Glucagon-Like Peptide 1 Attenuates the Acceleration of Gastric Emptying Induced by Hypoglycemia in Healthy Subjects

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OBJECTIVE

Exogenous GLP-1 slows gastric emptying in health and diabetes leading to diminished glycemic excursions. Gastric emptying is markedly accelerated by hypoglycemia. The primary objective was to determine whether GLP-1 attenuates the acceleration of gastric emptying induced by hypoglycemia.

RESEARCH DESIGN AND METHODS

Ten healthy volunteers were studied on four separate days in a randomized double-blind fashion. Blood glucose was stabilized using a glucose/insulin clamp at hypoglycemia (2.6 mmol/L on two occasions [hypo]) or euglycemia (6.0 mmol/L on two occasions [eu]) between *T* = −15 and 45 min before clamping at 6.0 mmol/L until 180 min. During hypoglycemia and euglycemia, subjects received intravenous GLP-1 (1.2 pmol/kg/min) or placebo. At *T* = 0 min, subjects ingested 100 g beef mince labeled with 20 MBq ^{99m}Tc-sulfur-colloid and 3 g of 3-*O*-methyl-glucose (3-OMG), a marker of glucose absorption. Gastric emptying was measured scintigraphically from *T* = 0 to 180 min and serum 3-OMG taken at 15-min intervals. The areas under the curve for gastric emptying and 3-OMG concentration were analyzed using one-way repeated-measures ANOVA with Bonferroni-Holm adjusted post hoc tests.

RESULTS

Gastric emptying was accelerated during hypoglycemia (hypo/placebo vs. eu/placebo; *P* < 0.001), as was glucose absorption (*P* < 0.03). GLP-1 slowed emptying during euglycemia (eu/placebo vs. eu/GLP-1; *P* < 0.001). However, hypoglycemia-induced acceleration of gastric emptying on placebo was markedly diminished by GLP-1 (hypo/placebo vs. hypo/GLP-1; *P* < 0.008), as was glucose absorption (*P* < 0.01).

CONCLUSIONS

Acute administration of exogenous GLP-1 attenuates, but does not abolish, the acceleration of gastric emptying by insulin-induced hypoglycemia in healthy subjects.

Glucagon-like peptide 1 (GLP-1) receptor agonists are now incorporated into standard treatment algorithms for the management of type 2 diabetes (1). Several GLP-1 receptor agonists are available and are increasingly used as monotherapy, as second-line therapy (particularly with metformin and/or sulphonylurea), and, more recently, in combination with basal insulin (1,2).

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The development of GLP-1 agonists was stimulated by characterization of the effects of exogenous GLP-1 to lower both fasting and postprandial glycemia (3). During fasting, the glucose-lowering effect of GLP-1 is predominantly mediated through effects on islet cell function to increase insulin and reduce glucagon secretion in a glucose-dependent manner (4,5). Accordingly, GLP-1 and its agonists are extremely unlikely to cause hypoglycemia when used as monotherapy.

In contrast to the fasted state, during the postprandial phase, glucose lowering by acute administration of GLP-1 is mediated primarily through its effect to slow gastric emptying (6). Indeed, postprandial insulin concentrations are reduced by administration of GLP-1 because of the delayed entry of nutrients into the small intestine (6). This also appears to be the case with "short-acting" GLP-1 agonists such as exenatide BD (7) and lixisenatide (8), which have a sustained effect to slow gastric emptying. Additionally, because the human stomach empties at an overall rate of 1–4 kcal/min in health (9), most humans are predominantly in the postprandial state with the duration of fasting limited to ~4 h before breakfast (10). Hence, the effect of GLP-1 and its agonists of slowing gastric emptying is of fundamental significance and provides a persuasive rationale for their combination with basal insulin, given that this approach targets both fasting and postprandial glycemia and doesn't lead to weight gain (10).

