Role of Fat Hydrolysis in Regulating Glucagon-Like Peptide-1 Secretion

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Context: Glucagon-like peptide-1 (GLP-1) is produced by specialized cells in the gut and secreted in response to carbohydrates and lipids. The mechanisms regulating fat-stimulated GLP-1 release have, however, not been clarified in detail.

Aim: We aimed to investigate the effect of intraduodenal (ID) fat hydrolysis on GLP-1 release and test whether the signal is mediated through cholecystokinin (CCK)-1 receptors.

Design and Setting: Thirty-four healthy, male ambulatory volunteers were studied in three consecutive, randomized, double blind, crossover studies.

Intervention: There were three interventions: 1) 12 subjects received an ID fat infusion with or without orlistat, an irreversible inhibitor of gastrointestinal lipases, in comparison with vehicle; 2) 12 subjects received ID sodium oleate (C18:1), ID sodium caprylate (C8:0), or ID vehicle; and 3) 10 subjects received ID sodium oleate with and without the CCK-1 receptor antagonist dexloxiglumide or ID vehicle plus iv saline (placebo). The effect of these treatments on GLP-1 concentrations and CCK release was quantified.

Results: The following results were reached: 1) ID fat induced significant increase in GLP-1 concentrations ($P < 0.004$), and inhibition of fat hydrolysis by orlistat abolished this effect; 2) sodium oleate significantly stimulated GLP-1 release ($P < 0.008$), whereas sodium caprylate was ineffective compared with controls; and 3) dexloxiglumide administration abolished the effect of sodium oleate on GLP-1. ID fat or sodium oleate significantly stimulated plasma CCK ($P < 0.006$ and $P < 0.004$) compared with saline, whereas sodium caprylate did not.

Conclusion: Generation of long-chain fatty acids through hydrolysis of fat is a critical step for fat-induced stimulation of GLP-1 in humans; the signal is mediated via CCK release and CCK-1 receptors. **(*J Clin Endocrinol Metab* 95: 879–886, 2010)**

Glucagon-like peptide (GLP)-1 is an incretin hormone secreted from enteroendocrine L cells that augment insulin secretion after oral intake of a meal (1, 2). The most potent secretagogues for GLP-1 release are carbohydrates and fat (3–5). Because iv glucose administration does not induce secretion of GLP-1 (3), it appears that nutrients within the lumen of the gut act on the luminal surface to stimulate GLP-1 secretion.

The effects of fat on digestive functions and hormone release are dependent on hydrolysis of triglycerides into free fatty acids (6). For example, in healthy subjects, inhibition of fat digestion by the lipase inhibitor orlistat attenuates the effects of duodenal triglyceride plasma cholecystokinin (CCK) and GLP-1 concentrations (7, 8). There is evidence that the gastrointestinal responses to fat are also dependent on the chain length of intraluminal

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Abbreviations: C8:0, Medium-chain fatty acids; C18:1, long-chain fatty acids; CCK, cholecystokinin; DEXLOX, dexloxiglumide; GLP, glucagon-like peptide; DEXLOX, dexloxiglumide; PYY, peptide YY.

fatty acids. In humans, it has been reported that oleic acid (C18:1), but not medium-chain fatty acids (C8:0), reduces energy intake (9), stimulates CCK and peptide YY (PYY) release, and suppresses ghrelin secretion (10, 11). Gastrointestinal peptides, including CCK and GLP-1, mediate, at least in part, the effects of fat on energy intake (12) and gastrointestinal motility (13, 14); hence, the role of fat hydrolysis on gastrointestinal hormone secretion is clearly of interest. Previous studies have shown that inhibition of fat hydrolysis attenuates the release of GLP-1 (7, 8). Furthermore, fatty acids with 12 carbons stimulated GLP-1, whereas fatty acids with 10 carbons did not (15). Finally, the generation of long-chain fatty acids through hydrolysis of fat was a critical step for fat-induced stimulation of PYY in humans; the signal was in part mediated via CCK release and CCK-1 receptors (11).

