Glucagon-like Peptide 1 Promotes Satiety and Suppresses Energy Intake in Humans

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Abstract

We examined the effect of intravenously infused glucagon-like peptide 1 (GLP-1) on subjective appetite sensations after an energy-fixed breakfast, and on spontaneous energy intake at an ad libitum lunch. 20 young, healthy, normal-weight men participated in a placebo-controlled, randomized, blinded, crossover study. Infusion (GLP-1, 50 pmol/kg·h or saline) was started simultaneously with initiation of the test meals. Visual analogue scales were used to assess appetite sensations throughout the experiment and the palatability of the test meals. Blood was sampled throughout the day for analysis of plasma hormone and substrate levels.

After the energy-fixed breakfast, GLP-1 infusion enhanced satiety and fullness compared with placebo (treatment effect: $P < 0.03$). Furthermore, spontaneous energy intake at the ad libitum lunch was reduced by 12% by GLP-1 infusion compared with saline ($P = 0.002$). Plasma GLP-1, insulin, glucagon, and blood glucose profiles were affected significantly by the treatment ($P < 0.002$). In conclusion, the results show that GLP-1 enhanced satiety and reduced energy intake and thus may play a physiological regulatory role in controlling appetite and energy intake in humans. (*J. Clin. Invest.* 1998. 101:515–520.) Key words: appetite regulation • hunger • satiety • glucagon • insulin

Introduction

The glucagon gene is expressed not only in the pancreas but also in endocrine cells of the intestinal mucosa. Here, but not in the pancreas, the primary translation product, proglucagon, is processed to release two peptides with a high sequence homology to glucagon, so-called glucagon-like peptides 1 and 2 (GLP-1 and -2),[1] whereas the glucagon sequence is confined within a larger, probably inactive molecule, glicentin (1–4). Of these two hormones, GLP-2, which is released in response to ingestion of meals (4), is a candidate as a trophic hormone playing a role in intestinal adaptation (5), whereas GLP-1 functions as one of the incretin hormones, the intestinal hormones that enhance insulin secretion stimulated by oral as opposed to parenteral nutrient administration (6–8).

However, GLP-1 is also believed to play an important role as one of the hormones of the "ileal brake mechanism," an endocrine mechanism that is activated by the presence of nutrients in the ileal lumen and which serves to inhibit gastric motility and secretion (4, 9, 10). It is well established that the same stimulus, i.e., nutrients in the ileum, may affect appetite and food intake (11), and therefore, it may be speculated that the hormones of the ileal brake may also participate in the regulation of energy intake. One mechanism whereby this might be accomplished could be inhibition of gastric emptying, because inhibition of gastric emptying by any agent would be expected to limit food intake (11). However, GLP-1 may act by additional mechanisms. Intracerebroventricular administration of GLP-1 has been shown to powerfully reduce food intake in rats, an effect that may be counteracted by the specific GLP-1 receptor antagonist exendin 9–39 (12–14). Intracerebroventricular GLP-1 presumably interacts with GLP-1 receptors, which have been shown to be present in a number of hypothalamic and extrahypothalamic nuclei in the brain (15). These receptors are probably not accessible to peripheral GLP-1, but seem to be targets for GLP-1 released from nerve endings of GLP-1–producing neurons located primarily in the nucleus of the solitary tract (16). In agreement with these observations, peripheral administration of GLP-1 to rats had no effect on food intake (13, 14). On the other hand, it has been shown that peripheral GLP-1 may gain access to two of the circumventricular organs of the brain, i.e., the subfornical organs and the area postrema, both of which may communicate to energy intake–regulating centers of the hypothalamus (17). In addition, it is known that GLP-1 is metabolized extremely rapidly (18), particularly in rats (19), which might explain the ineffectiveness of peripheral GLP-1 administration in this species. Of greater concern is the finding that mice, homozygous for a targeted deletion of the GLP-1 receptor gene, gained weight normally but were glucose intolerant (8). However, it is generally

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1. *Abbreviations used in this paper:* E%, energy percent; GLP, glucagon-like peptide; NIDDM, non–insulin-dependent diabetes mellitus; VAS, visual analogue scales.

believed that there is a redundancy of the mechanisms regulating energy balance and body weight; thus, other mechanisms may take over if one is impaired. Whether or not peripheral GLP-1 affects food intake remains undetermined.