Given that GLP-1 agonists may be combined with a sulphonylurea or insulin, there is, however, the potential for hypoglycemia. For this reason, it is reassuring that in health, pharmacological doses of GLP-1 have no effect on the counterregulatory hormonal response to insulin-induced hypoglycemia (5). Somewhat surprisingly, however, the effect of GLP-1 on gastric emptying during hypoglycemia has not been evaluated.

Acute glycemia per se is a major determinant of the gastric-emptying rate, and hypoglycemia potently accelerates gastric emptying in both healthy subjects (11) and patients with type 1 diabetes (12). Conversely, marked hyperglycemia slows gastric emptying (13,14), and even glycemic perturbations that are within the normal physiological range influence gastric emptying in health and patients with type 1 diabetes (14). Indeed, systemic glycemia has been shown to alter the effect of gastrokinetic drugs; for example, hyperglycemia potently attenuates the gastrokinetic effect of erythromycin (15). However, the interaction of hypoglycemia with drugs that slow gastric emptying has not yet been investigated in humans.

The acceleration of gastric emptying during hypoglycemia is likely to be a physiological protective mechanism that increases delivery and absorption of carbohydrate. Indeed, the current American Diabetes Association guidelines for the treatment of hypoglycemia emphasize the importance of carbohydrate ingestion (16). While the insulinotropic and glucagonostatic effects of GLP-1 and its agonists are established as glucose dependent, the effect of hypoglycemia on the GLP-1–induced slowing of gastric emptying is unknown. This is of particular importance considering the interest in combinations of GLP-1 agonists and basal insulin, which may increase the risk of hypoglycemia when compared with GLP-1 monotherapy. The primary aim of this study was to determine whether GLP-1 attenuates the acceleration of gastric emptying induced by acute hypoglycemia in health.

RESEARCH DESIGN AND METHODS

Subjects

Healthy volunteers aged 50–75 years were eligible, and those with diabetes (HbA_{1c} >6.5%; 48 mmol/mol), BMI ≥30 kg/m^2, impaired renal function or anemia, currently smoking, consuming >20 g/day of alcohol, receiving medication known to affect gastrointestinal motility or glycemia, or with a history of gastric or small intestinal surgery were excluded.

Protocol

All subjects were studied in random order on four separate occasions separated by a minimum of 4 days (Fig. 1). Each subject underwent concurrent measurements of gastric emptying, blood glucose concentrations, and glucose absorption on each of the four occasions: twice with blood glucose concentrations maintained at euglycemia (blood glucose of 6 mmol/L (108 mg/dL) [eu]) and twice with a period of insulin-induced hypoglycemia (blood glucose of 2.6 mmol/L [47 mg/dL] [hypo]) (Figs. 1 and 2). Randomization was performed by the Department of Pharmacy at the Royal Adelaide Hospital, which notified study investigators M.P.P., C.E.A., and C.E.C. of the glucose target on a particular

