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

Marzieh Salehi¹, Amalia Gastaldelli², and David A. D'Alessio^{1,3}

¹ University of Cincinnati, Department of Medicine, Division of Endocrinology, Cincinnati, OH

² Cardiometabolic Risk Unit, CNR Institute of Clinical Physiology, Pisa, Italy

³ Cincinnati VA Medical Center, Cincinnati, OH

Abstract

Background & Aims—Post-prandial glycemia excursions increase after gastric bypass surgery; this effect is even greater among individuals with recurrent hypoglycemia (blood glucose levels <50 mg/dL). These patients also have increased post-prandial levels of insulin and glucagon-like peptide 1 (GLP1). We performed a clinical trial to determine the role of GLP1 in post-prandial glycemia in patients with hyperinsulinemic hypoglycemia syndrome after gastric bypass.

Methods—Nine patients with recurrent hypoglycemia after gastric bypass (H-GB), 7 asymptomatic individuals with previous gastric bypass (A-GB), and 8 non-diabetic subjects who did not receive surgery (controls) were studied with a mixed-meal tolerance test (350 kcal) using a dual glucose tracer method on 2 days. On 1 day they received continuous infusion of GLP-1 receptor (GLP1R) antagonist, exendin-(9–39) (Ex-9), and on the other day, a saline control. Glucose kinetics and islet and gut hormone responses were measured before and after the meal.

Results—Infusion of Ex9 corrected hypoglycemia in all H-GB individuals. The reduction of post-prandial insulin secretion by Ex9 was greater in the H-GB group than other groups (H-GB, 50%±8%; A-GB, 13%±10%; and controls, 14%±10%) ($P<.05$). Meal-derived glucose (RaOral) was significantly greater among subjects who had undergone gastric bypass than controls, and in H-GB patients compared with A-GB subjects. Ex9 shortened the time to peak RaOral in all groups without any significant effect on the overall glucose flux. Post-prandial glucagon levels were higher among patients who had undergone gastric bypass than controls, and increased with Ex9 administration.

Conclusions—Hypoglycemia following gastric bypass can be corrected by administration of a GLP1R antagonist, which might be used to treat this disorder. These findings are consistent with reports that increased GLP1 activity contributes to hypoglycemia following gastric bypass.

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Corresponding author and the reprint requests should be addressed to: Marzieh Salehi, MD, MS University of Cincinnati College of Medicine Department of Internal Medicine Division of Endocrinology, Diabetes, & Metabolism 260 Stetson, Suite 4200 Cincinnati, OH 45219-0547 Phone: (513)558-0001 Fax: (513)558-8581 salehim@uc.edu.

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Keywords

Roux-en-Y gastric bypass surgery; hyperinsulinemic hypoglycemia syndrome; Glucagon-like peptide 1; islet function

Introduction

Roux-en-Y gastric bypass surgery (GB), now widely used as a treatment for obesity, alters glucose fluxes and metabolism^{1, 2}. GB leads to an earlier and higher peak of glucose as well as lower nadir glucose levels after food intake, and insulin and glucagon-like peptide 1 (GLP-1) secretion that is accentuated and occurs earlier in the postprandial period³. This pattern is due in part to more rapid transit of nutrients from the small gastric remnant into the small intestine, resulting in large fluxes of splanchnic glucose¹. 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. In fact, blockade of the GLP-1 receptor (GLP-1r) has a disproportionately greater effect on meal-induced insulin release in GB subjects⁶.

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 operation^{7, 8}. Affected patients have larger insulin and GLP-1 responses to meal ingestion compared to GB subjects without symptomatic hypoglycemia⁹. Examination of surgical specimens from patients with the hypoglycemia syndrome treated with partial pancreatectomy suggested islet cell hypertrophy⁸ although this has been disputed¹⁰. Despite the potential association of elevated GLP-1 with the post-GB hypoglycemia syndrome there is not yet conclusive evidence that these are directly linked. In a previous study with the GLP-1r antagonist exendin-[9–39] (Ex-9), we noted a trend towards a larger contribution of endogenous GLP-1 to postprandial insulin response in a group of GB subjects with postprandial hypoglycemia compared to an asymptomatic GB group⁶. However, in this study, focused on the effects of GLP-1-stimulated insulin secretion, blood glucose was clamped and 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 GB subjects with and without symptomatic hypoglycemia, and a group of non-surgical controls. We hypothesized that GLP-1 action has a greater effect on blood glucose in GB subjects with hypoglycemia compared to asymptomatic individuals.

