Lipid Infused into the Duodenum of Rats at Varied Rates Influences Food Intake and Body Weight Gain

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ABSTRACT We investigated the influence of luminal fat, including intestinal exposure time to fat, on food intake and body weight (BW) gain under conditions that resemble normal feeding. Male Wistar rats were divided into three groups: two groups received 30 mL Intralipid® and one group received 30 mL saline, in addition to having access to a nonfat diet. The saline-infused and one of the fat-infused groups were schedule fed (SF), i.e., allowed to eat for 7 h of the dark cycle; the other fat-infused group had diet available for 24 h. The three groups of rats were subdivided further by rate of infusion, i.e., 0.13 mL/min or 1.0 mL/min for a total of 6 groups. Rats infused with fat had lower BW gain than saline controls, except when diet was available for 24 h and rate of fat infusion was 1.0 mL/min (BW gain was similar to saline group). Daily energy intake corresponded to BW gain data. Pancreata and intestinal mucosa were examined (after 2 wk of treatment) for hypotrophic changes that may be related to specific factors stimulated by luminal fat; these may also be involved in the control of food intake. Rats infused with fat had heavier pancreata than saline-infused rats, and infusion of fat at 0.13 mL/min resulted in heavier pancreata than infusion at the faster rate. DNA and protein analysis indicated that hypotrophy rather than hyperplasia was responsible for this effect. Thus, prolonged fat exposure to intraluminal receptors resulted in reduced total daily energy intake, suppressed BW gain and heavier pancreata. The combined data support a connection among intraluminal fat, negative feedback signals that inhibit food intake and factor(s) associated with pancreatic stimulation. J. Nutr. 126: 2934–2939, 1996.

INDEXING KEY WORDS:
• fat • satiety • food intake • rats • infusion

fat has been shown to suppress short-term food intake in a variety of animals, including humans (reviewed in Greenberg 1992). Welch and co-workers (1985 and 1988) reported that instillation of fat into the small intestine of humans while they were consuming a meal resulted in premature feelings of satiety and a reduction in food intake. The presence of fat in the small intestine delays gastric emptying (Hunt 1968, Meyer 1987), and the resultant increase in gastric distension may inhibit further food intake. Although stimulation of gastric stretch receptors via gastric distension may play a role in the regulation of short-term food intake, it is not considered the primary regulating factor. This observation is supported by studies in sham feeding (gastric fistulated) animals in which the intraduodenal infusion of fat inhibits food intake, despite the lack of gastric distension (Greenberg et al. 1990). Additionally, lipid emulsions infused intravenously in humans (Welch et al. 1985) or rats (Greenberg et al. 1993) do not affect food intake, suggesting that feeding behavior is modulated by lipid acting at preabsorptive, intestinal receptors associated with the control of food intake.

The importance of intestinal receptors is illustrated by the interdigestive migrating motor complex (IDMMC), 1 which is the cyclic contractile activity that causes small intestinal motility in the absence of food. The presence of food in the small intestine suppresses the IDMMC, resulting in a "fed" pattern of contractile activity that is irregular and more intense. This fed pattern continues until the nutrient content of the intestine diminishes (Soffer and Adrian 1992). Sepple and Read (1989) reported a temporal association between the increase in hunger scores of human subjects and the return of the IDMMC, suggesting that the satiating

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4 Abbreviations used: BW, body weight; CCK, cholecystokinin; IDMMC, interdigestive migrating motor complex; SF, schedule fed.

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capacity of a meal and the duration of the fed pattern are related to intestinal receptor stimulation by the presence of nutrients.

Fat content of a meal is important in determining the duration of the fed pattern of small intestinal activity (Soffer and Adrian 1992). Because fat prolongs the fed pattern, independent of energy, suppresses food intake and affects gastric emptying, fat may also be responsible for sustaining satiety several hours after a meal has ended. If fat interacts with receptors in the small intestine that are involved in the regulation of food intake (i.e., satiety) and inhibits the migrating motor complex, then lengthening the time period in which fat is in contact with intestinal receptors may prolong the period of satiation following a meal. The purpose of this study was to determine if the inhibitory effects of fat on food intake are enhanced by increasing intestinal contact time of fat in the small intestine.

