Inhibition of food intake in response to intestinal lipid is mediated by cholecystokinin in humans

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Matzinger, Daniel, Jean-Pierre Gutzwiller, Jürgen Drewe, Amar Orban, Reto Engel, Massimo D’Amato, Lucio Rovati, and Christoph Beglinger. Inhibition of food intake in response to intestinal lipid is mediated by cholecystokinin in humans. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1718–R1724, 1999.—Intraluminal lipid inhibits gastric emptying and exerts early satiation in animals and humans, but it is not clear whether the effects are mediated by cholecystokinin (CCK) in humans. Here, we tested whether CCK-A receptors mediate the inhibition of fat on food intake. Two sequential, double-blind, crossover studies were performed in 24 male subjects. First, subjects received either intraduodenal fat or saline together with a preload of either water or banana shake. Second, 12 subjects received either intraduodenal fat or saline perfusion plus a concomitant infusion of saline or loxiglumide, a specific CCK-A receptor antagonist, together with a preload of banana shake. In both studies, subjects were free to eat and drink as much as they wished. Fat induced a reduction in calorie intake (P < 0.05) compared with controls. Furthermore, a decrease in hunger feelings was observed. Infusion of loxiglumide abolished the effects of fat. Duodenal fat interacts with an appetizer to modulate energy intake in humans. This effect is mediated by CCK-A receptors.

Intraluminal fat; gastric distension; appetite

TO DATE, ONLY INSUFFICIENT information is available about the processes that control food intake and satiation in humans. On the basis of animal experiments, it is assumed that food intake is suppressed by stimulation of specific receptors within the gastrointestinal tract (1, 3). Inspired by this hypothesis, Welch et al. (48) observed that the infusion of a lipid emulsion into the ileum reduces food intake in healthy volunteers; eating habits were, however, not influenced by intravenous administration of a similar fatty emulsion. Lipid infusion into the jejunum or the ileum showed, in addition to a decreased food consumption, early fullness (9, 49) and a delay in gastric emptying (34, 41). These effects were accompanied by a release in endogenous cholecystokinin (CCK) (9). These observations suggest that intraluminal fat induces changes in stomach fullness and/or distension or the release of CCK or both. CCK is normally released from endocrine cells of the duodenum and jejunum, contingent on intraluminal fat or amino acids (16, 23). In addition to stimulation of gallbladder contraction and exocrine pancreatic secretion, CCK causes a delay in gastric emptying and an inhibition of food intake (15). In laboratory animals outfitted with a gastric fistula that causes continuous drainage of food, CCK is able to inhibit food intake (15). These results are indications that the influence of this peptide on hunger and satiation is not solely caused by the fullness of the stomach. CCK can also cause satiation via CCK receptors in the brain (5). Along this line of investigations, an induction in the satiation response could be ascertained in humans using CCK infusions (22, 26, 45, 46), supporting the concept of a physiological effect of CCK in the regulation of food intake.

An investigation carried out a few years ago in humans examined the interaction between the influence of stomach distension in combination with exogenous CCK infusion on the satiation response (40); the results revealed an interaction (synergism) between exogenous CCK and stomach distension with regard to eliciting this response. The interaction between the effect of intraduodenal (ID) fat and stomach distension on food intake and satiation has, however, not been investigated in humans.

Further exploration of the interactions between ID fat, CCK, and the stomach appeared, therefore, to be a fruitful line of investigation to better understand the control circuits that regulate food intake in humans. The availability of potent and selective CCK-A-receptor antagonists has made it possible to continue these investigations. Loxiglumide (Lox) is one of these specific CCK-A-receptor antagonists available for human use (20, 33, 43). Lox, therefore, appeared to be a useful tool to test the hypothesis that endogenous CCK is responsible for mediating the interactions of ID fat with gastric distension via activation of peripheral CCK-A receptors in modulating food intake in humans.

METHODS
Overview

Two experimental series were sequentially performed. First, a randomized, double-blind, four-period, Latin square design was carried out in 12 healthy, paid, male volunteers. Each participant underwent tests on four experimental days, separated by at least 1 wk. On each of the experimental days, the intake of a test meal with related variables was measured. A continuous perfusion of either fat or saline (control) was given throughout the entire experiment. Forty minutes after starting the respective ID infusion, a preload of either 400 ml of water or 400 ml of banana shake was given. After an additional 20 min, subjects were invited to eat and drink as much as they wished (Fig. 1A).