The aim of this study was therefore to investigate whether CCK mediated the effect of intraduodenal fat on GLP-1 secretion via CCK-1 receptors. Three major issues relate to the present study: 1) the importance of adequate fat digestion on plasma GLP-1 secretion; 2) the role of fatty acid chain length in initiating the effects on GLP-1 secretion; and 3) the consequences of CCK-1 receptor blockade on GLP-1 release.

Subjects and Methods

Subjects

Thirty-two male subjects, aged 20–30 yr (mean 25.2 yr), participated in the study. Body weight of all subjects was within the normal range for age, sex, and height. Each subject gave written informed consent for the study. The human ethics committee of the University Hospital (Basel, Switzerland) approved the protocol. Before acceptance, each participant was required to complete a medical interview, receive a full physical examination, and participate in an initial laboratory screening. No subject was receiving any medications or had a history of food allergies or dietary restrictions.

Experimental procedures

Part I: effect of orlistat dissolved in olive oil on GLP-1 secretion

Three treatments, separated by at least 7 d, were performed in 12 subjects in a randomized order. On the evening preceding each treatment, subjects swallowed a radioopaque polyvinyl feeding tube (external diameter 8 French), which had openings at the tip of the tube. The tube was inserted through the nose because this procedure allowed the tube to be retained overnight and for the duration of the experiment. The tube was transported to the duodenum overnight. In the morning, the position of the tube was located fluoroscopically, and the tip of the tube was positioned 100 cm distal to the teeth. It was firmly attached to the skin behind the ear to prevent further progression of the tube during the experiment.

The treatments were identical in design except for the intraduodenal infusions. One treatment consisted of intraduodenal vehicle infusion (see below) for the duration of the experiment (120 min). In the second and third experiments, intraduodenal fat (olive oil) with or without orlistat (30 mg/h) was used instead of saline throughout the experiments. An infusion rate of 0.5 ml/min oil was chosen for the duration of the experiment; this rate was taken from previous experiments (6, 11). The intraduodenal fat infusion solution was indistinguishable in appearance from the control solution (vehicle). The treatments were given in a double-blind manner. Blood samples (7.5 ml each) were drawn at regular intervals into EDTA-coated tubes containing aprotinin (1000 kIU/ml blood) for plasma GLP-1 and CCK determinations.

Part II: effect of free fatty acids (C8:0 and C18:1) GLP-1 release

The design of the second series was similar to part I. The study was conducted in a randomized, double-blind, and three-period crossover fashion; 12 healthy male subjects participated in this part. One treatment consisted of intraduodenal infusion of medium-chain fatty acids. Medium-chain fatty acids in the form of sodium caprylate, C8:0, were infused at a rate of 0.5 ml/min, resulting in a load of 8 mmol/h sodium caprylate. In the second experiment, long-chain fatty acids in the form of sodium oleate, C18:1, were infused at a rate of 0.5 ml/min, resulting in a load of 8 mmol/h. On the third experimental day, volunteers received intraduodenal vehicle (control) instead of free fatty acids. The sodium caprylate (C8:0) and sodium oleate (C18:1) loads were chosen from previous experiments (11). During the experiments, 7.5-ml samples of blood were drawn into EDTA-coated tubes containing aprotinin (1000 kIU/ml blood) for plasma GLP-1 and CCK determinations.

Part III: effect of C18:1 with and without iv dexloxiglumide (DEXLOX) on GLP-1 secretion

The procedures in this series were similar to part II except for the iv and intraduodenal infusions (sodium caprylate was not infused in this part). Ten healthy male subjects participated in this randomized, double-blind study. Subjects received on 2 experimental days a continuous intraduodenal infusion of sodium oleate (C18:1; 8 mmol/h) together with either an iv infusion of isotonic saline (control) or an infusion of the CCK-1 receptor antagonist DEXLOX (5 mg/kg · h) for the duration of the study. The dose of DEXLOX was chosen from previous experiments (11). Infusions were started 30 min before the intraduodenal infusion. On the third experimental day, subjects received intraduodenal vehicle and iv saline for the duration of the study. Infusions were delivered via ambulatory pumps through a catheter inserted into a forearm vein. Blood was taken (7.5 ml samples) at regular intervals for GLP-1 and CCK determinations.