Methods

Subjects

Nine patients with recurrent hypoglycemia following GB (Hypoglycemic- GB, H-GB), 7 GB subjects with no prior history of hypoglycemic symptoms (Asymptomatic-GB, A-GB), and 8 healthy control subjects (CON) with normal glucose tolerance and no previous history of gastrointestinal (GI) surgery were recruited. The H-GB subjects 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's triad)¹¹. A-GB subjects denied hypoglycemic symptoms and had no documented episodes of low blood glucose. Seven H-GB and 3 A-GB had symptoms consistent with the dumping syndrome (nausea, diarrhea, weakness, sleepiness, palpitation, dizziness, headaches, feeling warmth, and

abdominal fullness¹²). 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 subjects had gastrointestinal obstruction, renal dysfunction, or liver disorders and none were taking any medications that interfere with glucose metabolism for at least one week prior to the studies. Two Subjects from the H-GB and 2 from 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 exendin-(9–39) (Ex-9) (C S Bio Co, Menlo Park, CA) was greater than 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 the U.S. Food and Drug Administration IND 65,837.

Experimental protocols

Subjects were instructed to maintain normal carbohydrate ingestion and not to 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 two separate days separated by 1–2 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 Ex9 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 [6,6-²H₂]glucose (22 μmol/kg prime and 0.22 μmol.kg⁻¹.min⁻¹ constant) was initiated and continued for the duration of the study¹³. At –60 minutes, subjects received either: a) a primed-continuous infusion of Ex-9 (7500 pmol/kg prime and 750 pmol.kg⁻¹.min⁻¹ constant) for the remainder of the study, or b) 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 got the saline treatment 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 gram of universally labeled glucose ([U-¹³C]glucose) was consumed within 10 minutes. Blood samples were drawn from 0–300 minutes (Fig.1), stored on ice and plasma separated within 60 minutes for storage at –80° C until assay.

Assays

Blood samples were collected as previously described⁶. Blood glucose concentrations were determined using an automated glucose analyzer. Insulin concentrations were determined with a previously described radioimmunoassay¹⁴. C-peptide and glucagon were measured by commercial RIAs (Millipore, Billerica, MA), and total GLP-1 (Meso Scale Diagnostics, LLC, Gaithersburg, MD) and total GIP (Millipore, Billerica, MA) using commercial ELISA, according to the manufacturers' specifications. Plasma enrichment of isotopes was determined using GC/MS.

Calculations and analysis

Fasting values of blood glucose and hormones were computed as the average of the 4 samples drawn from –130 to –60 min, and the pre-meal values as the average of the 5

samples drawn from -10 to 0 min. Insulin secretion rates (ISR) were derived from plasma C-peptide concentrations using deconvolution with population estimates of C-peptide¹⁶. Glucose, insulin, ISR, glucagon, and GLP-1 values from 0–180 min, and GIP levels from 0–150 min after meal ingestion, were used to compute incremental area under the curve (AUC) using the trapezoidal rule.

Rates of glucose appearance (R_{aTOT}), glucose disappearance (R_d), meal-derived glucose appearance (R_{aOral}) and endogenous glucose production (EGP) were derived from plasma [6,6-²H₂]glucose and [U-¹³C]glucose enrichments as previously described¹⁷ using an approach based on Steeles equations^{18, 19} (supplemental material). AUC values for R_{aTOT} , R_d , R_{aOral} , and EGP were calculated for 0–120 min. AUC for all parameters were also calculated for 0–30 and 0–60 min to evaluate the early response to meal ingestion since 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}. β -cell function during the meal studies was also compared using a previously validated model^{22, 23} that expresses ISR as the sum of two 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 rate of change in glucose concentrations on ISR, which represents principally early insulin release. β -cell glucose sensitivity, as the slope of ISR and blood glucose concentration was computed separately for the first part of the MTT as glucose increased to peak values, and the latter part of MTT as glucose levels declined towards fasting levels.

HOMA-IR was calculated as fasting glucose (mg/dL) $\times$ fasting insulin (pmol/L) / 8.66²⁴. MTT-derived insulin sensitivity ($OGIS_{120min}$) was measured as previously described^{25, 26}. A semiquantitative symptom questionnaire [adapted with modification from Sigstad¹²; supplementary material] was administered every 15 min starting from meal ingestion. Subjects scored 0 when they had none of symptoms and 1 if they had any at each time point. The sum of these symptoms during MTT studies constituted the total symptoms score. These values were also calculated for the time periods from the start of meal ingestion to the peak of glucose for each study as the early symptoms score.