**MATERIALS AND METHODS**

**Animals.** The study was approved by the Animal Use and Welfare Committee at the University of California, Davis. Thirty-six male Wistar rats (Simonsen Laboratory, Gilroy, CA), weighing 150–200 g, were surgically equipped with chronic duodenal cannulas. Under anesthesia [ketamine/rompun/acepromazine 50:5.0:0.75 mg/kg body weight (BW)], the abdomen was opened via a midline incision and the cannula (Dow Corning, Silastic Brand, medical grade tubing, Midland, MI) placed in the duodenum distal to the pancreatic duct and proximal to the ligament of Trietz. The tubing was secured with a purse-string suture and collar to make sure that neither the lumen of the cannula nor the intestine was occluded. Sufficient cannula was left free in the abdominal cavity to allow the gastrointestinal tract full mobility. The abdominal incision was closed with sterile braided silk suture (5-0 Ethicon, Somerville, NJ). The tubing was exteriorized mid-scapularly and then threaded through a coil spring which was attached to a swivel outside of the cage. This arrangement allowed rats relatively free movement in their cages and permitted daily nutrient or saline infusions without handling or disturbing the animals. Rats were allowed at least 1 wk postoperative recovery. During the recovery period, rats were infused daily with 3–5 mL saline to ensure that the cannula remained patent.

**Infusion treatments.** Treatments consisted of infusing 30 mL of physiological saline at 0.13 mL/min or 1.0 mL/min with restricted feeding only. Volume, osmolarity and pH were constant among the infusion treatments. There were six experimental groups with six rats in each group.

**Diet.** Rats receiving lipid infusions were fed a diet which contained (g/kg) 290 sucrose, 290 cornstarch, 240 casein, 100 α-cellulose, 60 mineral mixture (Richer and Schneeman 1987), 20 vitamin mixture (Richer and Schneeman 1987). Fat was provided only through the cannula so that rate and volume (intestinal exposure to fat) were controlled. Rats infused with saline were fed the diet described above plus a fat-supplemented diet. The details are outlined in the experimental protocol section.

**Experimental protocol.** After recovery from surgery, all rats were weighed and assigned randomly to an experimental group. Four groups of rats were adapted for 7 d to the scheduled feeding regimen (SF) which consisted of unlimited access to food for 7 h/d during the dark phase of the light:dark cycle. Scheduled feeding allowed us to coordinate the infusions with initiation of feeding. Rats allowed 24-h access to food (2 groups) required no adaptation period. Because food intake and BW of rats infused with saline and those with no infusion did not differ (data not shown), a saline-infused group with food available for 24 h was deemed unnecessary for the experimental design. Saline-infused SF rats were trained to eat, in the first 2 h of the feeding period, a small portion of nonfat diet (8 g) supplemented with fat [33 kcal = 138 kJ]. This amount of fat was comparable to the fat load delivered directly to the duodenum of the lipid-infused rats. Unlimited nonfat diet was given to the saline-infused rats for the remaining hours of the feeding period. All rats were adapted to a reverse 12-h light:dark cycle and an intestinal infusion of 30 mL of lipid or saline at either 0.13 mL/min or at 1.0 mL/min.

Once the experimental period began, lipid-infused SF rats were given food cups containing the nonfat diet at 1000 h. At the same time, saline-infused SF rats were given 8 g of the nonfat diet supplemented with fat. Two hours later, at 1200 h, the lipid or saline infusion began for all rats, and the fat-supplemented diet of the saline-infused group was switched to nonfat diet. The time at which the infusion stopped depended on the delivery rate of the 30 mL of lipid or saline. The faster rate (1.0 mL/min) lasted for 30 min while the slower rate (0.13 mL/min) lasted for 3 h 48 min. Food cups for SF rats were removed at 1700 h. These rates are high or low, respectively, relative to the rate of gastric emptying for lipid emulsions (Kalogeris et al. 1983).