Because of its insulinotropic and blood glucose lowering effects, GLP-1 is currently being considered as a potential therapeutic agent in the treatment of the hyperglycemia of non–insulin-dependent diabetes mellitus (NIDDM) (20). As many patients with NIDDM are also obese, it would be extremely interesting if peripherally administered GLP-1 also inhibits energy intake. Therefore, we undertook an investigation of the effects, on appetite perception after an energy-fixed breakfast and on energy intake in an ad libitum lunch, of GLP-1 infused intravenously at a rate that effectively reduces hyperglycemia in NIDDM patients yet elicits plasma levels similar to those observed after maximal stimulation of the endogenous production (21, 22).

Methods

Subjects. 20 healthy male subjects, 20–31 yr of age, normal weight (body mass index: 20.3–25.7 kg/m^2, body fat: 9.7–19.5%) nonsmokers who were not elite athletes, and had no history of obesity or diabetes, participated in the study. All subjects gave written consent after the experimental protocol had been explained to them. The subjects were tested on two different occasions in a placebo-controlled, randomized, blinded, crossover study. The two test days were separated by at least 3 wk and by not more than 7 wk. One subject was excluded from the data analysis because of a severe headache on the second test day. The study was approved by the Municipal Ethical Committee of Copenhagen and Frederiksberg, and is in accordance with the Helsinki-II declaration.

Diets. Two different test meals were used, one for a breakfast of fixed size and energy content and one for an ad libitum lunch. The breakfast consisted of yogurt, bread, butter, cheese, jam, kiwi fruit, orange juice, and water. Total available energy content of the meal was calculated to be 20% of each subject's individual energy requirements (22a), adjusted to the nearest 0.5 MJ. The lunch was a homogeneous mixed hotpot consisting of pasta, minced meat, green pepper, carrots, squash, onions, corn, and cream from which the subjects could eat ad libitum. For both meals, the distribution of energy was 50 energy percent (E%) carbohydrates, 37 E% fat, and 13 E% protein. Before each test day, the subjects followed a weight-maintaining standardized diet (50 E% carbohydrates, 37 E% fat, 13 E% protein, 3.5 g/MJ dietary fiber) consisting of ordinary food items, that was estimated to meet each subject's individual energy requirements, adjusted to the nearest 0.5 MJ. The food was prepared in the research department of human nutrition and delivered free of charge. All meals for 2 d were supplied, and the subjects were instructed to adhere strictly to the diet. If they were not able to consume all of the food in the first preexperimental period, they were instructed to bring the leftovers to the department for weighing and registration. This amount of food was then deducted from the standardized diet for the following preexperimental period. The subjects were also instructed to abstain from alcohol and strenuous physical activity for the 2 d before the test days in order to ensure equally filled glycogen stores and similar macronutrient balance on the test days (23).

The computer database of foods from the National Food Agency of Denmark (Dankost 2.0; Danish Food Tables 1989 [Levnedsmiddeltabeller]) was used in the calculations of energy and nutrient composition of the test meals and diets.

Experimental protocol. On the test day, the subjects arrived at the department in the morning, having used the least strenuous means of transportation. They had fasted for a minimum of 12 h from the evening before. After voiding and weighing, body composition (measured by bioelectrical impedance [24]) and height were measured. The subjects then rested in the supine position with slight elevation of the head on a bed covered with an antidecubitus mattress. A Venflon catheter (BOC Ohmeda AB, Helsingborg, Sweden) was inserted in an antecubital arm vein. After 45 min of rest, a fasting blood sample was taken. At 9:45 a.m., breakfast was served, and this was consumed within 15 min. Exactly the same time was spent on the two test days for each individual subject.

Blood samples were taken 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, and 300 min after the start of consuming the breakfast. Appetite ratings were made on 100-mm visual analogue scales (VAS) with text expressing the most positive and the most negative rating anchored at each end (25). VAS were used to assess hunger, satiety, fullness, prospective food consumption, and palatability of the test meals. Appetite sensations were recorded immediately before and throughout a 4.5-h period after breakfast and once after lunch. Palatability ratings (palatability, taste, aftertaste, smell, and visual appeal) of the meals were filled in immediately after consumption of the two meals using VAS scores.

