Reduced sensitivity to the satiation effect of intestinal oleate in rats adapted to high-fat diet

MIHAI COVASA AND ROBERT C. RITTER
Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, and Program in Neuroscience, Washington State University, Pullman, Washington 99164

Covasa, Mihai, and Robert C. Ritter. Reduced sensitivity to the satiation effect of intestinal oleate in rats adapted to high-fat diet. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R279–R285, 1999.—When rats are maintained on high-fat diets, digestive processes adapt to provide for more efficient digestion and absorption of this nutrient. Furthermore, rats fed high-fat diets tend to consume more calories and gain more weight than rats on a low-fat diet. We hypothesized that, in addition to adaptation of digestive processes, high-fat maintenance diets might result in reduction of sensitivity to the satiating effects of fat digestion products, which inhibit food intake by activating sensory fibers in the small intestine. To test this hypothesis we measured food intake after intestinal infusion of oleic acid or the oligosaccharide maltotriose in rats maintained on a low-fat diet or one of three high-fat diets. We found that rats fed high-fat diets exhibited diminished sensitivity to satiation by intestinal infusion of oleic acid. Sensitivity to the satiation effect of intestinal maltotriose infusion did not differ between groups maintained on the various diets. Reduced sensitivity to oleate infusion was specifically dependent on fat content of the diet and was not influenced by the dietary fiber or carbohydrate content. These results indicate that diets high in fat reduce the ability of fat to inhibit further food intake. Such changes in sensitivity to intestinal fats might contribute to the increased food intake and obesity that occur with high-fat diet regimens.

fat adaptation; maltotriose; cholecystokinin

DIGESTIVE FUNCTION changes to accommodate variations in the macronutrient composition of the diet. For example, increased dietary fat is associated with increased production and secretion of pancreatic lipase (27, 31). Secretion of amylase and various proteases also increases or decreases in relation to the relative predominance of carbohydrate and protein in the diet (11, 14). Changes in intestinal nutrient transporters also have been observed after changes in diet composition (15, 33, 34). The presumed survival value of such digestive adaptation could be to favor efficient digestion and absorption of specific nutrients, the dietary proportions and availability of which may change, independent of selections made by the animal itself.

Satiation, the behavioral manifestation of processes by which eating is terminated, controls how much food will be presented to the digestive tract during a meal. Nutrients in the intestine provide some of the signals that contribute to satiation. Hence, the digestive tract is in a position to control presentation of appropriate quantities of nutrients for efficient digestion and absorption. Reduction of food intake by intestinal infusions of specific nutrients has been demonstrated repeatedly in several species, including rats (12, 26, 39) and humans (36), and is illustrative of the control of ingestion by feedback signals from the digestive tract. Because the enzymatic and absorptive machinery of the digestive tract adapt to permit more efficient use of specific dietary macronutrients, we wondered whether control of food intake in response to nutrients entering the digestive tract might also exhibit adaptation in response to elevated dietary content of a particular macronutrient. Specifically, we hypothesized that rats maintained on diets high in triglyceride would be less sensitive to reduction of food intake by intestinal infusion of a lipolytic product, i.e., long-chain fatty acid (oleic acid). To test this hypothesis we examined the ability of oleic acid and maltotriose to reduce deprivation-induced food intake by rats fed either a low-fat diet or one of three high-fat diets.