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The design of the second series was almost identical: 12 healthy male subjects were studied in a double-blind, three-period crossover fashion. This second part was undertaken to demonstrate that the effects of fat are mediated by peripheral CCK-A receptors. Each participant underwent three tests separated by at least 1 wk. An ID perfusion of fat together with either intravenous saline (control) or, alternatively, Lox was given on two experimental days; on the third experimental day, ID and intravenous saline were administered (control experiment). On each day, a 400-ml preload (banana shake) was ingested 20 min before starting the test meal intake (Fig. 1B).

Subjects

Twenty-four male subjects, aged 20–44 yr (mean age 24.5 yr), participated in the study. The weight of all subjects was within a normal range for their age, sex, and height. Each subject gave written informed consent for the study. The protocol was approved by the Human Ethics Committee of the University Hospital in Basel. Before acceptance, each participant was required to complete a medical interview, received a full physical examination, and participated in an initial laboratory screening. No subject was taking any medication or had a history of food allergies or dietary restrictions.

Experimental Procedure

Part one. Four treatments, separated by at least 7 days, were performed in each subject. On the day preceding each experiment, subjects swallowed a radiopaque polyvinyl feeding tube (external diameter: 8 Fr) that had an opening 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, but it also allowed subjects to eat and drink with a minimum discomfort from the tube. The tube was allowed to be 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 distally to the teeth. It was firmly attached to the skin behind the ear to prevent further progression of the tube during the experiment. On the day of the experiment, each subject ate breakfast (if this was his normal custom), but no drinks were allowed after 8 AM. At noon, the experiment started with the first continuous perfusion. The treatments were identical in design except for the ID perfusions and the preloads (see Fig. 1A).

The first treatment consisted of an ID saline perfusion for the duration of the experiment. Forty minutes after starting the perfusion, a preload of 400 ml of water was given. After an additional 20 min, subjects were invited to eat and drink as much as they wished. The second treatment was similar: ID saline was given during the whole experiment, but 400 ml of banana shake were given instead of water. The third and fourth experiments used ID fat (corn seed oil) throughout the entire experiment instead of saline, combined with either water or banana shake as respective preloads. A perfusion rate of 0.375 ml/min for a total of 120 min (load 41 g of fat) was chosen from previous experiments (9, 48).

The preload used in this study is based on experiments performed by Muurahainen et al. (40). The shake was made of 100 g whey, 16 g sugar, and 100 g of blenderized banana mixed with water to a total amount of 400 ml. The fat content of this preload was very low to prevent stimulation of endogenous CCK (composition of preload: 35.8 g carbohydrates, 0.4 g fat, 1.7 g protein; total energy content: 150 kcal).

Twenty minutes after the preload, a standard meal was presented to the subjects, and they were invited to eat and drink as much as they wished for 60 min. The meal consisted of 1) orange juice, 2) ham sandwiches (60 g wheat bread, 10 g butter, and 25 g ham), 3) chocolate pudding, and 4) coffee with cream and sugar (coffee could be sweetened if desired; therefore, both cream and sugar were optional). Nonsparkling water could be taken during the meal as a noncaloric beverage. The order of food intake had to follow the above schedule. To reduce the participants’ awareness of the amount of food eaten, food was presented in small samples and in excess.

The ID fat perfusion solution was indistinguishable in appearance from the control solution (saline), and the person in charge of the experiments was unaware of the respective treatment, thereby making it possible to deliver treatments in a double-blind fashion. The amount of food eaten, the volume of fluid drunk, and the time for each subject to complete the meal were quantified. From these observations, the total calorie intake could be calculated. Before, during, and after the preload, blood was drawn for plasma CCK determinations in EDTA-coated tubes containing aprotinin (1,000 KIU/ml blood).