For the GLP-1 infusion, commercially available synthetic, human GLP-1 (7–36 amide) of GMP quality was purchased from Saxon Biochemicals (Hannover, Germany). The peptide was dissolved in a 0.9% saline solution containing 1% human serum albumin guaranteed to be free of hepatitis B surface antigen and HIV antibody (Novo Nordisk, Gentofte, Denmark), subjected to sterile filtration, checked for sterility, and kept at −20°C until use. The infusion (GLP-1 or saline) was started simultaneously with initiation of the test meals (energy-fixed breakfast and ad libitum lunch). An automatic pump (Infusomat; B. Braun Melsungen AG, Melsungen, Germany) provided a steady state infusion rate of 50 pmol·kg body wt^{-1}·h^{-1}. The infusion was stopped 30 min before lunch to allow plasma levels to return to baseline in order to mimic the normal physiological plasma profile (21).

During the experiment, the subjects could listen to the radio or watch television or video (light entertainment). Water consumption and toilet visits were allowed when necessary. The exact hour, time spent, type of activity, and amount of water consumed were noted and repeated on the second test day.

At 2:15 p.m., the lunch was served, and the subjects could eat ad libitum until "comfortably satisfied." Again, the time spent on the first occasion was noted and repeated on the second test day. Energy intake of the ad libitum meal was measured by weighing.

Laboratory analyses. Blood was drawn without stasis through the indwelling antecubital cannula into iced syringes. These contained EDTA (6 μmol/liter) and aprotinin (500 Kallikrein [KIU]/liter, final concentrations) for hormone samples. After centrifugation, plasma for hormone analyses was kept frozen at −20°C. Plasma glucose was analyzed by standard enzymatic methods (26). Insulin concentrations in plasma were measured against standards of human insulin by RIA according to the principles described by Albano et al. (27). The tracer was human insulin, monoiodinated in position A14 (a gift from Novo Nordisk A/S, Bagsvaerd, DK). The guinea pig antibody used (code 2004) cross-reacts with proinsulin and split insulin. Intraassay coefficient of variation was $< 5\%$, and sensitivity was < 5 pmol/liter. Glucagon and GLP-1 concentrations in plasma were measured after extraction of plasma with 70% ethanol (vol/vol, final concentration). The glucagon RIA was directed against the carboxy terminus of the glucagon molecule (antibody code 4305) and therefore measured mainly glucagon of pancreatic origin (28). The plasma concentrations of GLP-1 were measured (29) against standards of synthetic GLP-1 7-36 amide using antiserum code 89390, which is specific for the amidated carboxy terminus of GLP-1 and therefore reacts mainly with GLP-1 of intestinal origin. For these two assays, sensitivity was < 1 pmol/liter, intraassay coefficient of variation was $< 6\%$ at 20 pmol/liter, and recovery of standard, added to plasma before extraction, was $\sim 100\%$ when corrected for losses inherent in the plasma extraction procedure. There was no ($< 0.1\%$) cross-reaction of GLP-1 and glucagon in the glucagon and GLP-1 assays, respectively.

Statistical analyses. All results are given as means±SEM. Fasting values and energy intake were compared using a paired *t* test, while the postprandial response curves were compared by parametric ANOVA using repeated, paired measures with time and treatment as factors. The level of significance was set at $P < 0.05$. The Statistical Analysis package, version 6.08 (SAS Institute, Inc., Cary, NC), was used in the statistical calculations.

Results

The GLP-1 infusion increased the plasma concentration of GLP-1 from ~ 10 pmol/liter to 60–90 pmol/liter (Fig. 1). During the 30-min infusion intermission, the GLP-1 concentration dropped to 34±2 pmol/liter, but the resumed infusion restored levels to 86±6 pmol/liter. In the control experiment, GLP-1 concentrations increased in response to both the breakfast and the lunch, as expected.

The GLP-1 infusion influenced significantly the absolute, mean VAS scores (Fig. 2) for satiety ($P = 0.013$), hunger ($P = 0.012$), fullness ($P = 0.028$), and prospective food consumption ($P = 0.012$), with subjects feeling more satisfied and full and less hungry and indicating lower values of prospective food intake compared with saline infusion. There were no differences in the subjective ratings of taste, visual appeal, smell, aftertaste, and overall palatability of either of the two test meals on the two different experimental days.

During the ad libitum lunch, 15 of 19 subjects (Fig. 3) had a lower spontaneous energy intake during GLP-1 infusion compared with saline, averaging 3.7±0.3 and 4.2±0.2 MJ, respectively ($P = 0.002$), a 12% reduction.