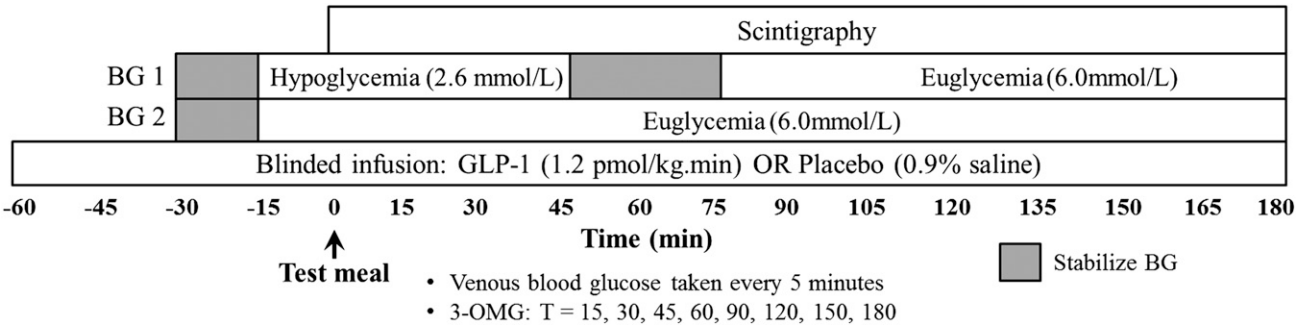

Figure 1—Schematic representation of the study protocol. Blood glucose concentrations (BG) were stabilized using a glucose/insulin clamp at hypoglycemia (2.6 mmol/L; BG 1) or euglycemia (6.0 mmol/L; BG2) between $T = -15$ and 45 min before clamping at 6.0 mmol/L until 180 min. During hypoglycemia and euglycemia, subjects received intravenous GLP-1 (1.2 pmol/kg · min) or placebo between $T = -60$ and 180 min. At $T = 0$ min, subjects ingested a labeled meal. Gastric emptying was measured scintigraphically from $T = 0$ to 180 min, and serum 3-OMG concentrations were taken at 15-min intervals for the first hour and then half hourly until study completion.

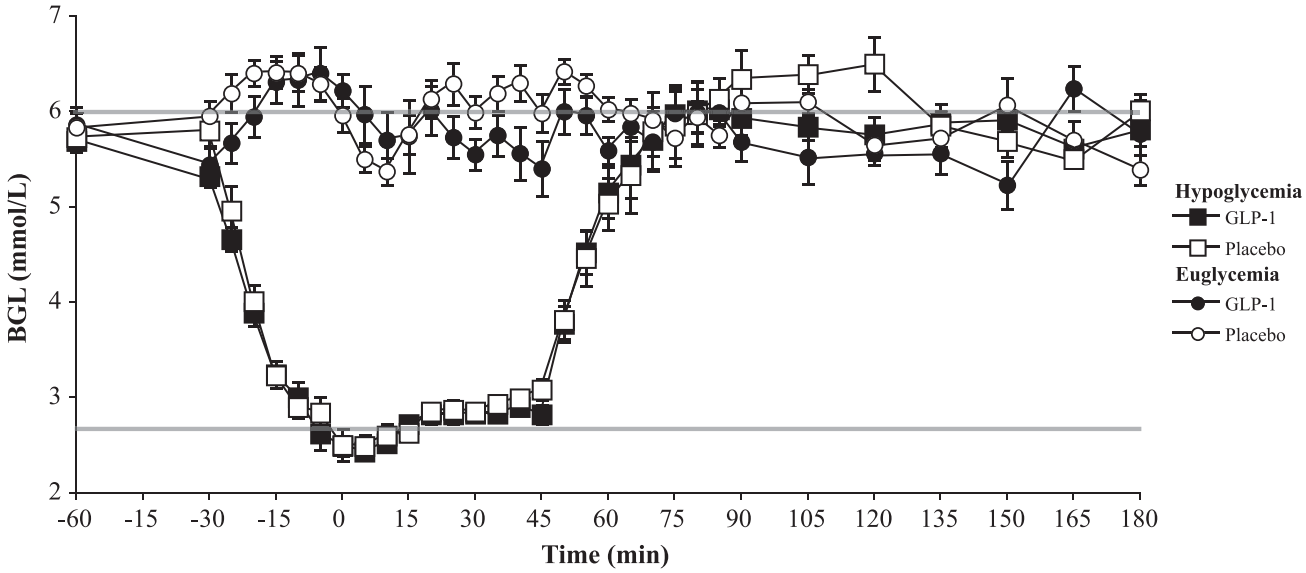

Figure 2—Blood glucose concentrations. Blood glucose (mmol/L) was stabilized using a glucose/insulin clamp at hypoglycemia (2.6 mmol/L) or euglycemia (6.0 mmol/L) between $T = -15$ and 45 min before clamping at 6.0 mmol/L until 180 min. Data are presented as mean (SE). BGL, blood glucose level.

study day. These investigators played no role in analysis of gastric-emptying data. Randomization of study drug, either GLP-1 or placebo, was also performed by the Department of Pharmacy, and allocation of the study drug was concealed to all investigators throughout the study. Either GLP-1 (1.2 pmol/kg/min) or placebo (0.9% saline) was administered intravenously during both euglycemia and hypoglycemia.

