The glucagon-like peptide-1 metabolite GLP-1-(9–36) amide reduces postprandial glycemia independently of gastric emptying and insulin secretion in humans

Juris J. Meier, Arnica Gethmann, Michael A. Nauck, Oliver Götte, Frank Schmitz, Carolyn F. Deacon, Baptist Gallwitz, Wolfgang E. Schmidt, and Jens J. Holst

The gut hormone glucagon-like peptide-1 (GLP-1) lowers postprandial glycemia independently of gastric emptying and insulin secretion in humans. The glucagon-like peptide-1 metabolite GLP-1-(9–36) amide reduces postprandial glycemia independently of gastric emptying and insulin secretion in humans.

Participants. Fourteen healthy male volunteers participated in the study. Their age was 24.2 (SD 2.0) yr, and the body mass index was 24.2 ± 2.2 kg/m². Mean HbA1c was 5.4 ± 0.2% (normal range: 4.8–6.0%), total cholesterol concentrations were 174 ± 24 mg/dl, triglyceride concentrations were 84 ± 6 mg/dl, and triglyceride concentrations were 84 ± 6 mg/dl. Mean HbA1c was 5.4 ± 0.2% (normal range: 4.8–6.0%), total cholesterol concentrations were 174 ± 24 mg/dl, triglyceride concentrations were 84 ± 6 mg/dl. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
21 mg/dl, and fasting glucose concentrations were 94 ± 7 mg/dl. None of the participants had a history of gastrointestinal disorders, had previously undergone abdominal surgery, or was taking any medication with a known modulating effect on gastrointestinal motility. All participants were advised to maintain their usual dietary habits and to avoid strenuous exercise before the experiments.

Blood was drawn from all participants in the fasting state to exclude anemia (hemoglobin <12 g/dl), an elevation in liver enzymes (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, γ-glutamyltransferase) to higher activities than double the respective normal value, or elevated creatinine concentrations (>1.5 mg/dl).

Study design. All participants were studied on four occasions. At a screening visit, blood was drawn in the fasting state for the determination of standard hematological and clinical chemistry parameters, and a physical examination was performed. If subjects met the inclusion criteria, they were recruited for the following tests. On separate occasions, either GLP-1(7–36) amide (1.2 pmol·kg⁻¹·min⁻¹), GLP-1(9–36) amide (1.2 pmol·kg⁻¹·min⁻¹), or placebo was administered intravenously over 390 min (−30 to 360 min). At 0 min, a mixed test meal (250 kcal) was ingested. Capillary and venous blood samples were collected frequently throughout the experiments for the determinations of glucose, GLP-1 (total and intact), insulin, C-peptide, and glucagon, and gastric emptying was determined over 360 min. The tests were carried out in randomized order. An interval of at least 2 days was kept between the tests to avoid carryover effects.

Parts of this study have previously been reported to evaluate the effects of GLP-1 on postprandial lipid concentrations (16).

Peptides. Synthetic GLP-1(9–36) amide was purchased from PolyPeptide Laboratories (Wolfenbüttel, Germany), and synthetic GLP-1(7–36) amide was a kind gift from Restoragen. Peptides were sterile filtered and processed for infusion as described (14).

Experimental procedures. The tests were performed in the morning after an overnight fast, in a supine position with the upper body lifted by 30° throughout the experiments. Two forearm veins were punctured with a Teflon cannula (Moskito 123, 18 gauge; Vygon, Aachen, Germany) and kept patent using 0.9% NaCl (for blood sampling and for peptide/placebo administration).

After basal blood samples were drawn (−45 and −30 min), the experiments were started with the infusion of GLP-1(7–36) amide, GLP-1(9–36) amide, or placebo at −30 min. At 0 min, a standard test meal (one egg, two slices of white bread, 5 g of margarine, 150 ml of water; 250 kcal), containing 100 mg [13C]sodium octanoate was served, and breath samples were collected at 15-min intervals during the first 180 min and at 20-min intervals during the last 180 min. Capillary and venous blood samples were collected at 30-min intervals.

Blood specimen. Venous blood was drawn in chilled tubes containing EDTA and aprotinin (Trasylo; 20,000 kallikrein inhibitor units/ml, 200 µl/10 ml blood; Bayer, Leverkusen, Germany) and kept on ice. After centrifugation at 4°C, plasma for hormone analyses was kept frozen at −28°C. This procedure has previously been shown to prevent in vitro degradation of incretin hormones in human plasma samples (4, 18). Capillary blood samples (~100 µl) were added to NaF (Microwette CB 300; Sarstedt, Nümbrecht, Germany) for the immediate measurement of glucose.