Drugs

Orlistat, also known as tetrahydrolipstatin (Xenical), was purchased from Roche Ltd. (Basel, Switzerland). Orlistat is poorly soluble in water; 120 mg orlistat was therefore dissolved in a solution containing 140 ml distilled water with addition of 4 ml lecithin-ethanol solution plus 1.8 ml saccharose solution. The lecithin-ethanol stock solution was made of 50 ml 96% ethanol plus 0.75 g lecithin. The saccharose stock solution was

prepared by adding 5 g saccharose to 10.0 ml distilled water; the mixture was gently vortexed until the saccharose was completely dissolved. An identical solution without orlistat was used as a control solution. The sodium caprylate (C8:0) solution was prepared as follows: 4.9 g of sodium caprylate were dissolved in distilled water by gently stirring for 2 h; after adding 1.2 ml 1 M hydrochloric acid, the solution was back-titrated to pH 7.2 with addition of 1.2 ml 1 M NaOH. The control solution was identical without adding sodium caprylate. Finally, the following procedure was used to prepare the sodium oleate (C18:1) solution: 8.6 g sodium oleate were dissolved in 100 ml distilled water by gently stirring for 2 h; the solution was stabilized at pH 9.5 by adding 1 M hydrochloric acid to titrate the pH to 9.5. All solutions were infused to the small intestine at a rate of 30 ml/h.

DEXLOX, the (R)-isomer of loxiglumide, is a selective and highly potent CCK1 receptor antagonist; the compound was a kind gift of Dr. Lucio Rovati (Rotta Pharma SpA, Monza, Italy).

Plasma hormone determinations

Plasma CCK concentrations were measured using a sensitive RIA based on an antiserum that recognizes the sulfated tyrosine residue of all CCK molecules but has little cross-reactivity with sulfated gastrin (<1%) and does not cross-react with unrelated gastrointestinal peptides. Plasma samples were extracted with ethanol. ^{125}I-CCK-8 was used as a label. The lowest concentration that could be measured by the assay was 0.6 pmol/liter plasma using CCK-8 as a standard. Details of the assay have been previously described (6, 11).

GLP-1 was measured with a commercially available ELISA kit (Linco Research Inc., St. Charles, MO). This kit is for non-radioactive quantification of biologically active forms of GLP-1 [*i.e.* GLP-1 (7-36 amide) and GLP-1 (7-37)] in plasma and other biological media. It is highly specific for the immunological measurement of active GLP-1 and will not detect other forms of GLP-1 (*e.g.* 1-36 amide, 1-37, 9-36 amide, or 9-37). This assay is based, sequentially, on the following: 1) capture of active GLP-1 from sample by a monoclonal antibody, immobilized in the wells of a microwell plate, that binds specifically to the N-terminal region of active GLP-1 molecule, 2) washing to remove unbound materials, 3) binding of an anti-GLP-1-alkaline phosphatase detection conjugate to the immobilized GLP-1, 4) washing off unbound conjugate, and 5) quantification of bound detection conjugate by adding methyl umbelliferyl phosphate, which in the presence of alkaline phosphatase forms the fluorescent product umbelliferone. Because the amount of fluorescence generated is directly proportional to the concentration of active GLP-1 in the unknown sample, the latter can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of active GLP-1. The intra- and interassay variability were less than 9 and 13%, respectively. The lowest level of GLP-1 that could be detected by this assay was 0.25 pmol/liter when using a 100-μl plasma sample.

Statistical analysis

Results are presented as means ± SEM unless indicated otherwise. Data were compared between the treatment groups by ANOVA. If the ANOVA revealed significant differences, pairwise comparisons were performed by Tukey-honestly significant difference test. When data did not show a normal distribution, both tests were performed on log-transformed data. All tests were performed as two-sided tests and the level of significance was set to 0.05. All statistical comparisons were performed using SPSS for Windows software (version 15.0; SPSS, Chicago, IL).