METHODS

Subjects. Adult male Sprague-Dawley rats weighing 280–300 g were subjects for these experiments. The animals were housed individually in a temperature-controlled room with a 12:12-h light-dark cycle (lights on from 0600 to 1800) and were given ad libitum access to water and one of four semipurified diets in spill-resistant feeding cups, as described previously (7). The rats were adapted to their respective diets for 3 wk before the beginning of behavioral testing. They were deprived of food for 17 h before each experimental trial. Water was available ad libitum throughout the experiments. Diets. Diets were prepared in our laboratory from commercially available macronutrient sources (Bio-Serv, Frenchtown, NJ), ICN Biomedicals, Cleveland, OH, and Sigma, St. Louis, MO) and were used as both maintenance and test diets. The diets formulated here are identical to those used in our previously published work (7) and are similar to diets used in dietary manipulation studies described by other investigators (22). The low-fat diet (LF, 3.86 kcal/g) contained the following nutrient categories, as percentages by weight: 65% starch, 5% fat, 20% protein, and 3% cellulose. A high-fat diet, which was isocaloric to LF (HF-1, 3.86 kcal/g) contained no starch, 34% fat, 20% protein, and 39% cellulose. Two additional high-fat diets, which were hypercaloric relative to LF, also were formulated. HF-2 (5.66 kcal/g) contained no starch, 54% fat, 20% protein, and 19% cellulose, whereas HF-3 (5.32 kcal/g) contained 36% starch, 34% fat, 20% protein, and 3% cellulose. All diets were balanced and equivalent with regard to mineral and vitamin content.

Surgical procedure. Rats were deprived of food overnight and anesthetized with 1 ml/kg of a mixture of xylazine (4.6 mg/ml; Haver-Mobay, Shawnee, KS) and ketamine HCl (76.9 mg/ml; Aveco, Ft. Dodge, IA) injected intraperitoneally. Via a midline incision, each animal was fitted with a chronic...
duodenal catheter, which consisted of a 22-cm length of silicone rubber tubing (0.025 in. ID, 0.047 in. OD, Dow Corning, Midland, MI). One end of the catheter was inserted through a needle puncture in the intestinal wall 2 cm aboral from the pylorus and advanced 6 cm aboral into the intestinal lumen. A small silicone nub placed 6 cm from the intraduodenal end of the catheter was passed through the intestinal puncture and resided against the intestinal mucosal surface. The catheter entry into the duodenum was surrounded by a ring of surgical polypropylene mesh (Marlex) (5 × 5 mm) that was sealed to the serosal surface of the intestine with a drop of cyanoacrylic cement. This procedure securely closed the ostomy through which the catheter passed and sealed the catheter in place. The other end of the catheter was passed subcutaneously and exited through a 5-mm skin incision at the back of the neck. The peritoneum and abdominal muscles were simultaneously closed with absorbable sutures. The abdominal skin incision was then closed with wound clips, which were removed 7 days after surgery. The exposed end of each catheter was occluded with stainless steel wire, which was removed immediately before each experiment when the catheter was connected to a syringe pump (Razel) to make intestinal infusions. With the use of this preparation we were able to maintain all catheters patent and the animals in good health for at least 4 mo.

Intestinal infusates. Oleic acid (Sigma), a monounsaturated fatty acid and fat digestion product of olive oil, which is a potent stimulus for CCK release, was dissolved in isotonic saline containing sodium taurocholate to achieve final fatty acid concentrations of 0.0, 0.03, 0.06, or 0.08 kcal/ml. Maltotriose, a glucose trisaccharide and starch digestion product, was prepared in distilled water to achieve final concentrations of 0.0, 0.03, 0.06, or 0.52 kcal/ml. Both oleate and glucose oligosaccharides, such as maltotriose, previously have been shown to reduce food intake when infused into the upper small intestine (12, 26, 35, 39). The pH of the infusates was adjusted to 7.4 by adding HCl or NaOH as necessary. The infusate solutions were then adjusted to a tonicity of 300 mosM by addition of NaCl, and the osmotic concentration was checked using a vapor pressure osmometer (Wescor 5130A). The infusates were drawn into 10-ml syringes that were connected to the intestinal catheters of the animals via PE-50 polyethylene tubing. Infusions of nutrients were delivered in descending order of nutrient concentration.