Fig. 4 shows blood glucose, plasma insulin, and glucagon levels. In the control experiment, there was a short-lived increase in blood glucose in response to the breakfast meal and a larger increase in response to the ad libitum lunch. With GLP-1, blood glucose decreased significantly for the first 60 min in spite of breakfast ingestion, and then slowly increased, reaching fasting levels at 180 min. A significant increase occurred in the course of the 30-min GLP-1 infusion intermission, followed by a slight decrease when the infusion was resumed. The break-

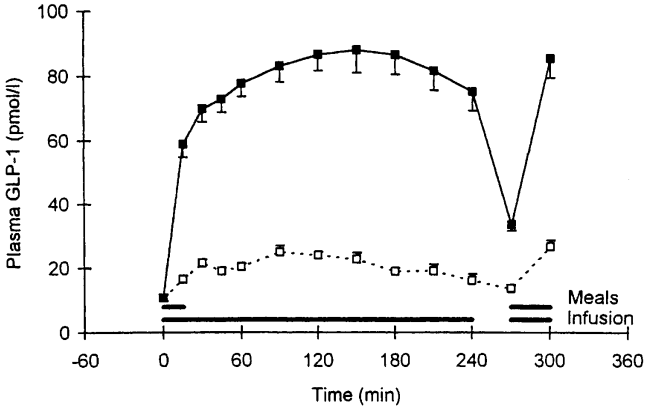

Figure 1. Plasma concentration of GLP-1 during GLP-1 (*filled squares*) and saline (*open squares*) infusions in 19 healthy, normal-weight male subjects. Data are means±SEM. By ANOVA, treatment, time, and treatment × time interaction effect: $P < 0.0001$. *Upper horizontal bar*, Time and duration of the test meals. *Lower bar*, Time and duration of the infusion.