Statistical analysis

Data are presented as Mean $\pm$ SEM. Baseline characteristics were compared using ANOVA or a Chi-squared 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 two-way repeated measure ANOVA 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

Subjects characteristics (Table 1)

The three groups had similar BMI, waist circumference, fat and lean mass, and HbA1C prior to study initiation. The H-GB and CON groups were almost all women but the A-GB included 4 men; control subjects were slightly younger than surgical subjects. The surgical groups had comparable age and rates of diabetes prior to GB. Total weight loss, and time since GB were similar in the surgical groups, although H-GB individuals had a trend towards greater weight regain from their postoperative nadir after surgery compared to the A-GB subjects. 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 (Table 2)

GB subjects had a lower fasting glucose compared to controls as well as a higher and earlier peak of glucose in response to meal ingestion (Fig.1). During the MTT, blood glucose fell to <50 mg/dL in 8 of the H-GB patients, all of who became symptomatic within 60–120 minutes. In contrast, none of the A-GB subjects had postprandial glucose levels <50 mg/dL or symptoms of hypoglycemia. While the early glucose response to meal ingestion (glucose peak and $AUC_{\text{Glucose}(0,60\text{min})}$) did not differ significantly between the two surgical groups, the average nadir glucose level was significantly lower in the H-GB patients compared to other groups, with no differences between the A-GB and CON individuals. Blockade of the GLP-1r with Ex-9 increased both fasting and postprandial glucose levels in all three groups (Fig.1). The postprandial glycemic effect of Ex-9 infusion was similar among the three groups for the first 60 min with increases in the $AUC_{\text{Glucose}(0,60\text{min})}$ of $18\pm10\%$ in H-GB, $14\pm6\%$ in A-GB, and $30\pm14\%$ in CON. However, over the entire course of the MTT the H-GB subjects had a significantly larger glycemic response to Ex-9 with an increase in the $AUC_{\text{Glucose}(0,180\text{min})}$ of $200\pm72\%$ compared to $37\pm12\%$ in A-GB, and $14\pm12\%$ in CON ($p<0.001$; Fig.1). None of the H-GB group had a blood glucose < 50 mg/dL or symptoms of hypoglycemia during administration of Ex-9. In all three groups the time to reach peak glucose became shorter and the time to reach nadir glucose levels longer as a result of GLP-1 receptor blockade.

The appearance of $U^{13}\text{C}$ -labeled glucose in the circulation paralleled that of blood glucose concentrations after the test meal. However, the rates of early Ra_{Oral} were significantly larger in the H-GB group compared to the A-GB subjects, and greater in the A-GB than the CON group ($AUC_{Ra_{\text{Oral}}(0,60\text{min})}$: 1548 ± 213 , 1137 ± 161 , and 544 ± 35 $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ in H-GB, A-GB, and CON; $p<0.01$; Fig.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 three groups, declined after the ingestion of glucose, and increased again within 30 min, with no significant differences among the groups (Fig.2). Infusion of Ex-9 showed a tendency to increase premeal EGP values ($p=0.1$) but had no significant effect on $AUC_{\text{EGP}(0,120\text{min})}$ in any groups. The early ($AUC_{Rd(0,60\text{min})}$) and overall ($AUC_{Rd(0,120\text{min})}$) rates of glucose disposal were significantly larger in GB subjects compared to the non-surgical controls ($p<0.05$). The difference in the rates of Rd between the saline and Ex-9 studies did not differ (Fig.2).

GLP-1 contribution to β - cell function, insulin sensitivity and insulin clearance (Table 2)

Fasting insulin was lower in the GB subjects compared to the non-surgical cohort consistent with their greater insulin sensitivity and fasting insulin clearance. The H-GB subjects had an earlier and more robust β -cell response after the test meal, although the overall AUC_{ISR} for the 3 hour postmeal period did not differ significantly among the groups (Fig.1). GLP-1r blockade diminished early and total insulin secretion in all groups with the largest effect in the H-GB group and no difference between the A-GB and CON (relative reduction in $AUC_{\text{ISR}(0,180\text{min})}$: $50\pm8\%$ in H-GB, $13\pm10\%$ in A-GB, and $14\pm10\%$ in CON; $p<0.05$).

