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

Marzieh Salehi,[1] Amalia Gastaldelli,[2] and David A. D'Alessio[1,3]

[1]Division of Endocrinology, Diabetes, & Metabolism, Department of Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio; [2]Cardiometabolic Risk Unit, CNR Institute of Clinical Physiology, Pisa, Italy; and [3]Cincinnati VA Medical Center, Cincinnati, Ohio

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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