**Analysis.** Food intake was measured daily for 14 d at 1000 h (2 h prior to start of infusion), 1200 h [when infusion began], and 1700 h [post-termination of infusion]. Body weight was measured at the beginning and the end of the 14-d experimental period.

Animals were anesthetized and killed by exsangui-
nation on day 15 after food was withheld overnight. The pancreas was removed, lyophilized, weighed and analyzed for protein by the modified Lowry method (Petersen 1977); DNA content was analyzed by a fluorometric method (Kisana and Robins 1958, Setaro et al. 1976). The small intestine was cut at the pylorus and the ileal/cecal junction and was removed, flushed gently with physiological saline, divided into thirds and then scraped with a glass slide to collect and weigh mucosa.

Statistical analysis. To determine if fat and/or the length of intestinal exposure to fat had a significant effect on body weight gain, total daily energy intake, pancreatic weight, small intestinal weight, and pancreatic protein and DNA, treatment groups were compared with one-way ANOVA (Neter et al. 1990) using the Statview 512+ statistical package (Brain Power, Calabasas, CA) for MacIntosh computers. If significant differences were detected, the ANOVA was followed by Fischer's protected LSD test for appropriate comparisons (Neter et al. 1990). Statistical significance was accepted at P-values <0.05.

RESULTS

Body weight. Rats were weighed prior to starting the experiment and again at the end of the 14-d experimental period. Lipid infusion significantly reduced body weight gain compared with saline infusion (Table 1). SF lipid-infused rats lost weight, whereas lipid-infused rats that had access to food 24 h/d gained weight, but not to the same extent as schedule-fed saline-infused rats.

Also displayed in Table 1 is the effect of infusion rate on body weight gain. No differences were detected in body weight gain between the two rates of saline infusion. In contrast, with 24-h feeding, rats receiving lipid infusion at the two different rates had a significant difference in weight gain: rats infused with lipid at 1.0 mL/min gained three times more weight than rats infused with lipid at the 0.13 mL/min rate. The weight gain of rats infused with lipid at the faster rate was similar to that of the saline-infused rats. With scheduled feeding, body weight gain for lipid-infused rats did not differ significantly [P = 0.08] between the two rates; however, rats infused at the 0.13 mL/min rate lost an average of 11.4 g during the experimental period and the rats infused at 1.0 mL/min gained only 2.05 g.

Energy intake. Total fat intake was 138 kJ in all groups. While fat available did not differ, total daily energy intake differed significantly among groups. When rate of nutrient delivery was not taken into account, lipid-infused rats allowed 24-h access to food had a significantly higher total daily energy intake compared with SF lipid-infused rats, and saline-infused rats consumed significantly more energy than lipid-infused groups with food available for 7 or 24 h.

Examination of data concerning rate of fat or saline delivery indicates that rate did not have a major influence on total daily energy intake of rats when food was available for 24 h (Table 1). However, in rats allowed to feed for 24 h, rate of lipid infusion had a marked effect on total energy intake. Rats infused with lipid at 1.0 mL/min consumed 25% more diet per day than rats infused with lipid at 0.13 mL/min.

In addition to measures of total energy intake, the pattern of food intake was examined relative to the lipid and saline infusions (i.e., 1000 h, 2 h prior to the start of infusions, 1200 h, when infusion began, 1700 h, post-termination of infusion). Lipid-infused SF (7 h/d) rats consumed nearly all of their total daily energy prior to the start of the infusion (i.e., between 1000 and 1200 h). Infusion of lipid at the slower rate caused complete cessation of feeding between 1200 and 1700 h, whereas rats infused with lipid at the faster rate consumed between 15 and 20% of total energy intake between 1200 and 1700 h. Observations of rat activity under black light indicated that this food was consumed during the last hour of the scheduled feeding period. These alterations were not observed in saline-infused rats.

