Glucagon-like peptide 1 increases the period of postprandial satiety and slows gastric emptying in obese men

Erik Näslund, Mark Gutniak, Staffan Skogar, Stephan Rössner, and Per M Hellström

ABSTRACT The gut peptide glucagon-like peptide 1(7-36) amide (GLP-1) is released into the circulation after food intake. GLP-1 has been shown to have an incretin effect and inhibits gastrointestinal motility in humans. In rats, intracerebral administration of GLP-1 results in reduced food intake. Obese humans have been found to have an attenuated plasma GLP-1 response to a mixed meal. To approximate the physiologic state, GLP-1 or saline was administered intravenously and randomly at the beginning of a test meal served on a universal eating monitor to 6 obese subjects to test our hypothesis that GLP-1 influences termination of food intake (and thus food intake during a meal) and feelings of satiety in humans. As a marker for gastric emptying, 1.5 g acetaminophen was given at the start of the meal. Blood samples for analysis of acetaminophen, insulin, glucose, glucagon, and C-peptide were obtained. Hunger, fullness, and food choice were assessed with visual analogue scales and food-choice questionnaires. GLP-1 infusion resulted in a prolonged period of reduced feelings of hunger, desire to eat, and prospective consumption after the meal. The rate of gastric emptying was slower during infusion of GLP-1. Postprandial blood glucose concentrations were reduced during the GLP-1 infusion, but the amount of energy consumed, eating rate, and plasma concentrations of insulin, glucagon, and C-peptide were unchanged. GLP-1 given exogenously at the start of a meal did not seem to affect meal termination or the amount of food eaten. However, postprandial feelings of hunger decreased, suggesting that exogenous GLP-1 may influence feelings of hunger and satiety in humans. Am J Clin Nutr 1998;68:525–30.

KEY WORDS Glucagon-like peptide 1, GLP-1, glucose, insulin, glucagon, C-peptide, satiety, gastric emptying, visual analogue scales, food intake, obesity, humans, men

INTRODUCTION

The regulation of food intake is a complex process involving psychologic, social, and physiologic components. Physiologically, it is generally assumed that food intake is regulated by a central feeding drive that is later counterregulated by peripheral satiety signals that are activated during a meal. These satiety signals have been suggested to include gut peptides and multiple signals via afferent signals from the gastrointestinal tract via the vagal nerves (1–4). GLP-1 is a peptide of 30 amino acid residues that is produced in L cells of the intestinal mucosa and secreted into the circulation after intake of a mixed meal. It has a sequence homology with glucagon of ~50% and arises as the result of proteolytic cleavage of proglucagon (6, 7). In the rat brain, GLP-1 immunoreactive cell bodies have been found in the caudal portion of the solitary tract and the dorsal and ventral parts of the medullary reticular nuclei, corresponding to regions that receive afferent signals from the gastrointestinal tract via the vagal nerves (8). GLP-1 immunoreactive nerve fibers have been identified in the hypothalamic paraventricular nucleus (PVN) and the periventricular strata (8–10). GLP-1 receptors have been found in thalamic and hypothalamic nuclei and in the brainstem (10, 11). In addition, GLP-1 binding sites have been found in the sensory circumventricular organs, including the subfornical organ and area postrema. These regions of the circumventricular organs lack the perivascular blood-brain barrier and allow a free exchange of molecules between blood and cerebrospinal fluid (12–14). Therefore, because of the high concentrations of GLP-1 immunoreactive fibers and cells in the PVN and periventricular areas, from where food intake is controlled (15), it seems reasonable that GLP-1 may influence food intake.

Several reports have shown that intracerebroventricular (ICV) injection of GLP-1 in rats inhibits food intake (16–18). In addition, central administration of GLP-1 has been shown to inhibit the intake of water in rats (17). The ICV administration of exendin (9-39) amide (exendin), a GLP-1 receptor antagonist, to satiated, but not fasted, rats results in an increased food intake (18). Administration of exendin twice daily for 10 d increased food intake, resulting in a significant weight gain in the treated rats (19).