Experimental procedure. Food cups were removed at 1700 the evening before the experiment and returned at 0900 the next morning. On experimental days the overnight-fasted rats were removed from their home cages and weighed. The stainless steel plugs were removed from the free end of the intestinal catheters, and the catheters were flushed with 0.5 ml of distilled water to assure patency. Then the catheters were connected to 10-ml syringes containing the intestinal infusates via PE-50 tubing. The rats were returned to their home cages, without food, and intestinal infusions were made at 0.48 ml/min for a total of 20 min using motorized syringe pumps (Razel). Five minutes after termination of the infusion, weighed cups of the maintenance diets were returned to the rats and intake and spillage were measured over the ensuing 30 min. At the end of the test, the animals once again were given access to the maintenance diet ad libitum until being deprived before the next infusion test. A minimum of 48 h elapsed between each intestinal infusion of either vehicle or intestinal nutrient. Each nutrient infusion was preceded and followed by an infusion of the 0.0 kcal/ml vehicle solution 48 h before and 48 h after nutrient infusion. Body weights of rats were recorded daily.

Data analysis and presentation. Results are graphically presented as percent reduction of 30-min food intake that was calculated for each rat and condition according to the following formula: \( \% \text{reduction} = 1 - \left( \frac{\text{experimental}}{\text{baseline}} \right) \times 100 \). Raw intake data as well as percent reduction of food intake were analyzed by appropriate repeated measures ANOVA, with diet and nutrient caloric concentration as main factors, followed by Bonferroni’s test.

Experiment 1: Reduction of food intake by intraintestinal oleic acid in rats maintained on low-fat and high-fat isocaloric diets. In this experiment we tested the hypothesis that when rats are maintained on isocaloric high-fat and low-fat diets, the high-fat diet-fed rats will exhibit reduced sensitivity to the satiating effects of intraintestinal oleic acid infusion. For this experiment nine rats were maintained on LF and an additional nine rats were maintained on HF-1 for 21 days before the beginning of intestinal infusion tests. Then both groups of rats received intestinal infusions of oleic acid (0.03, 0.06, and 0.08 kcal/ml) in descending order of caloric concentration. Each oleate infusion was preceded and followed by a test in which the isonicotic vehicle, containing 0.53% (wt/vol) taurocholate (sodium salt of taurocholic acid, 98% pure Sigma), was infused.

Experiment 2: Reduction of food intake by intraintestinal oleic acid in rats maintained on high-fat hypercaloric diets. In experiment 1 the HF-1 was made isocaloric with LF by addition of cellulose. Therefore, it is possible that changes in the response to intestinal oleic acid infusion were due to an effect of increased dietary fiber, rather than the fat content of the diet. Also, because HF-1 contained no soluble carbohydrate, it is possible that altered sensitivity to intestinal oleate infusion was an effect of the absence of digestible carbohydrate, rather than the high level of dietary fat. Therefore, in this experiment we examined the effects of oleic acid infusion on reduction of food intake by rats maintained on a diet that provided most calories as fat, but contained 36% by weight soluble carbohydrate and only 3% cellulose (HF-3). In addition, sensitivity to oleic acid-induced reduction of food intake was examined in rats maintained on a diet (HF-2) similar in caloric density to HF-3 but that provided no calories as starch and contained 19% cellulose. Four different groups of naive rats (n = 8/group) were adapted to each of the diets, LF, HF-1, HF-2, or HF-3, for 21 days before the beginning of infusion tests. General experimental procedures were the same as described for experiment 1.

Experiment 3: Reduction of food intake by intraintestinal maltotriose infusion in rats maintained on low-fat and high-fat diets. To determine whether high-fat maintenance diets selectively alter sensitivity to oleic acid infusion or whether high-fat maintenance also alters sensitivity to reduction of food intake by intestinal infusions of other nutrients, we examined reduction of food intake by intestinal infusion of 0.26 and 0.52 kcal/ml maltotriose in rats maintained on LF, HF-1, HF-2, and HF-3. Experimental procedures were the same as described for experiment 2, except that maltotriose was infused instead of oleic acid into the same group of rats from experiment 2.