Determination of gastric emptying. Gastric emptying was determined as described (14). Briefly, [13C]sodium octanoate (100 mg; Euriso-top, Saint-Aubin Cedex, France) was used to label the solid component of the test meal. At intervals of 15 or 20 min, breath specimens were sampled in gas-tight plastic bags, and the 13CO₂ content was determined within 24 h using nondispersive infrared spectrometry (Wagner Analysetechnik, Bremen, Germany).

To measure the proportion of the substrate given by mouth that is metabolized, the results were expressed as a percentage dose of 13C recovered (PDR) over time for each time interval from which the cumulative PDR (cPDR) for each time interval was calculated, according to Ghoos et al. (8).

The evaluation of the octanoate breath test for gastric emptying was done by nonlinear regression analysis (Graph PAD Prism, version 2, San Diego, CA) of the 13C excretion curves (PDR) to the formula: PDR(t) = at e⁻kt, which has been derived from the chi square distribution in statistics. The percentage of 13CO₂ cumulative values was fitted using a model given by cPDR(t) = M(1 − e⁻kt)β, where y is cPDR at time t in hours and m, k, and β are regression estimated constants, with M the total amount of 13CO₂ expired when time is infinite.

Gastric emptying was expressed as a percentage of the initial gastric contents (M = 100%) by computing the difference to this initial value at each time point according to the following formula: gastric content(t) = (M − cPDR(t))/M × 100 (%).

Laboratory determinations. Glucose was measured as described (14) using a Glucose Analyser 2 (Beckman Instruments, Munich, Germany).

Insulin was measured as described (14) using an insulin microparticle enzyme immunoassay (IMX Insulin; Abbott Laboratories, Wiesbaden, Germany). Intra-assay coefficient of variation was ~4%.

C-peptide was measured as described (14) using an ELISA from DAKO (Cambridgeshire, UK). Intra-assay coefficients of variation were 3.3–5.7%, and interassay variation was 4.6–5.7%. Human insulin and C-peptide were used as standards.

Immunoreactive glucagon was measured in ethanol-extracted plasma using antibody 4305 in ethanol-extracted plasma, as previously described (11). The detection limit was <1 pmol/l. Intra-assay coefficients of variation were 6.7%, and interassay coefficients of variation were 16%.

GLP-1 immunoreactivity was determined using two different assays. The COOH-terminal assay measures the sum of the intact peptide plus the primary metabolite GLP-1(9–36) amide using the antiserum 89390 and synthetic GLP-1(7–36) amide as standard. This assay cross-reacts <0.01% with COOH-terminally truncated fragments and 83% with GLP-1-(9–36) amide. The detection limit was 3 pmol/l. Intra-assay and interassay coefficients of variation were <6 and 15%, respectively, at 40 pmol/l. Intact GLP-1 was measured in unextracted plasma using an ELISA (36). The assay is a two-site sandwich assay using the following two monoclonal antibodies: GLP-1F5 as catching antibody (COOH-terminally directed) and Mab26.1 as detecting antibody (NH₂-terminally directed). It reacts <0.1% with GLP-1 precursors extended from the NH₂ terminus and NH₂-terminally truncated peptides, including GLP-1-(9–36) amide. The detection limit was 0.5...
GLP-1 plasma levels. During the administration of GLP-1-(7–36) amide, steady-state concentrations of 139 ± 15 pmol/l were reached for total and 21 ± 5 pmol/l for intact GLP-1. During the infusion of GLP-1-(9–36) amide, total GLP-1 levels were raised to 88 ± 9 pmol/l, whereas intact GLP-1 concentrations were not changed compared with placebo experiments (5 ± 3 pmol/l; Fig. 1).