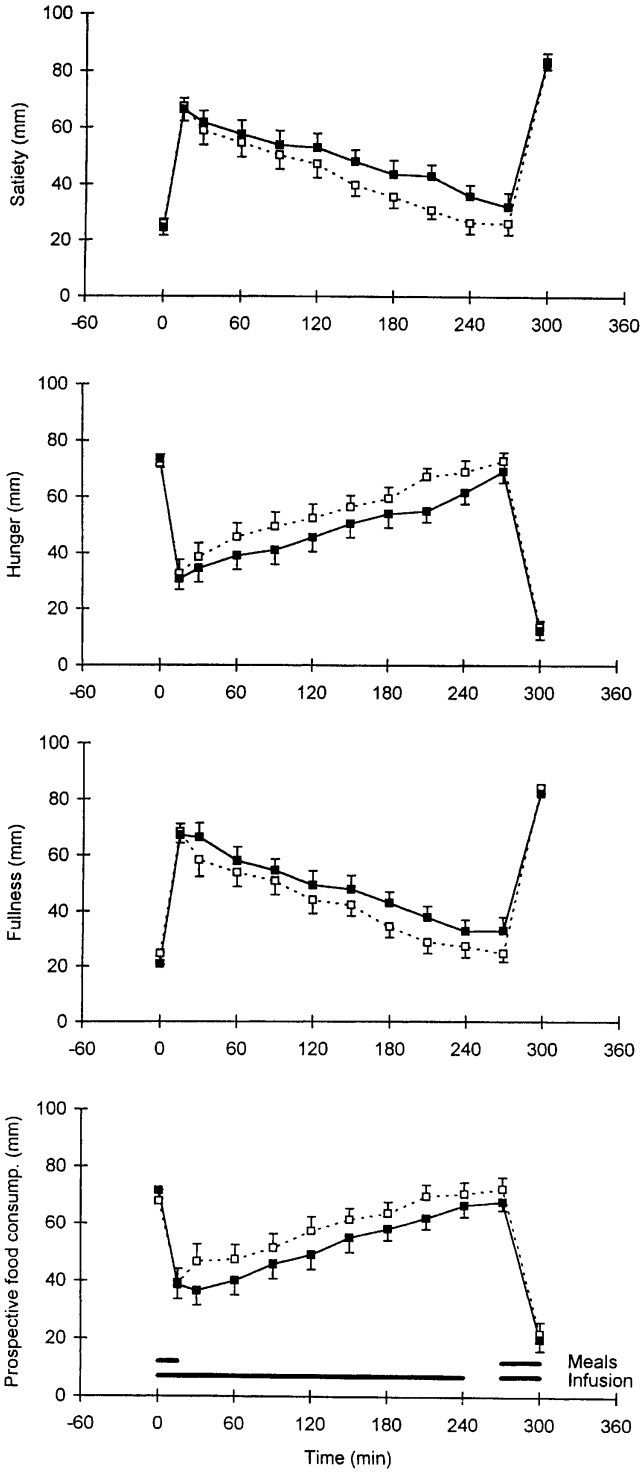

Figure 2. Appetite scores (satiety, hunger, fullness, and prospective food consumption) during GLP-1 (*filled squares*) and saline (*open squares*) infusions in 19 healthy, normal-weight male subjects. VAS equal to 100 mm correspond to "I cannot eat another bite" (satiety), "I have never been more hungry" (hunger), "I am totally full" (fullness), and "I can eat a lot" (prospective food consumption). *Upper horizontal bar*, Time and duration of the test meals. *Lower bar*, Time and duration of the infusion. Data are means±SEM. By ANOVA, time effect: $P < 0.0001$ (all parameters); treatment effect: $P = 0.013$ (satiety), $P = 0.012$ (hunger), $P = 0.028$ (fullness), $P = 0.012$ (prospective food consumption); and treatment × time interaction effect: $P = 0.0576$ (satiety), $P = 0.116$ (hunger), $P = 0.034$ (fullness), $P = 0.165$ (prospective food consumption).

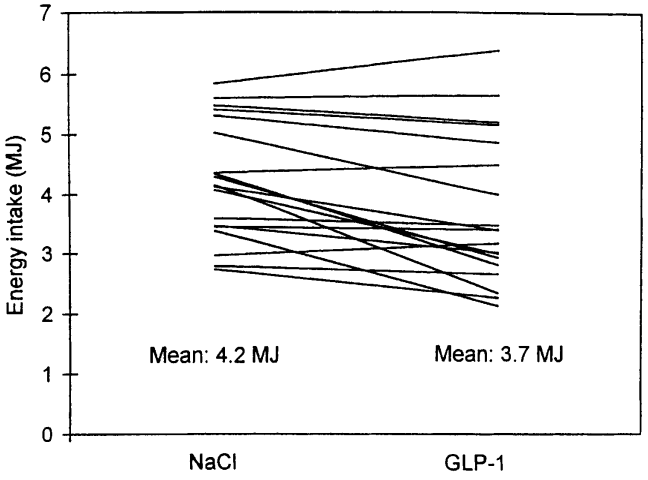

Figure 3. Spontaneous energy intake at an ad libitum lunch during GLP-1 or saline infusion in 19 healthy, normal-weight male subjects. By paired *t* test, $P = 0.002$.

fast-induced insulin response was reduced significantly ($P < 0.02$) with GLP-1 compared with saline infusion, whereas higher preprandial and equal postprandial concentrations were noted at the ad libitum lunch. The breakfast-induced glucagon response was also reduced significantly ($P < 0.0001$) with GLP-1 compared with saline, while pre- and postprandial values at lunch were equal on the two occasions.

Discussion

This investigation has shown that peripherally administered GLP-1 significantly influences subjective appetite sensations and reduces energy intake in healthy volunteers.

The effect on appetite was illustrated clearly by each of the four appetite parameters registered, and was apparent shortly after the start of GLP-1 infusion and seemed to last for the duration of the infusion, i.e., the interval between the meals. One subject reported a severe headache but no other symptoms of being ill or nauseated. With (much) higher doses of GLP-1, nausea (and, eventually, vomiting) have been reported (30, 31), effects that are thought to result from the powerful inhibitory effects of GLP-1 on gastric emptying. However, the palatability scores, which showed no significant differences between the two experimental days, support the conclusion that the effect is a true effect on appetite rather than food aversion. Although the subjects were not asked specifically about possible ill effects, nobody spontaneously expressed complaints or presented other indications of an altered state of well-being during the infusion.

At this stage, a number of important questions present themselves: (*a*) Is this a physiological effect? (*b*) What is the mechanism? and (*c*) Is there a clinical application?

