Effect of fat saturation on satiety, hormone release, and food intake

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ABSTRACT

Background: Ileal delivery of fat reduces hunger and food intake through activation of the ileal brake. Physicochemical properties of fat have been shown to affect satiety and food intake.

Objective: The objective of this study was to assess the effect of ileal fat emulsions with differing degrees of fatty acid saturation on satiety, food intake, and gut peptides (cholecystokinin and peptide YY). We hypothesized that long-chain triacylglycerols with diunsaturated fatty acids would increase satiety and reduce energy intake compared with long-chain triacylglycerols with monounsaturated or saturated fatty acids.

Design: We performed a double-blind, randomized, crossover study in which 15 healthy subjects [mean age: 24 y; mean body mass index (in kg/m²): 22] were intubated with a naso-ileal catheter and participated in 4 experiments performed in random order on 4 consecutive days. After consumption of a liquid meal, subjects received a fat or control infusion in the ileum. Fat emulsions consisted of 6 g of 18:0 (shea oil; mainly 18:0), 18:1 (canola oil; mainly 18:1), or 18:2 (safflower oil; mainly 18:2) oils. Food intake was measured during an ad libitum lunch. Satiety questionnaires (visual analog scale) and blood samples were collected at regular intervals.

Results: Compared with the control, only 18:2 and 18:1 significantly increased fullness and reduced hunger. No effect on food intake was observed. 18:1 and 18:2 increased cholecystokinin secretion significantly compared with the control. Fatty acid saturation did not affect peptide YY secretion.

Conclusions: When infused into the ileum, triacylglycerols with unsaturated fatty acids increase satiety, whereas triacylglycerols with saturated fatty acids do not. This trial was registered with the Dutch Trial Register as ISRCTN51742545.


INTRODUCTION

Fat in the gastrointestinal tract reduces hunger and impairs food intake by eliciting satiety signals (1). These signals are evoked by entry of triacylglycerols (after hydrolyzation to fatty acids) or fatty acids into the small intestine. Duodenal fat induces the release of cholecystokinin (CCK) and other gastrointestinal peptides involved in the regulation of satiety and food intake (1). When infused into the ileum, fat also increases satiety and reduces food intake (2). Studies in both animals (3, 4) and humans (1, 5) suggest that the satiating effect of fat from the ileum is even larger than that of the effect of fat from the duodenum. Infusion of fat into the ileum activates the ileal brake mechanism (6), an inhibitory distal to proximal feedback mechanism that regulates the transit and handling of a meal through the digestive tract to optimize nutrient digestion and absorption (7).

The satiating effect of fat is dependent on its physicochemical properties (8, 9). For instance, reduction of hunger and food intake increases with increasing fatty acid chain length (8). Feltrin et al (8) showed that a reduction in energy intake and in hunger was stronger after isocaloric infusion of lauric acid (12:0) than of decanoic acid (10:0).

Apart from fatty acid chain length, another physicochemical property of fat that may affect hunger and food intake is the degree of fatty acid saturation. The effect of fatty acid saturation on ileal brake–mediated satiety is not known, but data are available on the oral and duodenal administration of fats differing in fatty acid saturation. Lawton et al (10) showed that oral ingestion of triacylglycerol with unsaturated fatty acids induced a greater reduction in food intake than did triacylglycerols with saturated fatty acids. However, Flint et al (11) and Alfenas and Mattes (12) found no such difference in food intake or satiety.

French et al (9) measured food intake and satiety after various intraduodenally infused fat emulsions. Emulsions enriched with linoleic acid (18:2) reduced food intake more than did oleic (18:1) or stearic (18:0) acids without affecting satiety. Entry of fat into the small intestine induces the release of gut peptides [CCK and peptide YY (PYY)], and secretion of these peptides is partly responsible for the effects of fat on satiety and food intake (1).

The aim of the present study was to assess the effect of ileal fat emulsions with differing degrees of saturation of fatty acid chains on 1) satiety and food intake and 2) secretion of peptides known to affect satiety (CCK and PYY). We hypothesized that long-chain triglyceride (LCT) emulsions with diunsaturated fatty

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acids will enhance postprandial satiety and reduce energy intake compared with LCT emulsions with monounsaturated or saturated fatty acids.