Results

Part I

Fasting plasma GLP-1 concentrations were similar in the different treatments (Table 1). Fat infusion significantly stimulated both GLP-1 and CCK secretions (Table 1 and Fig. 1) compared with the control treatment (infusion of vehicle to the duodenum). Orlistat (60 mg, infused together with fat) reversed the effect of intraduodenal fat: the stimulation of GLP-1 release was virtually blocked by the lipase inhibitor ($P < 0.001$ fat *vs.* fat plus orlistat).

TABLE 1. Pharmacodynamics of CCK and GLP-1 during intraduodenal infusions of fat or vehicle (control treatment) with and without orlistat in 12 healthy male subjects

	Treatments		
	ID vehicle (control) (A)	**ID fat alone (B)**	**ID fat plus Orlistat (C)**
CCK			
Basal (pmol/liter)	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
AUC (0–120 min) (pmol/min · liter)	115.4 ± 4.18 (NA)	319.5 ± 46.63 ($P < 0.001$)	131.4 ± 5.6 (NS)
Cmax (pmol/liter)	1.3 ± 0.07 (NA)	3.9 ± 0.63 ($P < 0.001$)	1.4 ± 0.11 (NS)
GLP-1			
Basal (pmol/liter)	1.0 ± 0.1	1.0 ± 0.1	0.9 ± 0.1
AUC (0–120 min) (pmol/min · liter)	121.0 ± 6.9 (NA)	356.9 ± 16.9 ($P < 0.001$)	120.4 ± 6.7 (NS)
Cmax (pmol/liter)	1.3 ± 0.1 (NA)	4.3 ± 0.2 ($P < 0.001$)	1.3 ± 0.1 (NS)

Data are mean ± SEM (n = 12/group). *P* values represent statistically significant differences *vs.* control. ID, Intraduodenal; AUC, area under the curve; Cmax, maximum concentration; NA, not applicable; NS, not significant.

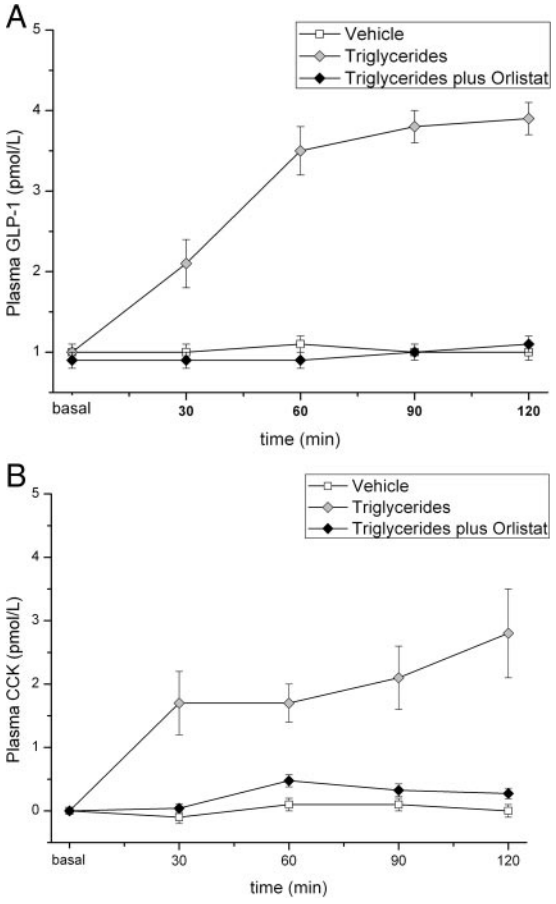

FIG. 1. Plasma concentrations of GLP-1 (A) and CCK (B), during intraduodenal perfusions of triglycerides with or without the lipase inhibitor orlistat (30 mg/h) or perfusion of vehicle (control treatment) in 12 healthy male subjects. Triglycerides perfused to the duodenum induced a treatment × time interaction for GLP-1 and CCK ($P < 0.01$ each). GLP-1 and CCK increased progressively during triglyceride perfusion; inhibition of fat hydrolysis completely abolished these effects: plasma GLP-1 and CCK concentrations were not different from control values. Data are mean ± SEM.