Obese subjects seem to have an attenuated release of GLP-1 in response to meals (20, 21). These findings and the fact that
GLP-1 influences food intake in rats suggest that obese subjects may have a defective GLP-1 response that perpetuates overeating. The aim of the present study was to examine whether GLP-1 administered intravenously to obese men at the start of a test meal affected termination of a meal and food intake during the meal, food preference, and feelings of satiety postprandially. Concomitantly, the rate of gastric emptying was studied by using an acetaminophen absorption technique.

SUBJECTS AND METHODS

Subjects

Six healthy, male, obese subjects aged 34.7 ± 3.3 years with a mean (± SEM) body mass index (BMI; in kg/m²) of 35.6 ± 1.8 were recruited from patients attending the outpatient Obesity Unit at the Karolinska Hospital and the waiting list for obesity surgery at Danderyd Hospital. None of the subjects had undergone previous gastrointestinal surgery or were taking any medication. Informed consent was obtained from the subjects and the study was approved by the Ethics Committee of the Karolinska Hospital.

Study protocol

Subjects were instructed to fast from midnight before the study day, to refrain from smoking from midnight before the study day, and to refrain from alcohol consumption and exercise for 24 h before the study day. Subjects reported to the Obesity Unit in the morning, at which time an indwelling catheter was placed in each antecubital vein. The study was performed in a randomized, double-blind fashion on 2 occasions 5 d apart. Randomization was performed by the Karolinska Hospital Pharmacy when preparing the solutions for intravenous administration. Immediately before the start of food intake, the intravenous infusion of either GLP-1 (0.75 pmol GLP-1 · kg⁻¹ · min⁻¹; Bachem AG, Bubendorf, Switzerland) or saline (9 g/L; Natriumklorid Pharmacia, Pharmacia & Upjohn, Stockholm) began and continued for 210 min.

Universal eating monitor

The test meal was served on a universal eating monitor (UEM) (VIKTOR; Huddinge University Hospital, Stockholm) at 1200. The UEM was described in detail elsewhere (22). Briefly, the UEM consists of a plate containing a large homogeneous meal that is placed on a scale connected to a computer. The total food intake, meal duration, eating rate, and relative rate of consumption, defined as food intake during the first half of the meal minus food intake during the second half of the meal divided by total food intake, was measured by the UEM. The test meal was an industrially produced Swedish hash with a standard energy content of 6.3 kJ (1.5 kcal)/g consisting of fried diced meat, onions, and potatoes (Nestlé AB, Helsingborg, Sweden). The meal contained 16% of energy from protein, 41% from carbohydrate, and 43% from fat.

Assessment of hunger and food choice

Food choice and feelings of hunger were assessed by the use of a questionnaire 15 min before, immediately after, and hourly for 4 h after food intake. The desire to eat, hunger, fullness, prospective consumption, and meal satisfaction were assessed with visual analogue scales (VASs) (23). After completion of the test meal, subjects were asked to assess how pleasant they found the meal on a VAS. Before and after the test meal, the subjects were prompted to check off on a list of 32 food items those that they would like to eat immediately. The food items represented 4 food groups, 8 food items from each of the following groups: high protein, high fat, high carbohydrate, and low energy. The subjects were also prompted to choose between 32 pairs of high-carbohydrate and high-protein items (23). The forced-choice list is designed to reveal a specific preference for proteins or carbohydrates. The results of the VAS and food-choice and forced-choice lists were interpreted by a registered dietitian not involved in the project.

Gastric emptying

The rate of gastric emptying was assessed by measuring acetaminophen absorption from the intestine after oral ingestion of a marker (24). Plasma concentrations of acetaminophen were measured 0, 15, 30, 60, 90, 120, and 180 min after ingestion of 1.5 g acetaminophen with the test meal. Plasma samples were stored at −20°C until analyzed for acetaminophen by fluorescence immunoassay (IMX; Abbott Laboratories, Chicago). The acetaminophen assay had a CV of 5%.

Assay for glucagon, insulin, C-peptide, and glucose

Blood samples were collected in heparin-containing tubes 30 and 15 min before food intake, at the start of food intake, and 15, 30, 45, 60, 90, 120, 150, 180, and 210 min after food intake for the analysis of glucose and plasma concentrations of glucagon, insulin, and C-peptide. Blood samples were placed on ice until centrifuged. The samples were centrifuged at 2000 × g at 4°C for 10 min. Plasma was collected and stored at −20°C for analysis in one series.