RESULTS

Experiment 1. As shown in Fig. 1 infusion of oleate reduced food intake as a function of oleate concentration delivered into the duodenum in both low-fat and high-fat diet-adapted rats (P < 0.001). However, rats maintained on HF-1 exhibited a significantly lower percent reduction of food intake after all three doses of oleate compared with rats maintained on LF.
Fig. 1. Oleate-induced reduction in food intake in rats adapted to low-fat (LF) and high-fat (HF-1) isocaloric diet. Data shown are percent reduction of 30-min food intake after 17 h food deprivation. Intestinal infusion of oleate caused significantly greater reduction of intake at all oleate doses (0.08, 0.06, and 0.03 kcal/ml) in low-fat diet-adapted rats than in high-fat diet-adapted rats. *Significantly different (P < 0.05).

Table 1. Low- and high-fat food consumption of rats 30 min after intraintestinal oleate infusion (experiment 1)

<table>
<thead>
<tr>
<th>Caloric Density, kcal/ml</th>
<th>LF-Fed Rats</th>
<th>HF-1-Fed Rats</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>Oleate</td>
</tr>
<tr>
<td>0.08</td>
<td>5.8 ± 0.4</td>
<td>3.1 ± 0.3*</td>
</tr>
<tr>
<td>0.06</td>
<td>6.1 ± 0.2</td>
<td>3.2 ± 0.4*</td>
</tr>
<tr>
<td>0.03</td>
<td>6.3 ± 0.4</td>
<td>4.5 ± 0.3*</td>
</tr>
</tbody>
</table>

Values are average 30-min food intake in g (mean of 2 tests per each infusate concentration ± SE). LF-1, high-fat diet isocaloric to low-fat diet (LF). *Significantly different (P < 0.05) compared with vehicle infusion. †Significantly different (P < 0.05) from same caloric concentration in LF.
gain associated with ingestion of the high-fat diets. However, this interpretation can be rejected, because in this study, as well as in our previously reported work, rats maintained on HF-1, which was made isocaloric with LF by addition of fiber, did not eat more or gain more weight than rats maintained on LF. This observation is consistent with our earlier report (7), which demonstrated that rats maintained on HF-1 and which were less sensitive than LF-fed rats to reduction of food intake by CCK, also did not eat more or gain more weight than rats fed LF. Therefore, increased food intake or weight gain does not account for decreased sensitivity to the satiation producing effects of intestinal oleate infusion in rats maintained on a high-fat diet. Other investigators also have reported that rats eating high-fat diets gain similar amounts of weight as rats fed an isocaloric low-fat diet (22, 29). Exactly why HF-1 rats do not eat more and gain more weight than rats fed LF, even though they are less sensitive to CCK- and intestinal oleate-induced reduction of food intake, is not known. However, we can speculate that the increased mechanical stimulation provided by the increased amount of fiber in HF-1 may increase mechno-receptive signals from the gastrointestinal tract and this increased mechanical stimulation may be sufficient to control food intake, even though CCK and oleate sensitivity are reduced. Thus the effects of high fat-induced desensitization of oleate-induced satiation may be most important when high-fat diets are low in fiber. We did not assess body adiposity of our rats fed LF and the various high-fat diets; therefore, we cannot rule out the possibility that HF-1- and LF-fed rats differed in adiposity, although they were comparable in body weight. Indeed, previous studies have demonstrated that increasing the level of dietary fat fed increased body fat stores without increasing body weight (3, 29).