Physiological effect? The infusion of GLP-1 was carried out at a rate of 50 pmol/kg·h, which resulted in elevations of plasma GLP-1 concentrations from ~ 10 pmol/liter to a level of ~ 80 pmol/liter. The plateau is ~ 40 pmol/liter above peak postprandial levels, determined with the same assay, in normal subjects given an appetizing high-energy mixed meal (range 32–76 pmol/liter) (21), but considerably below values obtained

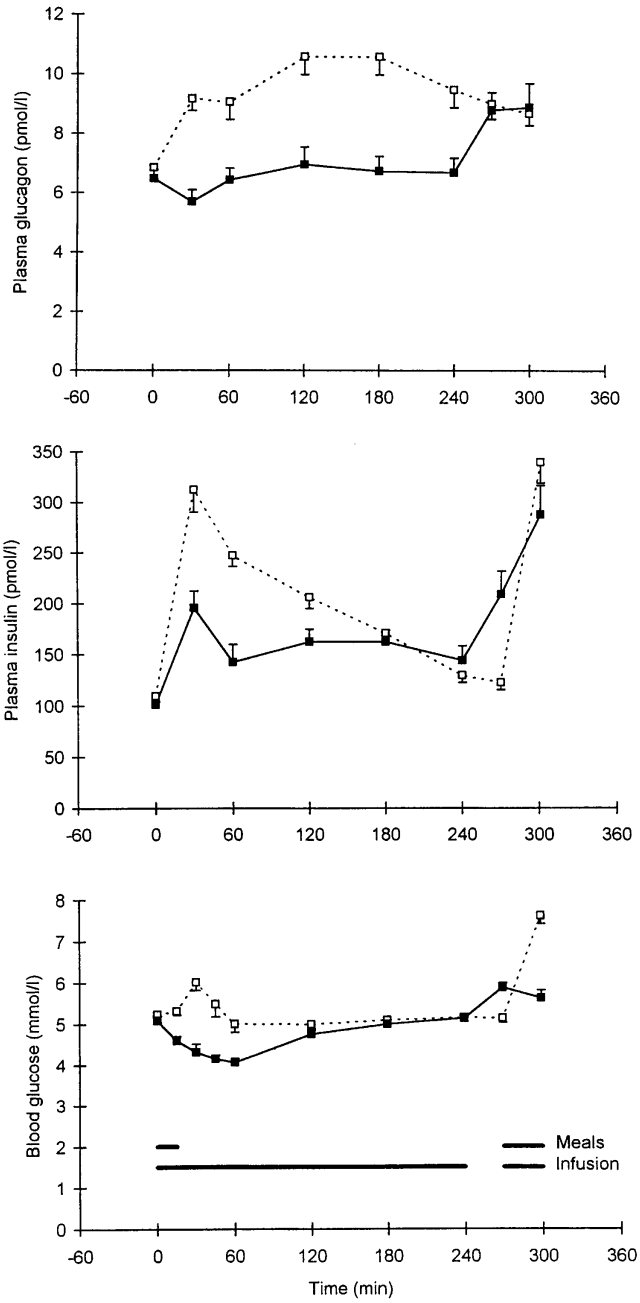

Figure 4. Plasma glucagon, plasma insulin, and blood glucose concentrations during GLP-1 (*filled squares*) or saline (*open squares*) infusions in 19 healthy, normal-weight male subjects. Data are means±SEM. By ANOVA, treatment, time, and treatment × time interaction effect: $P < 0.0001$, except treatment effect of insulin: $P < 0.002$. *Upper horizontal bar*, Time and duration of the test meals. *Lower bar*, Time and duration of the infusion.

in subjects with accelerated gastric emptying (22). Therefore, it may be concluded that the levels obtained are in the high physiological or perhaps slightly supraphysiological range. Further studies using lower infusion rates will be required to show if there is a dose–response relationship and whether lower doses are also effective. On the other hand, the levels reached here are clearly within the range obtained in previous studies in which the antidiabetic effects of GLP-1 were demonstrated

(32). Thus, the amount of GLP-1 required to normalize the blood glucose concentrations in patients with NIDDM would also be expected to influence appetite and reduce food intake. Whether this will, in fact, happen in diabetic subjects remains to be studied. It seems probable that high postprandial levels of GLP-1 may indeed influence appetite and further food intake, and that GLP-1 may, therefore, be one of the physiological regulators of food intake identified in studies of the effects of intraintestinal administration of nutrients on food intake and appetite (10). In addition, GLP-1 may interact with the other gastrointestinal hormones released in response to meal ingestion, which may influence food intake, such as cholecystokinin, gastrin-releasing peptide, and peptide YY. Thus, it has been shown recently that GLP-1 and peptide YY, which are released synchronously from distal small intestine, have additive inhibitory effects on gastric acid secretion (33). However, the precise relative role of endogenous GLP-1 in the regulation of food intake can be determined only when a specific and sufficiently potent antagonist of these actions of GLP-1 becomes available for human use.