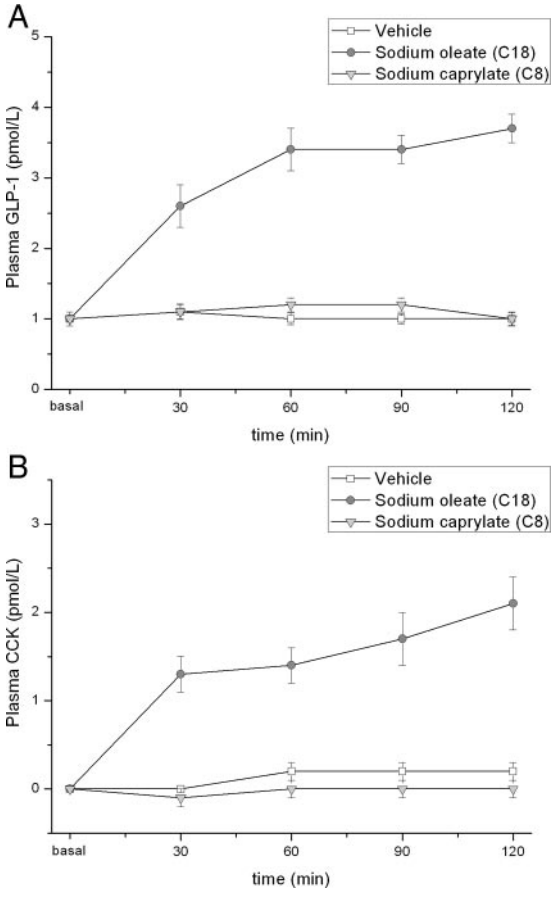

FIG. 2. Plasma concentrations of GLP-1 (A) and CCK (B) during intraduodenal infusions of C18:1 (sodium oleate, 8 mmol/h), C8:0 (sodium caprylate, 8 mmol/h), or perfusion of vehicle (control treatment) in 12 healthy male subjects. C18:1 increased GLP-1 and CCK progressively compared with the control. C8:0 did not affect hormone levels: plasma GLP-1 and CCK concentrations were not different from control values. Data are mean ± SEM.

Part II

Fasting GLP-1 concentrations were comparable in the different treatments (Table 2 and Fig. 2). During vehicle infusion (control treatment) and infusion of sodium caprylate (C8:0), plasma GLP-1 levels remained stable (Fig. 2). Intraduodenal administration of sodium oleate (C18:1) caused a significant increase in both plasma GLP-1 and CCK levels compared with controls (Table 2 and Fig. 2).

TABLE 2. Pharmacodynamics of CCK and GLP-1 during intraduodenal infusions of sodium oleate (C18:1), sodium caprylate (C8:0), or infusion of vehicle (control treatment) in 12 healthy male subjects

	Treatments		
	ID vehicle (control) (A)	**ID C8:0 (B)**	**ID C18:1 (C)**
CCK			
AUC (0–120 min) (pmol/min · liter)	114.2 ± 5.2 (NA)	113.1 ± 3.8 (NS)	272.3 ± 23.1 ($P < 0.001$)
Cmax (pmol/liter)	1.1 ± 0.1 (NA)	1.2 ± 0.1 (NS)	3.3 ± 0.3 ($P < 0.001$)
GLP-1			
AUC (0–120 min) (pmol/min · liter)	123.0 ± 7.2 (NA)	136.2 ± 7.2 (NS)	351.9 ± 11.5 ($P < 0.001$)
Cmax (pmol/liter)	1.3 ± 0.1 (NA)	1.5 ± 0.1 ($P = 0.072$)	4.3 ± 0.1 ($P < 0.001$)

Data are mean ± SEM (n = 12/group). *P* values represent statistically significant differences *vs.* control. ID, Intraduodenal; AUC, area under the curve; Cmax, maximum concentration; NA, not applicable; NS, not significant.