Each subject attended the Department of Nuclear Medicine, Positron Emission Tomography, and Bone Densitometry at the Royal Adelaide Hospital at 0830 h after an overnight fast. Two intravenous cannulae were inserted into the right arm: one in the antecubital vein for an infusion of insulin and 25% dextrose and another in the dorsal vein of the right hand for infusion of study drug. A third intravenous cannula was inserted into the left antecubital vein for blood sampling. Synthetic GLP-1 amide (Bachem, Weil am Rhein, Germany) was reconstituted by the Department of Pharmacy in 0.9% normal saline. After drawing of baseline blood specimens, both GLP-1 (1.2 pmol/kg/min) and placebo (0.9% saline) infusions were commenced 60 min prior to meal ingestion and infused at a rate of 1 mL/min for the duration of the study (i.e., $T = -60$ to 180 min) (17). The infusion rate of 1.2 pmol/kg/min was based on previous studies and was known to raise plasma GLP-1 concentrations into the pharmacological range (approximately three- to fourfold higher concentrations in comparison with those measured after oral glucose) (5,18,19) and is representative of GLP-1 receptor stimulation that occurs during administration of commercial agonists (4,20).

An insulin-glucose clamp was started 30 min later as described below. After blood glucose concentrations had been stabilized at the desired level for 15 min, subjects consumed the test meal within 5 min.

The study protocol was approved by the Royal Adelaide Hospital Human Research Ethics Committee and registered. Written informed consent was obtained from all subjects prior to their inclusion.

Stabilization of Blood Glucose Concentrations

An insulin-glucose clamp was started at $T = -30$ min with a continuous infusion of human insulin (Actrapid; Novo Nordisk Pharmaceuticals, Auckland, New Zealand). Insulin (50 IU) was drawn up into 50 mL normal saline. The protocol was modified from a hypoglycemic clamp algorithm administered to patients with type 1 diabetes (12). The infusion for the current study was commenced at 125 mU/m^2 per min and then titrated over 10 min to a maintenance rate of 40 mU/m^2 per min. A 25% dextrose infusion was given simultaneously at a varying rate to maintain the blood glucose at the desired level (12). On the euglycemic study days, blood glucose was maintained at 6.0 mmol/L (108 mg/dL) from $T = -30$ min to the completion of the study at $T = 180$ min with 25% dextrose infused at rates of 50–200 mL/h. During the hypoglycemic studies, the blood glucose was stabilized at 2.6 mmol/L (47 mg/dL) with infusion of 25% glucose at rates of 0–12 mL/h for 15 min prior to meal ingestion and then maintained at this blood glucose concentration for a further 45 min after completion of the meal (12). The blood glucose was then titrated to 6.0 mmol/L over a 30-min period (i.e., to $T = 75$ min) and maintained at 6.0 mmol/L for the remainder of the study (Fig. 2).

Starting immediately before the commencement of the insulin-glucose clamp, venous blood samples for measurement of glucose were taken every 5 min until $T = 90$ min and then every 15 min until study completion at $T = 180$ min. Blood glucose concentrations were measured using a portable glucose meter (Optium Xceed; Abbott Laboratories, Bedford, MA) (12).

Measurement of Gastric Emptying

The test meal comprised 100 g lean minced beef, labeled with 20 MBq ^{99m}Tc-sulfur-colloid (Pharmalucence, Inc., Bedford, MA) (12) and 3 g 3-*O*-Methyl-D-glucopyranose (3-OMG) (Sigma-Aldrich, Sydney, Australia) dissolved in 150 mL water. The solid meal was consumed over 5 min, followed by ingestion of the 3-OMG–labeled water. Scintigraphic

data were acquired with a γ camera (Digirad, Poway, CA) placed over the abdomen of the participant to obtain a left anterior oblique image. Subjects were lying in a supine position with the upper body at an angle of ~30 degrees. Data were acquired from meal completion (T = 0 min) in 1-min frames for 180 min and corrected for radionuclide decay, γ-ray attenuation (using the left anterior oblique view), and subject movement (15). Radioisotopic data were analyzed by a nuclear medicine scientist (K.L.J.) who was not present during studies and remained blinded to both the treatment arm and the glycemic period assigned. A region of interest was drawn around the total stomach, and a gastric-emptying curve, expressed as total retention over time, was derived from this region (15).