After the start of the perfusion, subjects scored their subjective feelings for hunger and fullness at 15-min intervals for the duration of each experiment using a visual analog scale from 1 through 10 and indicated their scores on a questionnaire. The scale and scores have previously been described in detail by Drewet et al. (9) and Welch et al. (48). In brief, a score of zero for hunger indicated that the subject was not hungry at all, two indicated “slightly hungry,” five indicated “moderately hungry,” eight indicated “very hungry,” and 10 indicated “absolutely ravenous.” The score for fullness was similar.

Part two. Procedures on all days were identical to part one, except for the intravenous infusions. Twelve healthy male subjects participated in this randomized, double-blind, three-period crossover study. Subjects received, on two experimen-
Results

Food Intake

Part one. The amount of food eaten and the corresponding energy intake (kcal), were compared between the treatments by ANOVA in case of significance followed by multiple paired t-tests with Bonferroni’s correction. Plasma CCK data were evaluated by calculating area under the plasma concentration/time curve (AUC). AUC was calculated by linear trapezoidal rule from T-60 to 0 min. Parameters were analyzed by ANOVA with row (subject) and column (treatment) effects. If significant differences were detected for the treatment effect, ANOVA was followed by Scheffé’s multicomparison test for pairwise comparisons. Scores for hunger and fullness were compared at the different time points before and after the meal between the different treatments using multiple paired t-tests with Bonferroni correction (42).

Table 1. Effect of ID fat or saline together with a preload of water or banana shake on eating behavior in 12 healthy male subjects

<table>
<thead>
<tr>
<th></th>
<th>ID Saline+Water</th>
<th>ID Saline+Banana</th>
<th>ID Fat+Water</th>
<th>ID Fat+Banana</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calorie intake, kcal</td>
<td>1,771 ± 85</td>
<td>1,491 ± 94*</td>
<td>1,568 ± 83*</td>
<td>1,197 ± 114†</td>
</tr>
<tr>
<td>Amount of food, g</td>
<td>625 ± 36</td>
<td>513 ± 38*</td>
<td>546 ± 37*</td>
<td>406 ± 45†‡</td>
</tr>
<tr>
<td>Amount of fluid, ml</td>
<td>758 ± 58</td>
<td>684 ± 52</td>
<td>690 ± 56</td>
<td>524 ± 52*</td>
</tr>
</tbody>
</table>

Data are means ± SE. ID, intraduodenal. *P ≤ 0.05 vs. control (ID saline + water) analyzed by ANOVA followed by multiple paired t-tests with Bonferroni’s correction. †P ≤ 0.05 vs. ID saline + banana. ‡P ≤ 0.05 vs. ID fat + water.

Part two. Food consumption and calorie intake were significantly (P < 0.05) reduced after ID fat plus intravenous saline administration (Table 2) in comparison to ID plus intravenous saline infusion (control). All applications were combined with a preload of banana shake. These results confirm the effectiveness of the combination of ID fat and banana shake administration shown in part one. ID fat plus intravenous Lox reversed the effect of ID fat plus intravenous saline, caused a similar calorie intake as the control application, and was significantly different from the ID fat plus intravenous saline infusion (P < 0.05). These data indicate that the inhibition in food intake induced by ID fat administration can be antagonized with the administration of a CCK antagonist. Fluid intake was increased with Lox treatment, but not changed with other treatments (Table 2). Eating time was not affected (data not shown).

Table 2. Effect of ID fat or saline together with a preload of banana shake and an IV infusion of saline or Lox on eating behavior in 12 healthy male subjects

<table>
<thead>
<tr>
<th></th>
<th>ID Saline+IV Saline</th>
<th>ID Fat+IV Saline</th>
<th>ID Fat+IV Lox</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calorie intake, kcal</td>
<td>1,900 ± 127</td>
<td>1,461 ± 110*</td>
<td>1,951 ± 119†</td>
</tr>
<tr>
<td>Amount of food, g</td>
<td>648 ± 57</td>
<td>499 ± 45*</td>
<td>682 ± 59†</td>
</tr>
<tr>
<td>Amount of fluid, ml</td>
<td>588 ± 28</td>
<td>551 ± 46</td>
<td>737 ± 52†</td>
</tr>
</tbody>
</table>

Data are means ± SE. IV, intravenous; Lox, loxiglumide. *P ≤ 0.05 vs. control (ID saline + IV saline) analyzed by ANOVA followed by multiple paired t-tests with Bonferroni’s correction. †P ≤ 0.05 vs. ID fat + IV saline.
nous Lox administration, an augmented CCK response was documented both in comparison to ID fat plus intravenous saline and to the control (ID saline plus intravenous saline) experiment \( (P < 0.0005, \text{ respectively}) \).