TABLE 3. Pharmacodynamics of CCK and GLP-1 during ID infusions of sodium oleate (C18:1) or infusion of vehicle (control treatment) with and without iv DEXLOX in 10 healthy male subjects

	Treatments		
	ID vehicle plus iv saline (control) (A)	**ID C18:1 plus iv saline (B)**	**ID C18:1 plus iv DEXLOX (C)**
CCK			
AUC (0–120 min) (pmol/min · liter)	164.0 ± 12.5 (NA)	277.4 ± 31.5 (NS)	1073.2 ± 138.8 ($P < 0.001$ *vs.* A) ($P < 0.001$ *vs.* B)
Cmax (pmol/liter)	2.1 ± 0.3 (NA)	3.5 ± 0.4 ($P < 0.01$)	14.7 ± 1.5 ($P < 0.001$ *vs.* A) ($P < 0.001$ *vs.* B)
GLP-1			
AUC (0–120 min) (pmol/min · liter)	132.6 ± 11.6 (NA)	356.8 ± 17.3 ($P < 0.001$)	175.8 ± 7.0 ($P = 0.055$)
Cmax (pmol/liter)	1.5 ± 0.1 (NA)	4.0 ± 0.2 ($P = 0.001$)	2.2 ± 0.1 ($P = 0.024$)

Data are mean ± SEM (n = 10/group). *P* values represent statistically significant differences *vs.* the respective treatment (ANOVA, Tukey-honestly significant difference test). ID, Intraduodenal; AUC, area under the curve; Cmax, maximum concentration; NA, not applicable; NS, not significant.

Part III

Similar to part II, intraduodenal infusion of sodium oleate (C18:1) with iv infusion of saline caused a significant increase in both GLP-1 and CCK levels. In contrast, intraduodenal sodium oleate (C18:1) infusion together with iv DEXLOX resulted in an attenuated GLP-1 response compared with intraduodenal sodium oleate (C18:1) plus iv saline (Table 3 and Fig. 3).

Finally, intraduodenal infusion of sodium oleate (C18:1) with iv infusion of saline caused a significant increase in plasma CCK compared with controls (ID vehicle plus iv saline). Intraduodenal sodium oleate (C18:1) infusion together with iv DEXLOX resulted in a significantly augmented CCK response (Table 3 and Fig. 3) in comparison with ID sodium oleate (C18:1) plus iv saline ($P < 0.004$).

Discussion

Three different approaches were used to establish that fat hydrolysis is a critical step in regulating GLP-1 secretion. Fat digestion is required for the triggering of gastrointestinal functions, such as gallbladder contraction and exocrine pancreatic secretion mediated by CCK release (6). There is also evidence that the gastrointestinal responses to fat are also dependent on the chain length of intraluminal fatty acids (5, 6, 8). Fatty acids with 12 or more carbon atoms are transported from the gut predominantly in lymphatic chylomicrons, a transport process that triggers a variety of gut signals including satiety and the slowing of gastric emptying (5). It has also been reported that oleic acid (C18:1), but not C8:0, reduces energy intake (12).

First, we used the lipase inhibitor orlistat as a tool to determine whether inhibition of fat hydrolysis affects the release pattern of GLP-1. Orlistat is known to inhibit fat hydrolysis from a meal (or in the present study, the duodenal fat infusion) with subsequent effects on different digestive functions (6–8). Stimulation of GLP-1 release in response to fat in the small intestine was completely abol-