Lipid-infused rats allowed access to food for 24 h/d consumed an average of 15% of total daily energy intake between 1000 and 1200 h (slow fat infusion, 19% and fast infusion, 12%) 0% between 1200 and 1700 h, and 43% between 1700 and 1000 h the following day (slow fat infusion, 37% and fast fat infusion, 48%).
tissue was 22% higher in lipid-infused rats than in saline-infused rats \( P < 0.05 \), but when protein was expressed relative to DNA, no differences were detected between the lipid- and saline-infused groups \( P = 0.45 \). DNA content did not differ among groups \( P = 0.60 \). Thus, the higher pancreas weight was related to hyperplasia rather than hyper trophy.

**DISCUSSION**

The presence of lipid in the upper small intestine had a significant inhibitory effect on energy intake. In the present study, infusion of fat reduced daily energy intake in SF rats, resulting in reduced growth or weight loss after 2 wk. The inhibitory effect of fat on energy intake was potent and appeared to override other physiological signals that should have encouraged the rats to eat, especially because most of them were in negative energy balance. When rats were infused with lipid but allowed unlimited access to diet for 24 h/d, they grew and gained weight. Food intake measurements revealed that 24-h fed, lipid-infused rats met their energy needs for growth during the noninfusion, nonlipid-suppressed part of the cycle. These data suggest that rats resumed eating once the suppressive effects of the fat on food intake subsided. It is interesting to note that the rats infused with lipid at the slower rate \( 0.13 \text{ mL/min} \) ate less food and gained significantly less weight than either the saline-infused rats or the rats infused with

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**TABLE 2**

Small intestine weight of rats receiving an intestinal infusion of fat or saline at two different rates and with food available for 7 or 24 h

<table>
<thead>
<tr>
<th>Infusate and feeding treatment (^2)</th>
<th>Total small intestine weight (^1) g/100 g BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow fat/SF</td>
<td>4.76(^{cd})</td>
</tr>
<tr>
<td>Fast fat/SF</td>
<td>5.44(^d)</td>
</tr>
<tr>
<td>Slow fat/24 h</td>
<td>4.45(^c)</td>
</tr>
<tr>
<td>Fast fat/24 h</td>
<td>3.56(^b)</td>
</tr>
<tr>
<td>Slow saline/SF</td>
<td>2.33(^a)</td>
</tr>
<tr>
<td>Fast saline/SF</td>
<td>2.26(^a)</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td>0.29</td>
</tr>
</tbody>
</table>

\(^1\) Values are the mean small intestinal weights [g/100 g body weight (BW)] of 6 rats receiving the described treatments. Means with different letters indicate significant differences \( P < 0.05 \) between treatments.

\(^2\) Infusate was either 30 mL 10% Intralipid\(^\text{®} \) (Clintec Nutrition, Deerfield, IL) or 30 mL physiological saline. Slow represents the 0.13 mL/min rate of rat or saline infusion. Fast represents the 1.00 mL/min rate of fat or saline infusion. SF (schedule fed) indicates that food was available 7 h/d; 24 h indicates food was available for 24 h.

**Tissue analysis. Small intestine.** Total small intestine weight expressed as g/100 g BW differed between the saline- and lipid-infused groups (Table 2). In the lipid-infused groups, the total weight of the small intestine was about twice that of the saline-infused groups. Mucosa scraped from sections of the small intestine was weighed and expressed in g/100 g BW (Table 3). Mucosal weight from the upper one third of the small intestine was greater in lipid-infused than in saline-infused rats (Table 3). Differences in mucosal weight between saline and lipid infusion were not evident in the middle \( P = 0.09 \) or lower one third of the intestine \( P = 0.26 \). The rate of infusion did not appear to affect mucosal weight expressed in relationship to BW in lipid- or saline-infused rats.