Mechanism of action. Recent studies have shown that GLP-1 dose-dependently, and certainly when infused at the rate used here, inhibits gastric emptying in healthy volunteers (11, 34). The glucose profiles obtained with and without GLP-1 in this study support the notion that gastric emptying was inhibited during the GLP-1 infusion. Thus, the fall in blood glucose occurring in spite of breakfast ingestion probably represents the combined effect of decreased hepatic glucose production (35), resulting from increased insulin and decreased glucagon secretion, and decreased entry of dietary carbohydrates into the small intestine. The latter also explains the smaller insulin response in spite of higher GLP-1 levels (34). The subsequent slow increase in blood glucose seems to result from glucose absorption, because glucagon concentrations remained low during the GLP-1 infusion. The cessation of GLP-1 infusion at 240 min was associated with an immediate increase in glucagon secretion that was probably responsible for the concomitant slight increase in blood glucose (not noted on the control day). A similar rebound glucose response was observed in a previous study in which hepatic glucose production was actually measured (35).

Presumably, inhibition of gastric emptying may in itself cause a limitation of food intake, through either neural or endocrine signaling pathways, perhaps associated with distention of the stomach (10). However, as mentioned in the Introduction, activation of brain GLP-1 receptors by intracerebroventricular GLP-1 administration causes pronounced inhibition of food intake in rats (12–14), and it has been shown that GLP-1 may access the brain via the subfornical organ and the area postrema of the circumventricular organs (17), in which the blood–brain barrier is leaky. Furthermore, in a series of recent studies of the inhibitory effects of GLP-1 on antral motility in anesthetized pigs, an effect that involves the vagus nerves, it was concluded that the effects of peripheral GLP-1 were transmitted by afferent vagal fibers reaching the brain (36). A similar conclusion was reached by Nakabayashi et al. (37), who were able to identify a vagal hepatopancreatic reflex evoked by the intraportal appearance of GLP-1. Thus, it remains possible that GLP-1, perhaps like cholecystokinin, exerts its effects via interaction with sensory nerve fibers in the periphery (38).

Therapeutic application of GLP-1? Several research groups and medical companies are trying to convert GLP-1 into a useful therapeutic agent for the treatment of diabetic hyperglycemia (20). As a peptide, it cannot be taken orally. In addition, GLP-1 is metabolized very rapidly, with a half-life for the intact molecule in the circulation of about 1.5 min (18). However, by a number of strategies, including design of metabolically stable analogs, inhibition of the degrading enzymes, and above all, screening for orally active agonists of the GLP-1 receptor, attempts are being made to solve these problems. Given the fact that obesity grossly aggravates NIDDM, and that most patients with this disease are, in fact, obese, our observation of the food intake–inhibiting effects of GLP-1 is of great clinical interest. This raises the question of whether the effects on food intake may exhibit tachyphylaxis. It is known that intravenously administered GLP-1 remains effectively hypoglycemic during continuous infusion for up to 7 d (31), i.e., its glucose-lowering effect does not exhibit tachyphylaxis. In view of the presumably complex and partly neural mechanisms whereby GLP-1 is likely to affect food intake, the regulation of which is equally complex, it seems premature to conclude that the effects of GLP-1 on food intake will not exhibit tachyphylaxis.

To date, there are only a few studies on the effects of GLP-1 in obese people. In a recent study, we were able to show that the glucose-lowering effect of GLP-1 was unimpaired in obese compared with lean diabetic subjects (39). Thus, the islet GLP-1 receptors seem to function normally in obese NIDDM patients. In other studies, it has been found that endogenous GLP-1 secretion is decreased or almost absent in morbidly obese patients (40–42). Such patients have been reported to exhibit accelerated gastric emptying (42). Both of these findings suggest that GLP-1 therapy could be effective in treating morbid obesity.

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