Measurement of Glucose Absorption

Serum 3-OMG was used as an index of intestinal glucose absorption (21,22) and was measured using liquid chromatography/mass spectroscopy, with an assay sensitivity of 0.0103 mmol/L (22). After the test meal, blood was collected at T = 15, 30, 45, 60, 90, 120, 150, and 180 min and, once clotted, centrifuged at 3,200 rpm for 15 min. Serum was then stored at −70°C for subsequent measurement of 3-OMG concentrations, with the rate of glucose absorption indicated by the area under the 3-OMG concentration curve (AUC) (22).

Statistical Analysis

Power calculations were performed using our data relating to the effect of exogenous GLP-1 on gastric emptying in health (23). Overall effects for both gastric emptying and 3-OMG absorption were calculated as $AUC_{0–180}$. Given the strict hypoglycemic period occurred in the first 45 min ($AUC_{0–45}$), this period was also predefined as of interest. Data were evaluated using one-way repeated-measures ANOVA, with Bonferroni Holm adjusted post hoc tests for multiple comparisons. Data are shown as mean ± SEM, with the difference between groups (Δ) reported as mean (SD). Given that we have reported an association between glucose absorption and gastric emptying (21,22), we tested for this relationship. This correlation was evaluated adjusted for repeated measures (24). The null hypothesis was rejected at the 0.05 significance level. Statistical analyses were performed using SPSS (version 16.0; SPSS, Chicago, IL). All analyses were supervised by an independent professional biostatistician.

RESULTS

All of the subjects tolerated the study without adverse effects. During hypoglycemia, all volunteers experienced a range of moderate symptoms including sweating, palpitations, and visual disturbance, but there were no major adverse events. One patient experienced mild nausea with GLP-1 during a euglycemic study day. Blood glucose concentrations were effectively clamped at hypoglycemic and euglycemic targets on both GLP-1 (2.7 ± 0.03 and 5.8 ± 0.05 mmol/L) and placebo (2.8 ± 0.04 and 5.9 ± 0.03 mmol/L) study days (Fig. 2).

To maintain the glycemic clamps, substantially less (intravenous) dextrose was required during hypoglycemia compared with euglycemia (hypo/placebo vs. eu/placebo, Δ36 (11) g; P = 0.018). GLP-1 increased intravenous dextrose required during euglycemia (eu/GLP-1 vs. eu/placebo, Δ25 (6) g; $P < 0.01$) and hypoglycemia (hypo/GLP-1 vs. hypo/placebo, Δ23 (8) g; P = 0.03).

Solid Gastric Emptying

Gastric retention (%) over time is shown in Fig. 3. The initial ANOVA for all four curves was significant at both 45 (P = 0.003) and 180 ($P < 0.001$) min. Gastric retention was less during hypoglycemia at both 45 min ($AUC_{0–45}$, hypo/placebo vs. eu/placebo; $P < 0.01$) and overall ($AUC_{0–180}$, $P < 0.001$), consistent with more rapid gastric emptying. Administration of exogenous GLP-1 increased gastric retention during euglycemia ($AUC_{0–180}$, eu/placebo vs. eu/GLP-1; $P < 0.001$) consistent with a profound slowing of gastric emptying. Despite GLP-1 administration, the induction of hypoglycemia accelerated gastric emptying at 45 and 180 min (hypo/GLP-1 vs. eu/GLP-1: $AUC_{0–45}$, P = 0.03, and $AUC_{0–180}$, $P < 0.01$). However, during hypoglycemia GLP-1 slowed gastric emptying at 180 min compared with placebo ($AUC_{0–180}$, hypo/placebo vs. hypo/GLP-1; $P < 0.008$) but not at 45 min ($AUC_{0–45}$, P = 0.10).