**Pancreas.** Pancreatic dry weight was also available, but only from the saline infusion group that had food available for 7 h and from the lipid infusion group that had food available for 24 h. In saline-infused rats, pancreatic dry weight [g/100 g BW] was 0.12 whereas in rats that received the fat infusion, pancreatic weight was 0.20 in the slow infusion group and 0.16 in the group with the fast infusion rate (pooled SEM = 0.01). Thus, pancreas weight in rats infused with lipid at the slow rate \( 0.13 \text{ mL/min} \) was \(^{~25}\%\) greater than that of pancreata from rats infused with lipid at the faster rate \( 1.0 \text{ mL/min} \), but infusion of lipid at either rate resulted in pancreas weights that were significantly higher than those of the saline-infused groups.

Pancreatic tissue protein and DNA content were measured to determine if the greater pancreas weight of lipid-infused rats was attributed to increased cell number (i.e., hyperplasia) or increased protein synthesis and storage (i.e., hypertrophy). Protein per milligram

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**TABLE 3**

Small intestine mucosal weight of rats receiving an intestinal infusion of fat or saline at two different rates and with food available for 7 or 24 h

<table>
<thead>
<tr>
<th>Infusate and feeding treatment (^2)</th>
<th>Upper mucosa ( g/100 \text{ g BW} )</th>
<th>Middle mucosa ( g/100 \text{ g BW} )</th>
<th>Lower mucosa ( g/100 \text{ g BW} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow fat/SF</td>
<td>0.62(^b)</td>
<td>0.72</td>
<td>0.36</td>
</tr>
<tr>
<td>Fast fat/SF</td>
<td>0.67(^{bc})</td>
<td>0.71</td>
<td>0.36</td>
</tr>
<tr>
<td>Slow fat/24 h</td>
<td>0.93(^d)</td>
<td>0.61</td>
<td>0.45</td>
</tr>
<tr>
<td>Fast fat/24 h</td>
<td>0.81(^{cd})</td>
<td>0.68</td>
<td>0.49</td>
</tr>
<tr>
<td>Slow saline/SF</td>
<td>0.42(^a)</td>
<td>0.44</td>
<td>0.31</td>
</tr>
<tr>
<td>Fast saline/SF</td>
<td>0.44(^a)</td>
<td>0.49</td>
<td>0.30</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td>0.07</td>
<td>0.08</td>
<td>0.07</td>
</tr>
</tbody>
</table>

\(^1\) The small intestine was removed, flushed gently with saline, divided into thirds and scraped with a glass slide to collect and weigh mucosa. Values represent the mean mucosal weight [g/100 g body weight (BW)] of 6 rats receiving the described treatments. Means with different letters indicate significant differences \( P < 0.05 \) between treatments.

\(^2\) Infusate was either 30 mL 10% Intralipid\(^\text{®} \) (Clintec Nutrition, Deerfield, IL) or 30 mL physiological saline. Slow represents the 0.13 mL/min rate of fat or saline infusion. Fast represents the 1.00 mL/min rate of fat or saline infusion. SF (schedule fed) indicates that food was available for 7 h/d; 24 h indicates food was available for 24 h.
lipid at the faster rate. Because volume, pH and osmolarity of the lipid and saline solutions did not differ, the duration of exposure of fat to the small intestine had a significant effect on subsequent food intake.

Suppression of the IDMMC and the resultant “fed” pattern of contractile activity that occurs from the presence of food in the small intestine greatly influences motility of food through the small intestine (Soffer and Adrian 1992). In turn, changes in the rate of food-dependent intestinal motility influence the rate of nutrient absorption, the period of time nutrients are in contact with the small intestine and the area of intestine exposed to nutrients. If the satiety actions of fat are initiated preabsorptively, then it is likely that slowing lipid digestion and absorption will allow fat more intraluminal contact time for stimulating fat-sensitive receptors involved in satiety. Data from the present study support this connection between increased intraluminal time for fat and enhanced satiety. When equivalent amounts of fat were infused at 2 different rates (0.13 mL/min or 1.0 mL/min), the rats allowed access to food for 24 h and receiving lipid at the slower rate consumed less diet per day and did not gain as much body weight as rats infused with lipid at the faster rate. Data from French and Read (1994) suggest that viscous polysaccharides, such as guar gum, affect satiety through a similar mechanism. Guar gum increases intestinal viscosity and slows the rate of lipid absorption. When human subjects were fed a high fat consomme soup supplemented with guar gum, the return of hunger and the decline of fullness were delayed compared with the results when subjects consumed the high fat soup without guar gum. Hunger and fullness scores were not correlated with the rate of gastric emptying, suggesting that the ability of guar gum to prolong nutrient exposure to intraluminal receptors is more critical than gastric distension in the control of food intake.