**Eating Behavior**

Part one. As expected, hunger ratings fell and fullness ratings rose after the subjects had either drunk the banana shake preload or received ID fat perfusion. The effect was most pronounced with the combination of ID fat plus a preload of banana shake, and, 15 min after a preload administration, a significant reduction of hunger ratings could be observed \( (P < 0.05; \text{ Fig. 4}) \). The administration of ID saline plus banana shake in comparison to ID saline plus a water preload was not significantly different, although a trend toward reduced hunger ratings was seen \( (P = 0.057) \).

Part two. As in part one of the study, hunger ratings fell and fullness ratings rose after the subjects had drunk the preload. The results were most pronounced with ID fat plus intravenous saline but did not reach a statistical significance. The data are given in Fig. 5.

**DISCUSSION**

The role of ID fat in the production of meal-ending satiation has been extensively explored in animals. In addition, it has been suggested that the effect of fat is mediated by CCK \( (7, 8, 24, 30) \). Solid evidence, largely based on studies with exogenous CCK, supports the following: 1) the brain-gut peptide CCK reduces meal size and energy intake \( (11–15, 35, 38, 47) \), and 2) type A CCK receptors seem to be critical. Information primar-
ily obtained in animals, using specific CCK-A receptor antagonists, indicate that fat-induced satiation signals are mediated via peripheral CCK-A receptors, thereby implying a physiological role for CCK (17, 18, 36, 51). Moran et al. (36) have recently provided additional experimental evidence in support of the hypothesis: rats that do not express the CCK-A receptor develop obesity, hyperglycemia, and noninsulin-dependent diabetes mellitus; in short-term feeding tests, the animals were completely resistant to exogenous CCK administration. In 24-h, solid food-access experiments, the rats consumed significantly more food than control animals. These results are consistent with the hypothesis that the lack of CCK-A receptors results in a satiety deficit leading to increases in meal size, overall hyperphagia, and obesity.

In humans, the evidence for CCK as a mediator of fat-induced stimulation of a peripheral satiety signal is less clear. In 1981, Kissileff et al. (22) reported that infusion of exogenous CCK-8 decreased liquid meal intake in human volunteers. Lieverse et al. (26) have extended these observations by showing that CCK at physiological plasma levels significantly increased satiation in humans. However, not all human studies have found consistent effects of exogenous CCK on food intake. In another study of Lieverse (28), infusion of exogenous CCK at plasma levels termed physiological (because they induced postprandial gallbladder contraction or pancreatic enzyme secretion) failed to affect food intake in healthy subjects. Conflicting results have also been obtained with different classes of CCK-A-receptor antagonists: oral administration of MK329 induced a significant increase in hunger feelings, suggesting that endogenous CCK is indeed involved in the physiological control of food intake (50). In contrast to these findings, no effect on satiety was obtained with the CCK-A-receptor antagonist Lox, neither in lean nor in obese subjects (29). In an additional study, the same group of investigators observed, however, that a small load of ID fat increased satiation in healthy volunteers mainly through the effects of endogenous CCK acting on CCK-A receptors (27).

In the present study, we have chosen a different approach by investigating the interaction of fat-induced reduction on food intake with a preload in the form of an appetizer in male volunteers. On the basis of studies of Muurahainen et al. (40) as well as Welch et al. (49), we used an experimental model that enabled us to investigate the interaction of a fat-free preload in combination with the administration of ID fat. Calorie and food intake were both significantly reduced by the preload or by ID fat in comparison with the control experiments. When applied in combination, the most pronounced and, moreover, synergistic inhibitory effect (reduction of calorie intake by 32% and amount of food by 35%) was observed. Along these lines, ID fat increased plasma CCK levels, whereas the preload did not have an effect on CCK release. Taken together, these results indicate that ID fat interacts with signals from the stomach to regulate food intake. The data suggest that signals from the stomach interact with signals from the small intestine. The results, furthermore, infer that CCK is the most likely candidate for mediating this effect. Muurahainen et al. (40) had previously suggested that exogenously administered CCK-8 was able to amplify neural signals induced by a soup preload. In agreement with this observation, gastric signals stimulated by the preload were amplified by fat-induced plasma CCK release in the present study. Our data, therefore, support the hypothesis of Muurahainen et al. that endogenous CCK amplifies gastric and/or intestinal signals in humans.