Serum 3-OMG Concentrations

Serum 3-OMG concentrations are shown in Fig. 4. The initial ANOVA for all four curves was significant at both 45 and 180 min ($P < 0.001$). During placebo and hypoglycemic GLP-1 studies, there was an initial linear rise in 3-OMG concentration tapering to a peak at 60 min followed by a gradual linear

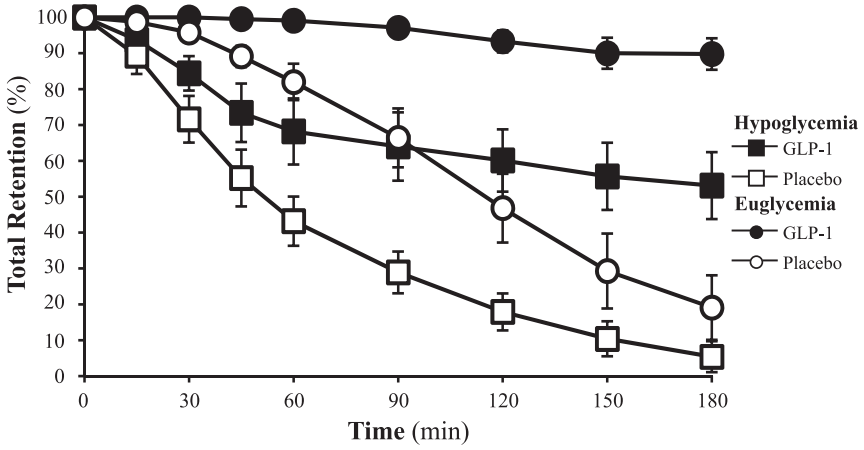

Figure 3—Gastric retention. Gastric retention (%) of test meal in 10 healthy volunteers with blood glucose clamped at either hypoglycemia (2.6 mmol/L) or euglycemia (6.0 mmol/L) with or without GLP-1 (1.2 pmol/kg · min i.v.). Data are presented as mean (SE).

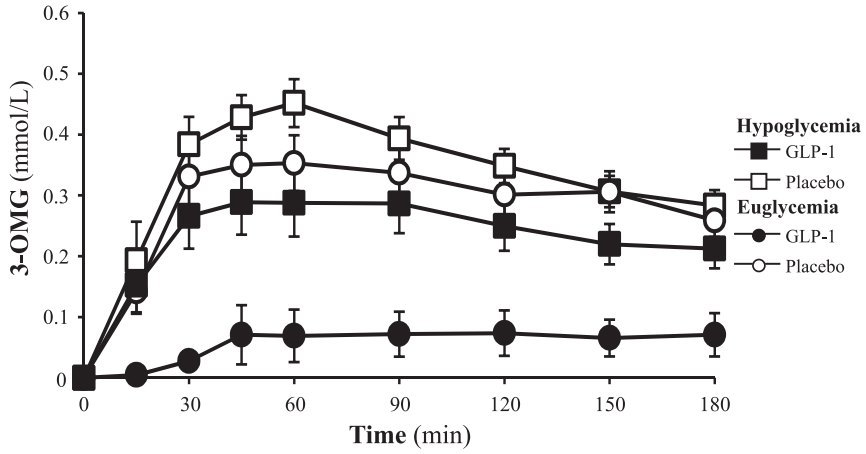

Figure 4—Glucose absorption (serum 3-OMG concentrations). Serum 3-OMG (mmol/L) concentrations in 10 healthy volunteers with blood glucose clamped at either hypoglycemia (2.6 mmol/L) or euglycemia (6.0 mmol/L) with or without GLP-1 (1.2 pmol/kg · min i.v.). Data are presented as mean (SE).