Glucagon was assayed with a previously described radioimmunoassay technique (25). The glucagon assay is directed against the carboxyl terminal of the glucagon molecule (antibody code no. 4305) and therefore measures glucagon of mainly pancreatic origin. The detection limit of the assay was 1 pmol/L and the CV was 5%. Insulin was analyzed with a sandwich enzyme immunoassay (Insulin Kit K6219; DACO, Copenhagen). The assay cross-reacts to 0.3% with proinsulin but not with C-peptide. The detection limit of the assay was 20.7 pmol/L and the CV was 8%.

C-peptide concentrations in plasma were determined with a radioimmunoassay (Euro-Diagnostica, Malmö, Sweden). The assay cross-reacts to 41% with proinsulin but not with insulin. The detection limit was 50 nmol/L, and the CV was 5%. Glucose was analyzed with an enzyme assay (aldose 1-epimerase and glucose dehydrogenase) with a Hitachi 917 automatic analyzer and reagents from Boehringer Mannheim GmbH (Mannheim, Germany).

Statistics

Values are given as means ± SEMs or medians and ranges as appropriate. For results of the desire to eat, hunger, prospective consumption, fullness ratings (VAS), and total number of items selected from the food-choice list, the difference between the results obtained immediately after and 4 h after food intake were used for statistical evaluation. The results from the UEM, the VAS, and the food-choice and forced-choice lists were analyzed by using Wilcoxon’s signed-rank test for matched pairs. Plasma concentrations of glucose, glucagon, insulin, and C-peptide were
analyzed by repeated-measures multivariate analysis of variance (MANOVA). The gastric emptying profile was estimated after conversion of acetaminophen plasma concentration to accumulated concentrations, ie, total absorption of the drug. Values are expressed in relation to maximal absorption under control conditions. In this way we obtained a gastric emptying curve from 0% to 100%, adapted for a third-degree polynomial for each subject. The actual plasma acetaminophen concentrations were used and analyzed by repeated-measures MANOVA. A $P$ value <0.05 was considered statistically significant. STATISTICA (StatSoft, Tulsa, OK) was used for the analyses.

RESULTS

No effect of GLP-1 was seen on the amount of energy consumed, eating rate, or prospective consumption (Table 1). Feelings of hunger, desire to eat, and prospective consumption of food decreased after food intake. The median difference between the ratings obtained immediately after food intake and those obtained 4 h after intake was significantly lower with GLP-1 than with saline infusion (Figures 1 and 2; data shown for hunger and prospective consumption only). Feelings of fullness increased after food intake, but the median difference between the rating obtained immediately after food intake and that 4 h after intake was not significantly different between GLP-1 (median: 13.5 mm; range: 9–43 mm) and saline (median: 32 mm; range: 12–83 mm) infusion ($P = 0.4$). The median difference in the total number of items selected from the food-choice list immediately after food intake and that 4 h after intake was significantly lower with GLP-1 (median: 0.5 mm; range: 1–3 mm) than with saline (median: 7.5 mm; range: 3–17 mm) infusion ($P = 0.03$). There was no significant difference in the proportion of food selected as carbohydrate, protein, fat, or low energy from the food-choice list between the GLP-1 infusion and the saline infusion (data not shown). The forced-choice list showed no significant difference in the ratio of high-carbohydrate to high-protein items selected between the GLP-1 infusion [carbohydrate (median: 3.5; range: −5 to 1) and protein (median: 3.5; range: −5 to 5)] and the saline infusion [carbohydrate (median: −1.5; range: −13 to 9) and protein (median: 2.0; range: 0–13)] ($P = 0.7$). There was no significant difference in the pleasantness rating of the meal between the GLP-1 infusion (median: 75 mm; range: 26–86 mm) and the saline infusion (median: 74.5 mm; range: 57–95 mm) infusion ($P = 0.1$).

Gastric emptying was significantly slower during GLP-1 infusion than during saline infusion, as reflected by a reduced absorption of acetaminophen (Figure 3; $P = 0.001$). Postprandial concentrations of blood glucose were significantly lower during GLP-1 infusion than during saline infusion (Figure 4; $P = 0.001$). There was no significant difference in plasma concentrations of glucagon, insulin, or C-peptide between the GLP-1 infusion and the insulin infusion.