The potential interaction of increased adiposity and reduced oleate sensitivity requires further experimental examination. However, there is some circumstantial evidence that changes associated with adiposity are not responsible for reduced sensitivity to intestinal oleate. For example, increased levels of circulating leptin, the ob protein, produced by the adipose tissue are associated with increased adiposity induced by ingestion of a high-fat diet (9). Furthermore, leptin gene expression is increased in the adipose tissue obtained from fat pads in rats fed a high-fat diet (18). However, there is no evidence to suggest that increased leptin levels would reduce the efficacy for intestinal oleate in the reduction of food intake. In fact there is evidence indicating that reduction of short-term food intake by acute administration of exogenous CCK actually is enhanced by injection of exogenous leptin (2). In addition, recent results indicate that injection of exogenous CCK enhances weight loss and reduction of food intake after systemic or intracerebral leptin injection (19, 20). Reduction of food intake by intestinal oleate infusion appears to be mediated by CCK (6, 38). Therefore, it seems unlikely that increased leptin levels, which might be associated with increased adiposity in high-fat diet-fed rats, could account for decreased sensitivity to intestinal oleate infusion.

Previously, we reported that rats fed high-fat diets were less sensitive to the satiation effects of CCK than rats fed low-fat diets (7). Brenner et al. (4) demonstrated that intraintestinal oleate releases CCK in the rat. Furthermore, high-fat diets also are associated with elevated levels of circulating CCK in both rats (5, 31) and humans (10). Finally, reduction of food intake by intestinal oleate and triglyceride infusion are attenuated by systemic injection of CCK-A receptor antagonists, such as devazepide or lorglumide (13, 37, 38).

![Percent suppression of food intake](http://ajpregu.physiology.org/)

### Table 2. Food consumption of rats 30 min after intraintestinal oleate infusion (experiment 2)

<table>
<thead>
<tr>
<th>Caloric Density, kcal/ml</th>
<th>LF</th>
<th>Oleate</th>
<th>LF</th>
<th>Oleate</th>
<th>LF</th>
<th>Oleate</th>
<th>LF</th>
<th>Oleate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.08</td>
<td>4.5±0.5</td>
<td>2.2±0.4*</td>
<td>4.4±0.8†</td>
<td>4.0±0.3</td>
<td>2.9±0.3*</td>
<td>4.1±0.4</td>
<td>3.0±0.6*</td>
<td></td>
</tr>
<tr>
<td>0.06</td>
<td>4.5±0.4</td>
<td>3.2±0.2*</td>
<td>4.6±0.4†</td>
<td>3.4±0.3</td>
<td>2.8±0.3</td>
<td>3.9±0.7</td>
<td>3.1±0.6</td>
<td></td>
</tr>
<tr>
<td>0.03</td>
<td>4.4±0.3</td>
<td>3.1±0.3*</td>
<td>4.9±0.4†</td>
<td>3.7±0.4</td>
<td>3.2±0.2</td>
<td>4.4±0.6</td>
<td>4.0±0.8</td>
<td></td>
</tr>
</tbody>
</table>

Values are average 30-min food intake in g (mean of 2 tests per each nutrient caloric concentration ± SE). HF-2, high-fat diet hypercaloric relative to LF (54% fat, 20% protein, and 19% cellulose); HF-3: high-fat diet hypercaloric relative to LF (36% starch, 34% fat, 20% protein, and 3% cellulose. *Significantly different (P < 0.05) compared with vehicle infusion. †Significantly different (P < 0.05) from same caloric concentration in LF.
Therefore, it is tempting to speculate that elevations of plasma CCK participate in reduction of food intake by intestinal oleate and that reduced sensitivity to endogenously released CCK is responsible for diminished sensitivity to oleate in high-fat diet-fed rats. A second possibility, which cannot yet be discounted, is that feeding high-fat diets may result in changes to both CCK and oleate sensitivity, but the change in CCK sensitivity might not be causally related to the change in oleate sensitivity. Therefore, although endogenous CCK may indeed play a role in reduced sensitivity to satiation by intestinal oleate infusion, the nature of that role remains to be experimentally elucidated.

