to promote insulin secretion in the H-GB group relative to the other groups, both of which had comparable responses. These results differ from our previous results[6] that showed comparable effects of GLP-1 to enhance insulin secretion in subjects who underwent GB with and without prior hypoglycemia. The apparent discrepancy in our 2 studies may be partly explained by differences in the characteristics of the subjects participating in these studies, with the groups reported herein more stringently selected for the extremes in prandial glucose regulation. In addition, our previous study measured GLP-1 action on beta cell function at stable hyperglycemia fixed by a glucose clamp, whereas in the current study glucose levels followed the usual variable course of meal absorption. In the present study, the beta cell sensitivity to the prandial increase in glucose levels did not differ among the H-GB and CON groups, although this parameter was more dependent on GLP-1 in the H-GB group than in the other groups. However, it is clear that the H-GB group had increased peak insulin secretion relative to peak glycemia and disproportionately high insulin secretion in the latter phases of

the meal when glucose levels were decreasing. Based on the effects of Ex-9, both of these beta cell responses were highly dependent on GLP-1. Although there was a trend toward higher postprandial plasma GLP-1 levels in the H-GB group compared with the A-GB group, there was no relationship between plasma levels of GLP-1 and the size of the GLP-1 effect on ISR. One possibility is that greater beta cell sensitivity to GLP-1 explains the propensity of some subjects to develop the postprandial hypoglycemia syndrome after GB. Another possibility is that subjects with H-GB are more susceptible to extra-islet actions of GLP-1, most likely on the nervous system, to cause abnormal glucose control.

Our secondary analyses implicate a greater effect of GLP-1 on ISR in the hypoglycemic response to meals. Other significant predictors of nadir glucose include the rapidity, but not the magnitude, of postprandial GLP-1 release, beta cell sensitivity to glucose, a parameter responsive to Ex-9, and Ra_{Oral}. Taken together, these findings support a model in which accelerated absorption of ingested glucose triggers an unusually large insulin response, mediated in great part by GLP-1. In this construct, increased rates of glucose flux into the intestine would promote both early GLP-1 secretion and more rapid glucose appearance. This combination, which would be present both in the systemic circulation and in the portal vein, seems to have potent effects on beta cell function that can contribute to hypoglycemia.

We have confirmed the distinct profile of postprandial glucagon concentrations reported previously in subjects who underwent GB.[1,3,6,32–34] Similar to those reports, the H-GB and A-GB groups both had much higher postprandial levels of glucagon than the CON group. Also consistent with previous studies,[6,35] there was a significant increase in glucagon levels when Ex-9 was administered, indicating that while alpha cell function differs between subjects who have undergone GB and control subjects, it is regulated by GLP-1 in both groups. However, what is not clear is why plasma glucagon levels did not increase substantially in the subjects with H-GB during the saline studies when they have significant hypoglycemia. This finding suggests that alpha cell and beta cell function is altered in subjects with the post-GB hypoglycemic syndrome.

There are several limitations to this study that warrant mention. The number of subjects in each of the groups was relatively small and highly selected, limiting applicability to the broad range of subjects who have undergone GB. Also, half of the subjects with A-GB were male. We are not aware of sex differences in glycemic regulation among subjects who have undergone GB, although this topic has not been directly studied; in the limited substudy comparison, the effect of Ex-9 on glucose or ISR response to meal ingestion was not affected by sex (Supplementary Figure 1). The A-GB group was slightly but more insulin resistant than the H-GB group. However, the lower insulin sensitivity in the subjects with A-GB should have contributed to larger meal-induced insulin secretion and is thus not likely to have biased our results. However, it is possible that, in the subjects with H-GB, increased insulin secretion in combination with greater insulin sensitivity contributed to postprandial hypoglycemia. We are aware that recent studies advocate using a triple-tracer protocol to measure glucose kinetics during meal tests.[36] We do not have access to this methodological advance and thus have been careful not to overinterpret the glucose turnover results reported here. However, we believe that the double-tracer method is adequate for measurement of Ra_{Oral}, which is the measure of flux we have emphasized.

In summary, we have shown that blocking the GLP-1R eliminates postprandial hypoglycemia in subjects who have undergone GB and are affected by the postprandial hypoglycemia syndrome. Our findings support enhanced beta cell sensitivity to GLP-1 in the hyperinsulinemia associated with symptomatic nadirs in postprandial glucose. The distinct pattern of ingested glucose appearance among subjects with H-GB suggests that altered GI function also contributes to glucose abnormalities in this syndrome.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2013.11.044.

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Conflicts of interest
The authors disclose no conflicts.