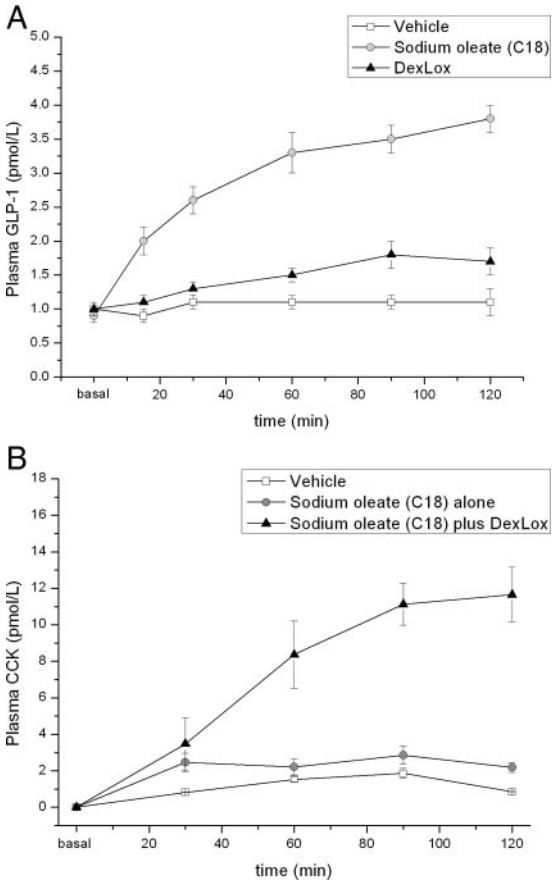

FIG. 3. Effect of DEXLOX, a specific CCK-1 receptor antagonist, on plasma concentrations of GLP-1 (A) and CCK (B) during intraduodenal infusion of sodium oleate (C18:1) or vehicle (control treatment) in 10 healthy male subjects. Sodium oleate (C18:1) increased GLP-1 and CCK progressively compared with controls. Intravenous administration of DEXLOX (5 mg/kg · h) attenuated the effects induced by sodium oleate (C18:1): plasma GLP-1 concentrations were reduced by the treatment. Data are mean ± SEM.

ished by orlistat administration. We did not include a formal control treatment (*i.e.* intraduodenal infusion of orlistat in vehicle alone) in the present study because we have seen in previous investigations that orlistat alone does not have any effect on the digestive system including hormone release (6, 11). The data are in line with two recent studies (8, 16): suppression of fat hydrolysis by orlistat inhibits the release of GLP-1.

The critical importance of fat hydrolysis on digestive functions is best illustrated by the products of fat digestion on exocrine pancreatic secretory responses in animals and humans: only duodenal infusion of long-chain free fatty acids can stimulate maximal pancreatic enzyme and bicarbonate secretion, whereas undigested long chain triglycerides are ineffective (17, 18). Inhibition of lipolysis by orlistat reduces the amount of free fatty acids in the small intestine with a subsequent reduction in CCK release (6). The reduction in CCK release prevents an adequate gallbladder contraction, results in a reduced postprandial exocrine pancreatic secretory response, and induces an attenuated inhibitory effect on further food intake (9, 12). The results imply that the generation of long-chain free fatty acids is a crucial step in the initiation of various digestive processes.

In the second part of the study, we analyzed the importance of chain length of fatty acids in stimulating GLP-1 secretion. In this part, medium-chain fatty acids in the form of C8:0 or long-chain fatty acids in the form of C18:1 were infused into the small intestine. Sodium oleate (C18:1) infusion resulted in a marked increase in GLP-1 concentrations, whereas sodium caprylate (C8:0) infusion was ineffective. Feltrin *et al.* (15) previously evaluated the effects of fatty acids of different chain lengths on GLP-1 secretion; fatty acids with 12 carbons but not fatty acids with 10 carbons infusions increased plasma GLP-1 concentrations. These observations are in line with our present results and suggest that in addition to fat hydrolysis, the chain length of free fatty acids is crucial for stimulating GLP-1 secretion.