The control of food intake is a complex, multifaceted network of interactions involving both pre- and postabsorptive mechanisms. The present study focused on the influence of intestinal contact time on food intake and body weight gain. The small intestine has an important role in the control of food intake presumably by the interaction of nutrients at preabsorptive receptor sites. Fat has been shown to be a potent nutrient in inducing satiety, probably by stimulating the release of chemical transmitters that mediate the signal for satiety. Cholecystokinin (CCK) has been implicated as a factor in mediating satiety (Smith 1984).

Cholecystokinin is synthesized and stored in mucosal endocrine cells in the upper one third of the small intestine and is released by the presence of fat and protein in the lumen (Green et al. 1989, Schneeman et al. 1977a and 1977b). Peripheral administration of exogenous CCK suppresses eating behavior, and peripheral injection of specific CCK antagonists increases food consumption in rats (Reidelberger et al. 1989) and humans (Wolkowitz et al. 1990). Cholecystokinin stimulates pancreatic growth hypertrophically (Johnson 1981, Mainz et al. 1973) and lipid has been shown to release CCK in rats (Green et al. 1989). The differences in pancreatic and intestinal weights observed among experimental groups suggest that CCK released from the intestine may be responsible, at least in part, for the feeding behaviors exhibited by rats in the present study. The pancreata of lipid-infused rats were consistently heavier than those of saline-infused rats, suggesting an enhanced release of CCK. In addition, rats receiving lipid at the slower rate (0.13 mL/min) had heavier pancreata than rats infused at the faster rate (1.0 mL/min). When lipid was infused for a longer time, total energy intake was less, body weight gain was less and pancreatic weight was higher. These data suggest that the total time plasma CCK was elevated was longer, thereby prolonging the satiety signal.

The negative feedback signals that inhibit food intake appear to be initiated at the level of the gastrointesti nal tract. Several studies, including the current study, emphasize the importance of nutrients such as fat interacting with small intestinal receptors to terminate feeding. In addition to terminating feeding, fat may also be involved in promoting the behaviors typical of post-prandial satiety (Antin et al. 1975). We observed that within 10 min of the start of the lipid infusion, rats stopped eating and began to display behaviors associated with ending a meal, i.e., grooming, drowsiness, resting. A similar sequence of behavior was documented in sham feeding rats that were intraduodenally infused with lipid (Greenberg et al. 1990). Studies on the infusion of lipid into the duodenum of humans indicated that the subjects felt more relaxed, drowsy and somewhat more friendly following the lipid infusion (Read et al. 1994). Thus, stimulation of fat-sensitive receptors appears to terminate feeding and induce comfortable sensations associated with satiety.

Fat, when present in the upper small intestine, stimulates a signal of satiety. The signal persists, independent of lipid quantity, as long as lipid is present intraluminally. Equivalent amounts of fat have different effects on daily energy intake and body weight gain depending on the rate at which fat is delivered into the small intestine. Perhaps a dietary strategy to maximize the satiating potential of a meal is to slow its transit through the small intestine so that intraluminal exposure to dietary fat is extended. Foods rich in viscous polysaccharides are known to slow meal transit time and delay lipid absorption (Schneeman 1986). Inclusion of foods rich in these polysaccharides may enhance satiety by allowing dietary fat more time in the small intestine to stimulate the fat-sensitive receptors involved in satiety.

LITERATURE CITED