β -cell glucose sensitivity during the first part of MTT tended to be higher in the H-GB and CON subjects compared to A-GB individuals (Fig.3) and there was a trend toward lower β -cell rate sensitivity in the GB subjects compared to the controls. Infusion of Ex-9 lowered β -cell glucose sensitivity in all three groups, with the maximum effect observed in the H-GB (relative reduction in glucose sensitivity: $60\pm8\%$ in H-GB, $15\pm44\%$ in A-GB, and $20\pm18\%$ in CON; $p=0.07$, Fig.3), but had no effect on β -cell rate sensitivity. The H-GB subjects also had higher rates of glucose-stimulated insulin secretion as blood glucose levels declined in the latter part of MTT compared to the A-GB and CON individuals, but blockade of GLP-1r

signaling reduced the insulin:glucose dose response in the latter part of MTT in all groups. C-peptide levels were modestly higher in the H-GB group as glucose levels rose after meal consumption, but were dramatically higher as glucose levels fell in the latter part of the test. Blockade of GLP-1r signaling almost completely eliminated this disparity in C-peptide levels (Fig.3).

Fasting insulin sensitivity, computed as $1/\text{HOMA-IR}$, and total glucose clearance during the MTT ($\text{OGIS}_{120\text{min}}$), were significantly greater in the H-GB and A-GB compared to the CON individuals. Ex-9 infusion reduced $\text{OGIS}_{120\text{min}}$ values in all three groups, but had no effect on $1/\text{HOMA-IR}$. While fasting insulin clearance was greater in surgical subjects compared to the controls, insulin clearance after eating was not different among the groups and was not significantly affected by Ex-9 in any group.

GI hormone and α -cell responses after meal ingestion and postprandial symptoms with and without GLP-1r blockade (Table 3)

Plasma concentrations of glucagon, GLP-1 and GIP before and after the test meal are shown in Fig.4. Fasting plasma glucagon was similar among the three groups; however, postprandial glucagon levels followed significantly different courses in GB and control subjects. Meal ingestion suppressed glucagon slightly in the CON, but increased both early and overall glucagon responses in the H-GB and A-GB groups. Infusion of Ex-9 had no influence on fasting glucagon but postprandial glucagon levels were increased in all three groups ($p<0.01$).

Postprandial plasma GLP-1 was substantially higher in the surgical subjects than the controls, with a trend towards larger responses in the H-GB compared to the A-GB group (Fig.4). Blocking the GLP-1r increased premeal levels of GLP-1 in surgical patients, and postmeal values in the A-GB and CON subjects with no effect in the H-GB group ($p<0.05$ for interaction between treatment and groups). Premeal GIP was similar in the three groups and meal ingestion increased the early response of GIP to meal ingestion in the surgical subjects compared to the controls although the overall postprandial GIP levels were similar in the three groups. GLP-1r blockade caused small but significant increases in the early and the overall GIP responses to meal ingestion in the three groups.

Consistent with a higher frequency of dumping symptoms in the past, H-GB subjects had higher scores for both GI and non-GI symptoms compared to the A-GB individuals during the MTT studies (GI and non-GI symptoms: 10 ± 0 and 17 ± 5 in H-GB vs. 3.9 ± 3 and 3 ± 0 in A-GB; $p<0.05$), 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 H-GB subjects (p for the interaction of group and treatment <0.05).

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 ($\text{AUC}_{\text{GLP-1}(0,60\text{min})}$; $r=-0.554$, $p=0.032$) and Ra_{Oral} values ($r=-0.524$, $p=0.037$), 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 ($\text{AUC}_{\text{ISR}(0,60\text{min})}$; $r=-0.48$, $p=0.06$) and β -cell glucose sensitivity ($r=-0.60$, $p=0.014$), but there was no association with the overall insulin response ($\text{AUC}_{\text{ISR}(0,180\text{min})}$) or β -cell rate sensitivity among the surgical patients. The magnitude of the GLP-1 effect on ISR (the difference in ISR with and without Ex9) also correlated inversely with nadir glucose values ($r=0.60$, $p=0.013$), but there was no 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 demonstrate that postprandial hypoglycemia in H-GB subjects 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 H-GB subjects, and administration of Ex9 reduces the abnormally high rate of insulin secretion these individuals have in the latter phase of meal absorption. Beyond these important differences in β -cell function, H-GB subjects have 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 affected by post-surgical hypoglycemia.