In the third part, we used the specific CCK-1 receptor antagonist DEXLOX to block the actions of CCK: the sodium oleate (C18:1)-stimulated GLP-1 response was abolished with CCK-1 receptor blockade, suggesting that the effect was, at least in part, mediated by CCK via its CCK-1 receptor. We did not include a formal control treatment (*i.e.* intraduodenal infusion of vehicle plus iv infusion of DEXLOX) in the present study for two reasons: 1) we have seen in previous investigations that DEXLOX alone does not have any effect on gastrointestinal hormone release in the fasting state (our unpublished observations); 2) iv DEXLOX is no longer available for human use and we had only limited amounts of drugs available. In conclusion, the present results together with previous observations document that adequate fat hydrolysis is required for stimulating GLP-1 release. The products of fat digestion stimulate CCK release, which in turn regulates GLP-1 secretion via CCK-1 receptors.

GLP-1 secretion is initiated by either direct luminal contact of nutrients with the endocrine cells and/or indirectly through neurohumoral signals such as CCK (1, 2). One potential pathway is a direct activation of CCK-1 receptors on afferent vagal fibers through sodium oleate (C18:1) stimulation (19–23). One type of afferent fibers is sensitive to long-chain fatty acids; furthermore, inhibition of food intake induced by intraduodenal sodium oleate is reversed by bilateral, subdiaphragmatic vagotomy or pretreatment with capsaicin (19–23). Because L cells are more abundant in the distal part of the small intestine (ileum) compared with the proximal part (duodenum and proximal jejunum), it has been suggested that the early rapid rise in GLP-1 after nutrient stimulation was not a direct effect on the L cells but rather an indirect mediated signal (proximal to distal model) (24). A second potential pathway is therefore stimulation of GLP-1 secretion via circulating CCK. The present study provides further evidence for this model because blockade of CCK-1 receptors by a specific antagonist (DEXLOX) markedly reduced fatty acid stimulated GLP-1 secretion.

This stimulatory pathway might be specific for fatty acid-stimulated, but not glucose-stimulated, GLP-1 release because there is considerable experimental evidence suggesting that luminal glucose can directly lead to GLP-1 secretion from the proximal gut: 1) although there are more L cells in the distal gut than in the proximal part in humans, there are enough cells in the proximal part to stimulate GLP-1 release (25, 26); 2) the time course of glucose-stimulated GLP-1 secretion (onset, peak, and duration) is consistent with glucose-stimulating GLP-1 release from the proximal part, whereas the time course of fatty acid stimulated GLP-1 release would suggest a stimulation of GLP-1 over the entire small intestine (8, 24); 3) infusion of small amounts of glucose to the duodenum stimulates GLP-1 release in healthy subjects (13); and finally 4) studies with isolated tissues from wild-type and a-gust$^{-/-}$ mice suggest that direct sensing of glucose in the proximal part of the gut stimulates GLP-1 release from proximal L cells (27, 28); unfortunately, similar studies have not been performed with fatty acids.

The present and previous observations have physiological implications: they suggest a mechanism through which CCK plays a role in regulating food intake and appetite with fat hydrolysis and the generation of free fatty acids as triggers to further stimulate GLP-1 and PYY or

both. These peptides in turn inhibit further food intake and decrease appetite.

The plasma CCK levels were markedly higher with DEXLOX compared with the control treatment. This phenomenon has been observed before with CCK-1 receptors (29, 30): the explanations include activation of a negative feedback mechanism due to low concentrations of bile and/or pancreatic juice constituents in the duodenal lumen and interference with autoregulatory, inhibitory CCK-1 receptors on the I cell.

In summary, the three major issues that relate to the present study are: 1) the importance of adequate fat digestion on plasma GLP-1 secretion; 2); the role of fatty acid chain length in initiating the effects on GLP-1 secretion, and 3) the consequences of CCK-1 receptor blockade on GLP-1 release. The data support the following concept: fat hydrolysis in the proximal small intestine plays a crucial regulatory function for digestive processes; adequate fat hydrolysis is required to start the process; the specific products of fat digestion, long-chain fatty acids, then stimulate the release of CCK; CCK in turn acts on CCK-1 receptors, which then initiate a series of digestive functions including modulation of GLP-1 secretion.

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