decline. In contrast, during euglycemic GLP-1 studies there was minimal 3-OMG absorption over the first 30 min with a mild rise to 60 min, which then plateaued for the remainder of the studies. Overall, hypoglycemia increased 3-OMG concentrations, ($AUC_{0–180}$, hypo/placebo vs. eu/placebo; $P = 0.03$). However, GLP-1 markedly reduced 3-OMG concentrations during hypoglycemia (hypo/placebo vs. hypo/GLP-1; $AUC_{0–45}$, $P = 0.02$; and $AUC_{0–180}$, $P < 0.01$), as well as during euglycemia ($AUC_{0–180}$, eu/placebo vs. eu/GLP-1; $P < 0.001$).

Relationships Between Glucose Absorption and Gastric Emptying

There was a strong association between 3-OMG absorption and gastric emptying ($AUC_{0–180\%}$ retention and $AUC_{0–180}$ 3-OMG; $r = -0.81$, $P < 0.001$).

CONCLUSIONS

The key finding of this study is that in health, exogenous GLP-1 (1.2 pmol/kg/min) administered acutely attenuates the acceleration of gastric emptying secondary to insulin-induced hypoglycemia and, thereby, the rate of small intestinal carbohydrate absorption.

It is established that hypoglycemia markedly accelerates gastric emptying in healthy subjects (11) and patients with insulin-dependent diabetes (12). The magnitude of the acceleration of emptying that we observed during hypoglycemia was consistent with these previous studies, and while the exact mechanism(s) underlying this effect is incompletely understood, vagal stimulation appears to be important (25).

Furthermore, it is unequivocally established that acute administration of GLP-1 at pharmacological doses profoundly slows gastric emptying at "normal" blood glucose concentrations in health and patients with diabetes (18,23,26). While the underlying mechanisms also remain to be fully elucidated, vagal cholinergic pathways (3,27) and direct centrally mediated effects (28) are probably important.

While the islet cell effects of GLP-1 are glucose dependent, such that with increasing blood glucose concentrations the insulinotropic effects are more pronounced (29), during hypoglycemia the secretion of the counterregulatory hormones are unaffected by GLP-1 (5). Nauck et al. (5) reported that during insulin-induced hypoglycemia (2.3 mmol/L) glucagon, catecholamines, and cortisol concentrations were the same regardless of whether exogenous GLP-1 (1.2 pmol/kg/min) or placebo was infused. Similar results have been reported using the commercial GLP-1 agonists exenatide and liraglutide at therapeutic concentrations in both health and type 1 diabetes (30,31). Furthermore, in health, GLP-1 and exenatide have no effect on C-peptide concentrations during insulin-induced hypoglycemic clamp conditions (5,30). Accordingly, during hypoglycemia even pharmacological doses of GLP-1 have no effect on islet cell secretion. This is the first study to evaluate the effect of a drug that slows gastric emptying during hypoglycemia in humans. A particular strength of our study is that we achieved glucose concentrations during hypoglycemia that are below the glycemic threshold for the glucagon and epinephrine counterregulatory response in older adults and comparable with a "severe" hypoglycemic episode in the community (32). At these glucose concentrations, and in contrast to the glucose-dependent β-cell response described above, we observed that during severe hypoglycemia (2.6 mmol/L), GLP-1 continued to slow gastric emptying, albeit less potently than during euglycemia.

The mechanism(s) underlying the effect observed in our study can only be speculated upon. It is possible that GLP-1–induced slowing of gastric emptying and retardation of delivery of carbohydrate to the small intestine share the same efferent vagal pathways as the "normal physiological" response to hypoglycemia, which is to accelerate gastric emptying and increase delivery of carbohydrate to the small intestine (11). Accordingly, the effects on gastric emptying that we observed in health may not be reproducible in patients with autonomic dysfunction. However, it should also be recognized that the acceleration of gastric emptying by insulin-induced hypoglycemia is still evident in patients with type 1 diabetes who have autonomic dysfunction (12). Further animal or human studies using specific cholinergic antagonists will be required to define the exact contribution of peripheral and central mechanisms (27,33,34).