Plasma glucose concentrations increased significantly after the test meal during placebo administration (P < 0.0001; Fig. 2). This was accompanied by a significant rise in plasma concentrations of insulin and C-peptide (P < 0.0001; Fig. 2). Administration of GLP-1-(7–36) amide led to an increase in insulin and C-peptide levels before meal ingestion (−15 to 0 min), which caused a reduction in glucose concentrations. The meal-related increases in glycemia and in insulin and C-peptide concentrations were markedly influenced by the administration of GLP-1-(7–36) amide. When GLP-1-(9–36) amide was infused, no differences in insulin or C-peptide concentrations were observed compared with the placebo experiments, but postprandial glucose excursions were lower during the administration of placebo and GLP-1-(9–36) amide, respectively; Fig. 2]. Likewise, the incremental area under the glucose curve was significantly lower during the infusions of GLP-1-(7–36) amide (265 ± 112 mg·kg⁻¹·min) and GLP-1-(9–36) amide (686 ± 127 mg·kg⁻¹·min) compared with placebo administration [1,229 ± 215 mg·kg⁻¹·min, P = 0.0012 vs. GLP-1-(7–36) amide and P = 0.041 vs. GLP-1-(9–36) amide]. However, the overall reduction in postprandial glycemia was much smaller.
during the administration of GLP-1-(9–36) amide than with GLP-1-(7–36) amide (Fig. 2).

To further evaluate whether glucose-stimulated insulin secretion was affected by the GLP-1 metabolite, plasma insulin concentrations were expressed in relation to the ambient glucose concentrations (Fig. 3). No differences in the time pattern of the glucose-to-insulin ratio were found between the experiments with the administration of GLP-1-(9–36) amide and placebo (Fig. 3).

Glucagon plasma concentrations were significantly lower during the administration of GLP-1-(7–36) amide compared with placebo at t = 0 min, as well as from 210 to 300 min, and significantly higher at t = 60 min (Fig. 4). Even though the overall time pattern of glucagon concentrations appeared to be similar between the experiments with the administration of GLP-1-(9–36) amide and placebo, a significant difference was found at the 60-min time point (Fig. 4; P = 0.016).

Gastric emptying was almost completely assessed during the 360-min observation period in the placebo experiments (Fig. 5). As expected, GLP-1-(7–36) amide administration led to a marked deceleration of gastric emptying (P < 0.0001). In contrast, the time pattern of gastric emptying was unchanged by the administration of GLP-1-(9–36) amide. The proportion of the test meal retained in the stomach after 360 min was 22 ± 4% during placebo administration, 50 ± 7% during the infusion of GLP-1-(7–36) amide (P = 0.0019 vs. placebo), and 18 ± 3% during GLP-1-(9–36) amide administration (P = 0.43 vs. placebo).

**DISCUSSION**

The present studies were undertaken to investigate the influence of the primary glucagon-like peptide 1 metabolite GLP-1-(9–36) amide on gastric emptying and on postprandial glucose homeostasis compared with intact GLP-1-(7–36) amide and placebo. Consistent with previous reports (14, 23, 34), administration of intact GLP-1-(7–36) amide led to a marked inhibition of gastric emptying and stimulated insulin secretion in the fasting state. After meal ingestion, insulin secretory responses were reduced significantly by intact GLP-1, most likely because the entry of nutrients in the circulation was delayed (14). In contrast, gastric emptying and insulin secretion were not affected by the administration of GLP-1-(9–36) amide. However, despite the lack of GLP-1 effect on gastric motility and insulin secretion, the postprandial rise in plasma glucose concentrations was significantly lower during GLP-1-(9–36) amide infusion than during the placebo experiments, suggesting that the GLP-1 metabolite exerts independent glucose-lowering effects.

One obvious question arising from these studies is what mechanism mediated the reduction in postprandial glucose concentrations during GLP-1-(9–36) amide infusion. The most likely explanation seems to be increased glucose disposal induced by the GLP-1 metabolite. Indeed, even though the overall impact of GLP-1 and its metabolites on insulin action and glucose disposal has been discussed controversially (29), a number of observations tend to support this notion. Thus GLP-1 binding has been shown in muscle and adipose tissue (7, 9, 28, 32), and Yang et al. (37) demonstrated enhanced insulin-induced glycogen synthesis in isolated myotubes. Moreover, some investigators found glucose effectiveness during an intravenous glucose tolerance test to be increased by GLP-1 in humans (2, 10), and one study reported a significant increase in glucose disposal during a hyperglycemic clamp (19). Furthermore, regarding the metabolite GLP-1-(9–36) amide, one previous study described increased glucose elimination rates in pigs (5), and a recent study by Nikolaidis et al.
(24) demonstrated enhanced myocardial glucose uptake during intravenous infusion of GLP-1-(9–36) amide in dogs. Taken together, these findings suggest that, under certain circumstances, GLP-1 exerts a modest effect on glucose disposal that may be mediated by the primary metabolite GLP-1-(9–36) amide.