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Supplementary Methods

Glucose fluxes were determined using methods described previously[1] based on measures of tracer enrichments and plasma glucose concentrations. Total glucose rate of appearance (Ra_{TOT}) was calculated from the tracer-tracee ratio ($TTR_{6,6}$) using Steele's equation,

$$Ra_{TOT}(t) = \frac{IR - C(t) \times V \times \left(dTTR_{6,6}(t)/dt\right)}{TTR_{6,6}(t)},$$

where *IR* is the [6,6-$^2H_2$]glucose infusion rate, *C* is the plasma glucose concentration, and *V* is the volume of distribution (162.5 mL/kg). Plasma $TTR_{6,6}$ data were smoothed using a spline fitting approach to stabilize the calculation of the derivative of enrichment before applying Steele's equation. The plasma glucose concentration resulting from the absorption of ingested glucose (exogenous glucose concentration) was calculated from the ratio of plasma [U-^{13}C] glucose concentration to the [U-^{13}C]glucose enrichment of the ingested glucose. The plasma glucose concentration resulting from endogenous glucose output (endogenous glucose concentration, C_{end}) was computed as the difference between the total and exogenous glucose concentrations. TTR_{end} of endogenous glucose and EGP were calculated as follows:

$$TTR_{end}(t) = TTR_{6,6}(t) \times C(t)/C_{end}(t)$$

$$EGP(t) = \frac{IR - C_{end}(t) \times V \times (dTTR_{end}(t)/dt)}{TTR_{end}(t)}.$$

Ra_{Oral} was measured as the difference between Ra_{TOT} and EGP. Rd as a measure of insulin-mediated total body glucose disposal was calculated as

$$Rd(t) = Ra_{TOT}(t) - (dC(t)/dt) \times V.$$

For validation, the tracer/glucose data were also analyzed with a 2-pool model and comparable results were obtained; only the data for the primary analysis are presented.

Methodology

A semiquantitative symptom questionnaire (adapted with modification from Sigstad[2]) was administered every 15 minutes starting from meal ingestion. Non-GI symptoms were breathlessness, weakness, sleepiness, palpitation, restlessness, dizziness, headache, feeling warmth or sweating, lightheadedness, and syncope, and GI symptoms were nausea, abdominal fullness, growling sound, belching, and vomiting.

Supplementary References

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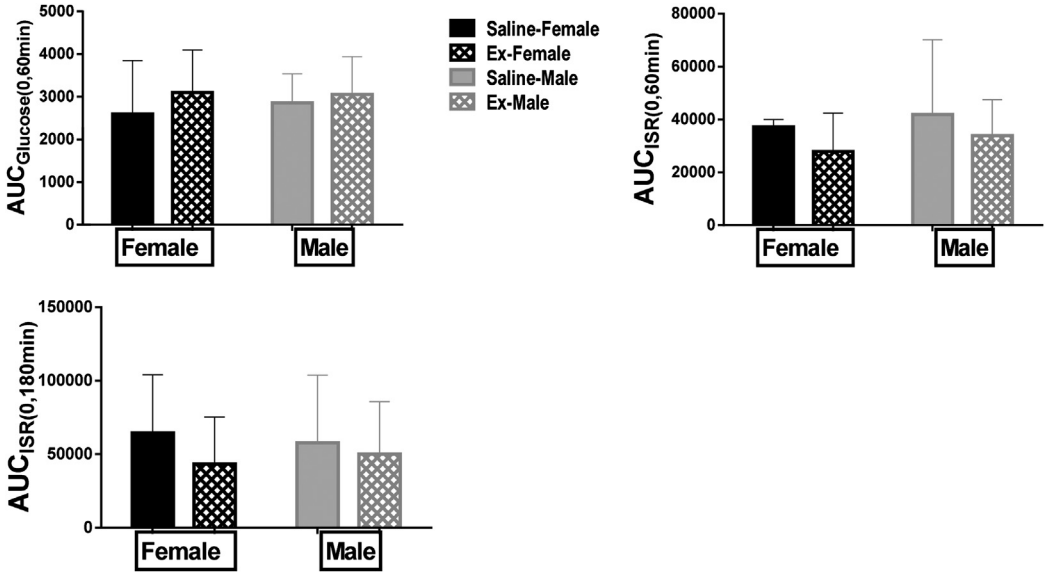

Supplementary Figure 1. The effect of Ex-9 on glucose and ISR response to meal ingestion is not affected by sex in the A-GB group. Data are presented as mean ± SD.

Supplementary Table 1. Effect of Meal Ingestion on CP Levels in the H-GB, A-GB, and CON Groups in Studies With and Without Intravenous Infusion of Ex-9

	H-GB group (n = 9)		A-GB group (n = 7)		CON group (n = 8)		Statistical tests (*P* values)		
	Saline	Ex-9	Saline	Ex-9	Saline	Ex-9	Ex-9 vs saline	Group status	Interaction
Fasting CP (*ng/mL*)[a]	0.9 ± 0.1	0.9 ± 0.1	1.4 ± 0.5	1.4 ± 0.4	1.7 ± 0.2	1.4 ± 0.1	.18	.34	.19
$AUC_{CP(0,60min)}$ ($ng \cdot mL^{-1} \cdot min$)	461 ± 66	218 ± 41	217 ± 60	194 ± 35	145 ± 41	139 ± 24	.00	.00	.01
$AUC_{CP(0,180min)}$ ($ng \cdot mL^{-1} \cdot min$)	807 ± 123	395 ± 70	537 ± 127	417 ± 89	678 ± 119	516 ± 68	.00	.03	.60

NOTE. Data are presented as mean ± SEM.
CP, C-peptide.
[a]Fasting values from 110 to 120 minutes of study (immediately before meal ingestion). Statistical effects (treatment [saline/Ex-9], group status [H-GB/A-GB/CON], and their interaction) are provided in the last 3 columns.