Although the incretin-based therapies have an inherently low risk of

hypoglycemia, our findings may have important safety implications in patients for whom GLP-1 agonists are prescribed as "add-on" therapy to a sulphonylurea or basal insulin. While these combinations with GLP-1 agonists are associated with a low risk of hypoglycemia (35), our data highlight that the subsequent management of hypoglycemia, should it occur, is potentially problematic. Indeed, our findings related to gastric emptying and glucose absorption suggest that the response to treatment of hypoglycemia with carbohydrate ingestion will be delayed in these patients. While only a trend toward attenuation of solid gastric emptying was observed during GLP-1 and the "strict" hypoglycemic clamp period (0–45mins), the end point of primary clinical relevance is small intestinal glucose absorption, which was substantially reduced compared with placebo on the hypoglycemic days on GLP-1 at both 45 min and for the duration of the study period. While we urge against overinterpreting these data, the observation that GLP-1 slows gastric emptying even during hypoglycemia may have implications for the prescription of other drugs that influence gastric emptying, such as opiates (36), when coadministered to patients who are receiving insulin. Studies of these other agents are now required.

There are, however, limitations to our study. We elected to evaluate the effects of GLP-1 on the response to hypoglycemia in subjects that were not diabetic, as patients with diabetes have the potential for abnormally slow gastric emptying at baseline (37), and it is known that the effect of GLP-1 to slow gastric emptying is dependent on the underlying rate of emptying (22). The effect of hypoglycemia on gastric emptying in patients with type 2 diabetes is yet to be fully elucidated, but the hormonal counterregulatory response is similar, albeit occurring at higher blood glucose concentrations when compared with health (38), so the effect on gastric emptying is unlikely to be diminished.

A continuous infusion of synthetic GLP-1 was administered at a representative pharmacological concentration rather than subcutaneous administration of a commercially available GLP-1 agonist. This ensured predictable GLP-1 concentrations, but the response to the commercially available drugs may not be so consistent. Furthermore, the study design was, of necessity, somewhat "artificial" so that the effects in patients may potentially differ from those observed using a sustained period of hyperinsulinemic hypoglycemia and a predominantly protein "meal." This, of course, does not reflect clinical practice where hypoglycemia is treated with a carbohydrate load and the blood glucose allowed to normalize. It is reassuring that in large clinical trials of combined GLP-1 receptor agonist and basal insulin, the occurrence of severe hypoglycemia (loss of consciousness, seizure, or hypoglycemia requiring third-party intervention) has been very low (0.0–2.5%) (2,39). The extent to which findings account for the low but variable rate of minor hypoglycemia (4–53%) (2,39) is unknown and warrants further investigation.

In conclusion, this study establishes that acute administration of exogenous GLP-1 attenuates, but does not abolish, the acceleration of gastric emptying induced by hypoglycemia in healthy subjects. This is in contrast to the well-documented glucose-dependent glucose-lowering effects of GLP-1 on the pancreatic islet cells. Our data highlight the potential safety implications for the combination of GLP-1 agonists with agents known to induce hypoglycemia, in particular sulphonylureas and insulin. Our observations support ongoing evaluation of the gastrokinetic properties of GLP-1 and its commercially available agonists during hypoglycemia in patients with type 2 diabetes.

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Author Contributions. M.P.P. was responsible for study conception and design, acquisition of data, statistical analysis, interpretation, and drafting the manuscript. K.L.J. was responsible for analysis and interpretation of the scintigraphic data and contributed to the study design and critical revision of the manuscript for important intellectual content. C.E.A. and C.E.C. contributed to the acquisition and interpretation of data. J.J.M., M.J.C., and M.H. contributed to the study design and critical revision of the manuscript for important intellectual content. A.M.D. was responsible for the study conception and design, obtaining funding, acquisition of data, interpretation, and critical revision of the manuscript for important intellectual content. M.P.P. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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