The present study is an extension of a previous assessment of GLP-1-stimulated insulin secretion in GB subjects with and without symptomatic hypoglycemia⁶. This earlier study focused on β -cell function assessed during clamped plasma glucose levels, and thus could not determine effects of GLP-1r blockade on glycemia. In this study we infused Ex9 during an MTT to replicate the mealtime setting in which some GB subjects have hypoglycemia, and used changes in blood glucose as the primary outcome. Subject selection for this study was highly focused to obtain individuals who fit unequivocally as either H-GB or A-GB; we took 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 two groups was successful, and while only representative of extremes in glucose regulation among GB subjects, 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 subjects had clearly faster Ra_{Oral} compared to the non-surgical controls, 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 compared to the A-GB subjects, with and without Ex-9. While 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 of the H-GB subjects 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 subjects, it seems likely that these individuals had higher intraportal glucose concentrations during the MTT. Variation in portal glucose concentrations contributes to hepatic and extrahepatic glucose uptake³¹ and could conceivably have a role in the differences in postprandial glucose regulation described here. The results described here support more in depth study of the role of gastrointestinal 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 subjects relative to the other groups, both of which had comparable responses. These results differ from our previous results⁶ that showed comparable effects of GLP-1 to enhance insulin secretion in GB subjects with and without prior hypoglycemia. The apparent discrepancy in our two studies may be partly explained by differences in the characteristics of the subjects participating in the two 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 β -cell function at stable hyperglycemia fixed by a glucose clamp, while in the current study glucose levels followed the usual variable course of meal absorption. In the present study the β -cell sensitivity to the prandial rise of glucose did not differ among H-GB and control subjects, although this parameter was more dependent on GLP-1 in the H-GB subjects than the other groups. However, it is clear that the H-GB subjects 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 falling. Based on the effects of Ex-9, both of these β -cell responses were highly dependent on GLP-1. Although there was a trend toward higher postprandial plasma GLP-1 levels in the H-GB subjects compared to the AGB individuals, 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 β -cell sensitivity to GLP-1 explains the propensity of some individuals to develop the postprandial hypoglycemia syndrome after GB. Another possibility is that H-GB subjects 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 GLP-1 effect 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, β -cell sensitivity to glucose, a parameter responsive to Ex9, and Ra_{Oral} . Taken together these findings support a model whereby 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 β -cell function that can contribute to hypoglycemia.

We have confirmed the distinct profile of postprandial glucagon concentrations reported previously in subjects with GB^{1, 3, 6, 32–34}. Similar to those reports the H-GB and A-GB subjects both had much higher postprandial levels of glucagon than the non-operated controls. Also consistent with previous studies^{6, 35} there was a significant increase in glucagon when Ex-9 was given indicating that while α -cell function differs between GB and control subjects it is regulated by GLP-1 in both groups. However, what is not clear is why plasma glucagon does not rise substantially in the H-GB subjects during the saline studies when they have significant hypoglycemia. This finding suggests that α -cell and well as β -cell function is altered in subjects with the post-GB hypoglycemic syndrome.

There are several limitations to this study that warrant mention. The numbers of subjects in each of the groups was relatively small and was highly selected, limiting applicability to the broad range of individuals who have had GB. Also, half of the A-GB subjects were males. We are not aware of sex differences in glycemic regulation among GB subjects although this topic has not been directly studied; in the limited substudy comparison we were able to do with this cohort the effect of Ex-9 on glucose or ISR response to meal ingestion was not affected by gender (supplemental material). The A-GB cohort was slightly but more insulin resistant than the H-GB group. However, the lower insulin sensitivity in the A-GB subjects should have contributed to larger meal-induced insulin secretion and it thus not likely to have biased our results. However, it is possible that in the H-GB subjects increased insulin secretion in combination of 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 tests³⁶. We do not have access to this methodologic advance and so have been careful not to over-interpret the glucose turnover results reported here. However, we think 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 demonstrated that blocking the GLP-1r eliminates postprandial hypoglycemia in GB subjects affected with the postprandial hypoglycemia syndrome. Our findings support enhanced β -cell sensitivity to GLP-1 in the hyperinsulinemia associated with symptomatic nadirs in postprandial glucose. The distinct pattern of ingested glucose appearance among H-GB subjects suggests that altered GI function also contributes to glucose abnormalities in this syndrome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

A-GB	asymptomatic surgical subjects with glucose nadir 50mg/dL during MTT
AUC	incremental area under the curve
BMI	body mass index
CON	non-operated controls with normal glucose tolerance
Ex-9	Exendin-(9–39)
GB	Roux-en-Y gastric bypass surgery
GI	gastrointestinal
GIP	glucose dependent insulintropic peptide
GLP-1	glucagon-like peptide 1
H-GB	Surgical subjects with prior history of recurrent neuroglycopenia associated with blood glucose nadir <50 mg/dL and relief of symptoms with carbohydrate intake
HbA1C	glycosylated hemoglobin A1C
EGP	endogenous glucose production
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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Figure 1.