On the other hand, studies employing the pancreatic clamp technique, in which any confounding influences of increased insulin secretion are eliminated because of the action of somatostatin, failed to reveal any effects of GLP-1 on peripheral glucose uptake (30, 31). However, because the insulinotropic and glucagonostatic effects of GLP-1 are known to be strictly glucose dependent (21, 22), it appears possible that the GLP-1 effects on glucose disposal would only become apparent at hyperglycemic conditions as well. This would also explain why, in the present studies, a glucose-lowering effect of GLP-1-(9–36) amide could only be detected in the immediate postprandial period. In light of these considerations, it seems possible that the lack of GLP-1 effect on glucose disposal observed in some previous studies was because of the use of euglycemic conditions (30, 31). Moreover, given the multiple metabolic effects of somatostatin, minor changes in glucose metabolism, such as those observed in the present studies, could have easily been obscured because of the complexity of such experimental systems (29).

The present results also seem to be at variance with a previous study by Vahl et al. (27), who found no effects of GLP-1-(9–36) amide on glucose elimination in healthy human subjects. It is therefore important to point out some differences in the study designs of these two studies. In the experiments by Vahl et al. (27), glucose elimination was assessed after an insulin-modified intravenous glucose tolerance test, whereas in the present studies plasma glucose levels were measured after a mixed test meal. Moreover, the total GLP-1 plasma levels achieved during the exogenous infusion of GLP-1-(9–36) amide were approximately twofold higher in the present experiments (~90 pmol/l) than in those previous studies (~50 pmol/l; see Ref. 27). Because the glucose-lowering activity of GLP-1-(9–36) amide was modest in the present studies (~6 mg/dl at t = 90 min), it seems plausible that any effects of the GLP-1 metabolite would only become relevant at higher plasma concentrations.

Similar to previous studies (10, 22), we observed a significant suppression of glucagon secretion during the infusion of intact GLP-1. In contrast, although the overall concentration time pattern during the administration of GLP-1-(9–36) amide was rather similar to the placebo experiment, a significant increase in glucagon levels was detected at the 60-min time point. Given the lower plasma glucose concentrations measured at the same time, this transient increase in glucagon levels is difficult to explain. Thus the regulation of α-cell secretion is complex and can be influenced by circulating fuel substrates (especially glucose and amino acids), as well as by different paracrine (e.g., via somatostatin), endocrine (e.g., via insulin, GLP-1, gastric inhibitory polypeptide, gastrin, and CCK), and neuronal factors (11, 13, 15, 26). The present data therefore do not allow any conclusions to be made regarding the potential mechanisms that mediated the GLP-1-(9–36) amide effects on glucagon concentrations. However, because glucagon levels were somewhat higher rather than lower during GLP-1-(9–36) amide administration, the glucose-lowering effect of the GLP-1 metabolite cannot be attributed to any changes in glucagon secretion.

In light of the current attempts to employ inhibitors of DPP IV for the pharmacotherapy of type 2 diabetes (1, 6), one important question is whether a reduction in GLP-1-(9–36) amide plasma concentrations induced by DPP IV inhibition would bear any consequences for the regulation of glucose metabolism and gastrointestinal motility. With regards to gastric emptying, our data do not suggest a role for the GLP-1 metabolite. Arguably, any antagonistic effect of GLP-1-(9–36) amide on the gastric GLP-1 receptor may only be relevant after the ingestion of a larger test meal more capable of rising endogenous GLP-1 secretion than the 250-kcal test meal chosen for the present studies (33). However, because the affinity of GLP-1-(9–36) amide to the gastric GLP-1 receptor is ~100-fold lower than that of intact GLP-1-(7–36) amide (12), these effects seem to be of minor importance under physiological conditions.

In conclusion, the present studies support the notion that GLP-1-(9–36) amide exerts independent glucose-lowering effects in humans. The reduction in postprandial glycemia by the GLP-1 metabolite is independent of changes in gastric emptying and insulin or glucagon secretion. However, the overall magnitude of the glucose reduction was rather modest despite approximately fivefold supraphysiological plasma concentrations of GLP-1-(9–36) amide. Therefore, the glucose-lowering potential of GLP-1-(9–36) amide seems rather small compared with that of intact GLP-1-(7–36) amide.

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REFERENCES