From the current results, we cannot specify what modification of neural or intestinal sensitivity accounts for high-fat diet-induced reduction of sensitivity to intestinal oleate. However, reduction of food intake by intestinal oleate (35, 39, 40, 41) and CCK (30) are mediated by sensory fibers in the abdominal vagus nerve. In addition, it is likely that reduction of food intake by maltotriose also is vagally mediated (41). Therefore, it is of interest that in other recent work we have demonstrated that intestinal infusion of maltotriose induces high levels of expression of the immediate early gene protein Fos in the vagal sensory nucleus (nucleus of the solitary tract) of both high- and low-fat diet-fed rats (8). On the other hand, injection of exogenous CCK or intestinal infusion of oleate induced strong Fos expression in the nucleus of the solitary tract of low-fat diet-fed rats, but not in rats adapted to a high-fat diet (8). Thus it is possible that reduced sensitivity to intestinal oleate, as well as CCK, is due to a change in CCK binding or signaling in vagal sensory neurons.

Reduced sensitivity to the satiation-producing effects of CCK or intestinal oleate conceivably could contribute to increased body weight gain by enhancing food intake of animals chronically exposed to fatty foods. In this regard it is interesting that other investigators also have reported behavioral and metabolic changes associated with high-fat feeding, which could amplify assimilation of calories as fat. For example, rats maintained on a high-fat diet exhibit a greater preference and acceptance of fat (24), as well as increased capacity to absorb and oxidize fat (25). Furthermore, Lucas and Scafani (16) demonstrated that high-fat diet feeding enhances conditioning of a flavor preference by intragastric fat infusions. Collectively, these results indicate that chronic feeding of a high-fat diet is associated with a variety of behavioral and physiological changes that may contribute to increased food intake and weight gain.

In summary, we have shown that rats fed high-fat diets exhibit reduced sensitivity to reduction of food intake by intraintestinal oleate infusion but not by intraintestinal maltotriose infusion. Our results suggest that high-fat diets might favor increased fat intake by diminishing satiation in response to intestinal fat. Reduced sensitivity to oleate-induced reduction of food intake is similar to reduced sensitivity to satietogenic effects of CCK, which we have previously reported. Although studies exactly comparable to ours have not been performed in humans, French et al. (10) reported that humans adapted to high-fat diets exhibit delayed meal termination and increased hunger rating compared with those adapted to low-fat diets. Therefore, it is conceivable that diet-induced desensitization of satiation signals could play a role in overeating in populations where diets rich in fats and poor with regard to fiber are readily available.

**Perspectives**

The alimentary tract of omnivores is capable of digesting and absorbing all three macronutrient classes: protein, fat, and carbohydrate. However, omnivore diets often do not contain the individual macronutrients in equal proportions. In fact, many foods may provide the majority of metabolizable calories in the form of a single macronutrient. Consequently, the alimentary tract deals with diets that contain high
proportions of a particular nutrient by increasing the production of nutrient-specific hydrolytic enzymes and transporters. For example, animals maintained on high-fat diets exhibit increased production and secretion of pancreatic lipase (27) and increased absorptive rate for lipolytic products (1, 28). Presumably, these adaptive alimentary changes ensure that animals can take advantage of rich sources of calories, regardless of their macronutrient form, when they are encountered in nature.

The amount of nutrients presented for digestion and absorption is determined, in part, by satiation signals, which terminate ingestion and thereby limit meal size. Therefore, if animals were to benefit by increased assimilation from diets that are rich in fat, it would be important not to unduly limit ingestion of a fat-rich diet. In other words, reduced sensitivity of fat-induced satiation would operate in concert with increased digestive capacity for fat. We submit that in the context of maximally exploiting available dietary nutrients in a natural environment where rich sources of calories are at best uncertain, reducing the sensitivity of fat-induced satiation signals may be beneficial. Although we often think of satiation mechanisms and other controls of food intake as operating in the interest of body weight regulation, this may not always be the case. In fact, control of food intake in the interest of processes that are not directly linked with body weight could be the rule, rather than the exception. In this regard, reducing sensitivity of intestinal satiation to fat may confer an advantage in the harsh environment of caloric scarcity. However, in an environment where calories are not scarce, such desensitization could work against control of food intake in the interest of body weight regulation.

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