Blood glucose (A), plasma insulin (B), and insulin secretion (C) responses to meal ingestion in GB subjects, with (Hypoglycemic-GB, left) and without (Asymptomatic-GB, middle) recurrent hypoglycemia, and non-surgical controls (right) during studies with (dashed line, white bar) and without (solid line, black bar) Ex-9 infusion

Corresponding AUC for 0–60 and 0–180 min are shown as inset figures, * $p < 0.05$ compared to Ex-9 study, # $p < 0.05$ compared to non-surgical controls, § $p < 0.05$ for the interaction of group and treatment.

Figure 2.

The rates of meal-derived glucose appearance (A), endogenous glucose appearance (B), and glucose disappearance (C) in GB subjects, with (Hypoglycemic-GB, left) and without (Asymptomatic-GB, middle) recurrent hypoglycemia, and non-surgical controls (right) during studies with (dashed line) and without (solid line) Ex-9 infusion. AUC_{RaOral} for 0–30 and 0–120 min are shown as inset figures, # $p < 0.05$ compared to non-surgical controls, † $p < 0.05$ compared with A-GB.

Figure 3.

Circulatory C-peptide levels across blood glucose values and β -cell glucose sensitivity in GB subjects with (A, D) and without (B, E) hypoglycemia syndrome, and non-surgical subjects (C, F) during MTT studies with (open circle, dashed line) and without (close circle, solid line) Ex-9 infusion (0–120 min for GB subjects and 0–180 min for healthy controls). Black arrows show the initial phase of the MTT (increasing glucose) and white arrow the latter phase of the MTT (decreasing glucose); * $p < 0.05$ compared to Ex-9 study, § $p = 0.07$ for the interaction of group and treatment.

Figure 4.

Plasma glucagon (A), GLP-1 (B), and GIP (C) responses to meal ingestion in GB subjects, with (Hypoglycemic-GB, left) and without (Asymptomatic-GB, middle) recurrent hypoglycemia, and non-surgical controls (right) during studies with (dashed line, white bar) and without (solid line, black bar) Ex-9 infusion

Corresponding AUC are shown as inset figures, * $p < 0.05$ compared to Ex-9 study, # $p < 0.05$ compared to non-surgical controls, § $p < 0.05$ for the interaction of group and treatment.

Table 1

Baseline characteristics of study subjects

	H-GB (9)	A-GB (7)	CON (8)	P value
Age (y)	44.6±4.5	47.6±3.0	35.1±3.3	0.08
BMI (kg/m ²)	30.9±2.5	33.8±3.4	32.8±1.1	0.69
gender (F/M)	9/0	3/4	7/1	0.02
DM (yes/no)	2/7	2/5	0/8	0.70
Dumping symptoms (yes/no)	7/2	3/4	NA	0.15
Waist circumference (cm)*	95.5±4.8	105.0±8.6	108.9±2.7	0.30
Total fat mass* (kg)	32.9±5.8	34.6±8.3	35.0±1.6	0.90
Total lean mass* (kg)	49.7±2.5	60.9±6.8	52.7±2.1	0.20
HbA1C (%)	5.2±0.2	5.2±0.1	5.1±0.1	0.80
Time since surgery (y)	3.9±0.5	3.6±0.7		0.72
Preop BMI (kg/m ²)	48.0±2.6	55.0±2.6		0.09
Total weight loss (kg)	45.4±4.4	60.6±10.0		0.15
Weight regain (kg)	17.3±6.0	-4.5±10.0		0.07

Data are presented as mean ± SEM unless specified otherwise; H-GB, hypoglycemic gastric bypass patients; A-GB, asymptomatic gastric bypass patients; CON, non-surgical controls;

* measured in 7 subjects from each group; statistical p values for ANOVA or X² analysis are provided in the last right-hand column

Table 2

Effect of meal ingestion on glucose and β cell response in studies with and without intravenous Ex-9 infusion in hypoglycemic gastric bypass patients (H-GB), asymptomatic gastric bypass patients (A-GB), and non-surgical controls (CON)