In the second series of experiments, we investigated the interaction between a preload and ID fat by using a CCK-A-receptor antagonist as a tool. We were able to show that the reduction on food intake induced by ID fat administration plus preload could be reversed by the CCK-A-receptor antagonist Lox. ID fat plus intravenous Lox caused a similar food and calorie intake as in the control experiment. We interpret these findings as convincing evidence that CCK is the mediator of this interaction. The results, furthermore, support the importance of CCK as a physiological signal in the control of food intake in humans. Additional evidence comes

![Graph](http://example.com/graph.png)
from a study performed by Feinle et al. (10), who found that lipid-induced CCK release is involved in the induction of meallike sensations of fullness during gastric distension and that these effects are mediated via activation of CCK-A receptors.

Plasma CCK concentrations during the premeal period were significantly increased by 1D fat and three-fold augmented by Lox, confirming previous studies (9, 20, 33, 43). The augmented CCK response has been explained by a negative feedback control of CCK release by pancreatic enzymes and/or bile acids. Because Lox markedly suppresses bile and enzyme secretions in the small intestine, it may counteract the postulated negative control of CCK release by bile acids and/or pancreatic enzymes and thereby increase circulating plasma CCK concentrations (33, 43).

Recent evidence has suggested that leptin, the product of the obese gene, may be involved in satiety pathways originating from the gastrointestinal tract. Bado et al. (4) have detected leptin gene expression and immunoreactive leptin in the gastric fundus. Furthermore, food ingestion caused a rapid stimulation of gastric leptin secretion, an effect that was reproduced by CCK administration. In mice, leptin enhances the satiety-inducing effect of CCK (6), suggesting that CCK-induced leptin secretion may amplify the intestinal regulation of food intake (21). In the present study, we did not, however, observe any changes in circulating leptin concentrations (data not shown).

In conclusion, we have shown that 1) endogenous CCK mediates fat-induced reduction on food intake, 2) CCK is involved in the interaction of 1D fat with the stomach to regulate food intake in humans, and 3) the effects of CCK are mediated by type A receptors.

Perspectives

Inhabitants of western countries are frequently urged to reduce their intake of dietary fat, because fat has been linked to cardiovascular disease, obesity, diabetes mellitus, and some forms of cancer. It is therefore of interest that fat in the small intestine reduces food intake and induces early satiety (47, 48). Welch et al. (48) reported in 1985 in humans that infusion of a lipid emulsion into the small intestine reduced food intake; an intravenous infusion of a similar lipid emulsion had no effect on eating. These findings form the basics of the hypothesis that fat acts in the gut as a preabsorptive site to decrease food intake.

The major products of luminal lipid digestion are monoglycerides and fatty acids. Fatty acids are potent secretagogues of CCK release (23, 24). Thus there is a link between fat digestion and the ability of fat digestion products to initiate a feedback response on food intake.

It is well known that the fat content of food provides a pleasing texture, flavor, and odor that, in turn, enhance food acceptability. Moreover, there is evidence for a positive correlation between body fatness and the proportion of calories eaten as fat. In other words, the fat content of food makes it appealing to fat people with further consequences for overeating. On the basis of the available results, it should be further investigated whether specific fat substitutes that cannot be absorbed are able to reduce food and energy intake and function as a satiety signal. Understanding the appetite control system is a key factor for developing strategies for modifying dietary fat intake. The role of intestinal fat in the regulation of food intake is only one piece of the puzzle. Elucidation of this complex control system will increase the understanding of the role of dietary nutrients in the regulation of appetite and body weight and the development of overweight and obesity.

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