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### TABLE 1

Results from the universal eating monitor during infusion of saline or glucagon-like peptide 1 (GLP-1) in obese men

<table>
<thead>
<tr>
<th></th>
<th>Saline ($n = 6$)</th>
<th>GLP-1 ($n = 6$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total food intake (g)</td>
<td>493 (216–687)</td>
<td>464 (207–685)</td>
</tr>
<tr>
<td>Meal duration (min)</td>
<td>8.4 (3.8–18.3)</td>
<td>8.2 (3.7–13.8)</td>
</tr>
<tr>
<td>Eating rate (g/min)</td>
<td>47 (26–118)</td>
<td>55 (24–120)</td>
</tr>
<tr>
<td>Relative rate of consumption (g)$^2$</td>
<td>0.15 (0.09–0.22)</td>
<td>0.10 (0.03–0.22)</td>
</tr>
</tbody>
</table>

$^2$Median; range in parentheses. There were no significant differences between groups by Wilcoxon’s signed-rank test for matched pairs.

$^3$Food intake in the second part of the meal minus food intake in the first part of the meal divided by total food intake.

### FIGURE 1

Median (horizontal bar within box) hunger ratings on visual analogue scales before and after a test meal during infusion of saline (A) or glucagon-like peptide 1 (GLP-1) (B) in 6 obese men. The median difference between the rating obtained immediately after food intake (5 min) and that 240 min after food intake was significantly lower during GLP-1 infusion ($P = 0.03$, Wilcoxon’s signed-rank test for matched pairs). 75th percentile, upper limit of box; 25th percentile, lower limit of box; 90th percentile, edge of upper limit; 10th percentile, edge of lower limit.
DISCUSSION

This study showed that GLP-1 administered to 6 obese men at the beginning of a meal retarded gastric emptying and simultaneously evoked lower postprandial hunger ratings. The characteristics of food intake were similar to those previously obtained for obese men (22), but we did not find any influence of GLP-1 on food intake during the test meal when GLP-1 was administered at the start of the meal. This finding differs with the findings of 2 other studies of the effect of GLP-1 on food intake in normal-weight humans (26, 27). In the first study (26), GLP-1, infused at 0.375, 0.75, or 1.5 pmol·kg\(^{-1}\)·min\(^{-1}\) into normal-weight subjects for 60 min before and continuing for 60 min after a test meal, shortened the duration of the meal and decreased the amount of energy consumed in a dose-dependent manner. The authors also noted a reduction in feelings of hunger and early fullness in the fasting state with the higher dose given. In the second study (27), infusion of GLP-1 or saline began at the start of an energy-fixed breakfast in normal-weight males and continued for 4 h. The infusion was discontinued for 30 min and then resumed at the start of an ad libitum lunch and continued for the duration of the meal. Appetite ratings were lower during GLP-1 infusion than during saline infusion as was food intake at lunch. One explanation for the difference between our results for food intake and those of the 2 studies cited above was the loading period used in these 2 studies, ie, the infusion took place for 60 min (26) and 4 h (27) before the test meal began, thereby allowing a period of satiety to be induced before the test meal, whereas in the present study the infusion began at the same time as the test meal. Our results indicate that GLP-1 administered in a manner that approximates the physiologic state—ie, given at the beginning of a meal, when GLP-1 normally rises in plasma (21)—does not influence eating behavior nor acts as a meal terminator. Yet, it is possible that exogenous GLP-1 reduces food intake in obese humans as well if administered for a period before a meal.