	H-GB (9)		A-GB (7)		CON (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) *	74.9 $\pm$ 2.4	80.5 $\pm$ 2.2	81.0 $\pm$ 3.8	88.8 $\pm$ 4.9	83.7 $\pm$ 3.1	88.6 $\pm$ 2.1	0.00	0.10	0.62
Time to reach peak glucose (min)	33.3 $\pm$ 2.2	30.6 $\pm$ 2.1	39.3 $\pm$ 8.9	30.7 $\pm$ 2.8	62.5 $\pm$ 12.2	41.3 $\pm$ 3.7	0.02	0.02	0.21
Time to reach nadir glucose (min)	98 $\pm$ 8	155 $\pm$ 14	141 $\pm$ 18	165 $\pm$ 15	154 $\pm$ 14	158 $\pm$ 12	0.01	0.12	0.08
Nadir glucose (mg/dL)	42.3 $\pm$ 3.7	70.8 $\pm$ 4.1	77.4 $\pm$ 8.3	88.5 $\pm$ 8.9	86.6 $\pm$ 3.3	95.1 $\pm$ 3.6	0.00	0.00	0.00
peak glucose (mg/dL)	170.9 $\pm$ 7.4	185.0 $\pm$ 9.3	157.7 $\pm$ 9.1	169.9 $\pm$ 8.6	119.0 $\pm$ 4.3	128.4 $\pm$ 4.0	0.00	0.00	0.87
AUC _{Glucose} (0,60min) (mg.dL ⁻¹ .min)	3289 $\pm$ 339	3785 $\pm$ 426	2747 $\pm$ 332	3075 $\pm$ 321	1004 $\pm$ 73	1255 $\pm$ 95	0.00	0.00	0.64
AUC _{Glucose} (0,180min) (mg.dL ⁻¹ .min)	1317 $\pm$ 721	4615 $\pm$ 757	3693 $\pm$ 579	4879 $\pm$ 790	3739 $\pm$ 564	3940 $\pm$ 456	0.00	0.33	0.00
Fasting insulin (pmol/L) *	27.2 $\pm$ 3.7	23.9 $\pm$ 3.5	47.1 $\pm$ 14.4	44.8 $\pm$ 11.6	77.1 $\pm$ 14.7	78.0 $\pm$ 16.1	0.83	0.00	0.97
AUC _{Insulin} (0,30min) (pmol.L ⁻¹ .min)	18.6 $\pm$ 3.4	10.7 $\pm$ 2.1	10.2 $\pm$ 1.9	10.1 $\pm$ 1.6	4.9 $\pm$ 1.9	4.7 $\pm$ 0.9	0.02	0.01	0.01
AUC _{Insulin} (0,60min) (pmol.L ⁻¹ .min)	67.0 $\pm$ 11.6	25.8 $\pm$ 6.3	24.3 $\pm$ 5.2	20.7 $\pm$ 3.6	18.6 $\pm$ 6.1	15.3 $\pm$ 2.7	0.00	0.01	0.00
AUC _{Insulin} (0,180min) (pmol.L ⁻¹ .min)	88.3 $\pm$ 15.6	33.3 $\pm$ 8.0	40.2 $\pm$ 10.5	32.5 $\pm$ 8.9	55.7 $\pm$ 13.5	43.3 $\pm$ 6.5	0.00	0.25	0.01
Fasting ISR (pmol/min) *	91.8 $\pm$ 13.3	80.0 $\pm$ 9.1	150.7 $\pm$ 65.4	151.8 $\pm$ 54.7	166.0 $\pm$ 24.5	141.5 $\pm$ 10.8	0.19	0.24	0.51
AUC _{ISR} (0,30min) (pmol)	34.6 $\pm$ 5.6	22.5 $\pm$ 5.7	17.9 $\pm$ 4.0	18.4 $\pm$ 2.6	9.5 $\pm$ 3.2	11.3 $\pm$ 2.5	0.06	0.02	0.00
AUC _{ISR} (0,60min) (pmol)	80.1 $\pm$ 10.9	40.5 $\pm$ 9.7	39.9 $\pm$ 9.6	31.3 $\pm$ 5.0	30.1 $\pm$ 8.6	24.9 $\pm$ 4.3	0.00	0.02	0.00
AUC _{ISR} (0,180min) (pmol)	90.3 $\pm$ 11.4	48.0 $\pm$ 11.3	60.8 $\pm$ 15.0	47.2 $\pm$ 11.9	80.4 $\pm$ 14.8	62.2 $\pm$ 9.1	0.00	0.55	0.05
B-cell rate sensitivity (pmol.m ⁻² .mM ⁻¹)	1692 $\pm$ 605	1656 $\pm$ 522	1615 $\pm$ 555	1633 $\pm$ 465	3381 $\pm$ 1020	3122 $\pm$ 666	0.80	0.10	0.96
B-cell glucose sensitivity (pmol.min ⁻¹ .mM ⁻¹)	367 $\pm$ 43	155 $\pm$ 34	186 $\pm$ 30	160 $\pm$ 28	394 $\pm$ 95	279 $\pm$ 64	0.00	0.10	0.07
Fasting insulin clearance	2.9 $\pm$ 0.2	3.0 $\pm$ 0.4	2.7 $\pm$ 0.5	2.8 $\pm$ 0.3	1.9 $\pm$ 0.2	1.9 $\pm$ 0.2	0.70	0.03	0.96
Postprandial insulin clearance	1.4 $\pm$ 0.3	1.4 $\pm$ 0.3	1.6 $\pm$ 0.2	1.8 $\pm$ 0.3	1.6 $\pm$ 0.1	1.7 $\pm$ 0.1	0.89	0.81	0.88
HOMA-IR	0.7 $\pm$ 0.1	0.7 $\pm$ 0.1	1.4 $\pm$ 0.5	1.5 $\pm$ 0.5	2.3 $\pm$ 0.5	2.5 $\pm$ 0.6	0.83	0.00	0.95
OGIS _{120min} (ml.min ⁻¹ .m ⁻²)	537 $\pm$ 17	486 $\pm$ 16	472 $\pm$ 30	439 $\pm$ 29	406 $\pm$ 19	393 $\pm$ 12	0.00	0.00	0.14

Data are presented as Mean $\pm$ SEM. Ex-9, exendin-[9-39] (GLP-1r antagonist); AUC, incremental areas under the curve; ISR, insulin secretion rate; HOMA-IR, homeostatic model assessment of fasting insulin resistance; OGIS, oral glucose insulin sensitivity index from 0-120 min after meal ingestion;

* fasting values from 110–120 min 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 three right-hand columns.

Table 3

Effect of meal ingestion on GI hormone and α -cell response in studies with and without intravenous Ex-9 infusion in hypoglycemic gastric bypass patients (H-GB), asymptomatic gastric bypass patients (A-GB), and non-surgical controls (CON)

	H-GB (9)		A-GB (7)		CON (8)		Statistical tests (p-values)		
	Saline	Ex-9	Saline	Ex-9	Saline	Ex-9	Ex-9 vs. saline	Group status	Interaction
AUC _{CgIP(0.30)} (ng.mL ⁻¹ .min)	7.9±0.8	9.4±1.2	6.9±0.9	8.3±1.1	2.9±0.5	4.1±0.7	0.01	0.00	0.96
AUC _{CgIP(0.45)} (ng.mL ⁻¹ .min)	12.6±1.0	14.6±1.7	11.1±1.5	12.7±1.7	5.9±1.0	8.1±1.0	0.02	0.00	0.95
AUC _{CgIP(0.150)} (ng.mL ⁻¹ .min)	23.5±1.8	25.4±2.8	22.5±2.7	24.9±4.8	21.6±3.0	27.6±2.6	0.04	0.97	0.54
AUC _{CgIP-1(0.30)} (ng.mL ⁻¹ .min)	2.7±0.4	2.5±0.3	1.5±0.4	2.2±0.5	0.1±0.0	0.3±0.0	0.11	0.00	0.07
AUC _{CgIP-1(0.60)} (ng.mL ⁻¹ .min)	7.7±1.3	6.4±0.7	4.0±1.3	5.6±1.4	0.3±0.1	0.7±0.1	0.77	0.00	0.05
AUC _{CgIP-1(0.180)} (ng.mL ⁻¹ .min)	11.9±2.0	9.8±1.2	5.6±1.6	9.0±2.5	1.0±0.3	2.0±0.2	0.43	0.00	0.05
AUC _{Cgucagon(0.20)} (ng.mL ⁻¹ .min)	0.2±0.1	0.4±0.1	0.2±0.0	0.4±0.1	0.0±0.0	0.1±0.1	0.00	0.01	0.37
AUC _{Cgucagon(0.60)} (ng.mL ⁻¹ .min)	1.5±0.5	2.0±0.4	1.1±0.0	1.7±0.4	-0.1±0.2	0.5±0.2	0.00	0.01	0.56
AUC _{Cgucagon(0.180)} (ng.mL ⁻¹ .min)	4.8±1.2	4.6±0.6	2.3±0.3	3.5±0.5	-0.9±0.7	1.2±0.7	0.03	0.00	0.12

Data are presented as Mean±SEM. Ex-9, exendin-[9-39] (GLP-1r antagonist); AUC, incremental areas under the curve; Statistical effects (treatment [saline/Ex-9], group status [H-GB/A-GB/CON], and their interaction) are provided in the last three right-hand columns.