Our results indicate that infusions of GLP-1 result in a longer period of decreased feelings of hunger, a decreased desire to eat, and a diminished feeling of prospective consumption after a test meal. The central administration of leptin and GLP-1 both elevate c-fos-like immunoreactivity in the PVN and central amygdala. However, leptin selectively elevated c-FLI in the dorsomedial hypothalamus, whereas GLP-1 selectively elevated c-fos-like immunoreactivity in the nucleus of the solitary tract, area postrema, lateral parabrachial nucleus, and arcuate hypothalamic nucleus (28). These findings suggest the possibility that leptin and GLP-1 activate different regions of the central nervous system and have different roles in the regulation of food intake.
Leptin has been proposed to have a physiologic role in maintaining long-term energy balance (5). Our results indicate that GLP-1 may increase short-term postprandial satiety. The mechanisms by which GLP-1 influences postprandial satiety can be central or peripheral. In rats, GLP-1 has been shown to cross the blood-brain barrier (29) and an ICV injection of GLP-1 was found to increase c-fos expression in the PVN (18), an area known to influence food intake in rats. However, no effect on food intake was found after intraperitoneal injection of GLP-1 in rats (17, 18), suggesting a central rather than a peripheral mode of action. For comparison, GLP-1 infused into humans inhibits acid secretion, but in vagotomized humans this inhibitory effect was not seen (30). This suggests that the effect of GLP-1 may be mediated centrally or via GLP-1 receptors on vagal afferent pathways in a manner similar to that of vagal cholecystokinin receptors (31).

Our results showed a reduced rate of gastric emptying during GLP-1 infusion in obese subjects concomitantly with decreased feelings of postprandial hunger. This inhibitory effect of GLP-1 on gastric emptying has been reported in both normal subjects and in patients with diabetes and it was reported recently that the inhibitory effect of GLP-1 on gastric emptying outweighs its insulinotropic effects (32–34). GLP-1 has been suggested to be a putative candidate hormone for the ileal-brake function and inhibits upper gastrointestinal tract motility in response to nutrient stimulation of the lower gut (32). In humans, satiety is induced if emulsified lipids are infused into the duodenum at the same time that the stomach is distended (35), and hunger is suppressed if lipid is infused into the jejunum without stomach distention (36). The gastrointestinal tract is equipped with mechano- and chemosensitive receptors that relay information to the central nervous system via the vagus nerve (3, 37). It is possible that the decreased rate of gastric emptying seen after infusion of GLP-1 results in a prolonged stimulation of chemosensitive receptors in the stomach and small intestine, which in turn may lead to a prolonged period of satiety after food intake.

It has been suggested that GLP-1 is an incretin hormone (38), and another possible peripheral mechanism for GLP-1 on satiety could be effects on blood and plasma concentrations of glucose and insulin. Intraportal infusions of glucose have been shown to decrease food intake in animals; however, in the present study blood concentrations of glucose decreased after the meal, making it unlikely that this is a mechanism inducing satiety for GLP-1. Insulin has been shown to cross the blood-brain barrier (39) and to induce a decrease in food intake during ICV administration (40). We did not show a significant difference in plasma insulin concentrations between subjects who received the GLP-1 infusion and those who received the saline infusion, a result that was shown by others (41). Therefore, it is unlikely that an effect of GLP-1 on food intake is mediated by changes in plasma insulin concentrations.

It has been suggested in some animal studies that ICV administration of GLP-1 results in taste aversion (42). There was no significant difference in how pleasant the subjects found the test meal during GLP-1 or saline infusion. This rating of meal satisfaction was performed after the meal and we did not specifically address nausea in this study. However, in a subsequent study performed at our institution, in which GLP-1 or saline was infused at the same doses as in the present study in 8 obese subjects for 8 h, no significant difference was seen in nausea ratings on a VAS that was completed hourly during the day (unpublished observation, 1997). In the present study, we were unable to detect any specific change in nutrient selection in the food-choice list or any difference in the selection of high-carbohydrate or high-protein items selected in the forced-choice list. These findings suggest that GLP-1 exerts a global postprandial satiety effect, not specific for any type of nutrient and independent of inducing nausea.

In agreement with others (20), we found previously that obese subjects have an attenuated release of GLP-1 after a test meal (21). Thus, it is possible that the lower GLP-1 response in obese subjects after a meal may result in a shorter period of postpran-
dial satiety, which should require more frequent food intake to maintain a reasonable individual satiety level. Therefore, GLP-1 administered to obese subjects may have a weight-reducing and slimming effect if given over a prolonged period of time. In summary, this study showed that GLP-1 administered to obese subjects induced a prolonged period of postprandial satiety and slower rate of gastric emptying than did saline infusion. This effect of GLP-1 on hunger may be mediated through central mechanisms or vagal afferent pathways in concert with prolonged gastric emptying.

REFERENCES