**SUBJECTS AND METHODS**

### Subjects

Healthy volunteers aged 18–55 y with a body mass index (in kg/m²) between 18 and 32 were recruited by advertisement. Restrained eaters (as assessed by the Dutch Eating Behavior Questionnaire) and subjects who reported that they were following either a weight-reduction diet or a medically prescribed diet were excluded from participation. Subjects who were taking medication that could influence appetite and sensory function or who reported metabolic or endocrine disease, gastrointestinal disorders, or a history of medical or surgical events that may have affected study outcome were also excluded. Informed consent was obtained from each individual. The study protocol had been approved by the Medical Ethics Committee of the Leiden University Medical Center, and the procedures followed were in accordance with the ethical standards of the Institution on Human Experimentation. This study was registered as ISRCTN51742545, and subject recruitment started in September 2005.

Eighteen subjects met the inclusion criteria. Three volunteers dropped out during the study: 1 because of discomfort during catheter positioning and 2 because of failure to position the tip of the catheter beyond the ligament of Treitz (flexura duodenojejunalis). Fifteen (13 women) healthy volunteers (mean age of 24 y and mean BMI of 22) participated in the study and completed the protocol.

### Catheters

The catheter for ileal intubation was a 290-cm long, rubber silicon, 9-channel (8-lumen, 1 balloon inflation channel, 3.5 mm in diameter) catheter custom-made by Dentsleeve International Ltd (Mississauga, Ontario, Canada). The functional length of the catheter was 240 cm; there was a 50-cm connection segment. The catheters contained side holes at 80, 95, 110, 125, 210, 220, 230, and 240 cm from the proximal junction and had an inflatable balloon (maximum inflation capacity: 10 mL) at the distal tip. The 3 distal side holes (220, 230, and 240 cm) reaching into the ileum were used to administer placebo or fat emulsion.

### Experimental protocol

In this double-blind, placebo-controlled, crossover design, 4 different perfusions were administered into the ileum: 1) saline (control) or a 6-g fat emulsion consisting of 2) mainly unsaturated fats (18:0), 3) mainly monounsaturated fat (18:1) or 4) mainly diunsaturated fat (18:2) (Table 1).

On Monday, subjects arrived at 1200 after eating a light breakfast (ingested before 0900). Through an anesthetized nostril, the catheter was introduced into the stomach and allowed to pass through the pylorus to the ileum by peristalsis. After passing the ligament of Treitz, a small balloon at the tip was inflated to facilitate passage of the catheter to the ileum. During the day, the subjects were offered small snacks and sugared tea or coffee to stimulate peristalsis. The tip was placed in the ileum (&gt;120 cm distal from the pylorus; 13), so 3 infusion ports were available in the ileum. In our study, we perfused the ileum a distance of &gt;175–195 cm from the nose. During the positioning and before each test day, the position of the catheter was checked fluoroscopically.

### Test day

On each study day, the subjects arrived at the Gastrointestinal Unit at 0800 after fasting overnight. After the position of the catheter was checked, a venous catheter was placed in a forearm vein for the collection of blood samples. At 0845, a basal visual analog scale (VAS) score was determined, and a basal blood sample was collected. The experiment then began. At 0900 h (0 min), a fat-free liquid meal was ingested.

The ileal infusion started at 105 min. The infusion rate was 1 mL/min (0.9 kcal/min) for 60 min. Each day the ileum was perfused with a fat emulsion or the fat-free emulsion. The 3 most distal catheter ports were used for this perfusion, so the emulsion was spread out over a length of 30 cm.

At 1300 (240 min), the ad libitum lunch was served. At 1445, the intravenous canula was removed, and the subjects were allowed to go home. They received an evening meal and snackbox from which they were allowed to consume freely until 2200.

### Satiety

Scores for satiety feelings (fullness, hunger, prospective feeding, desire to eat a meal, and desire to eat a snack) were measured with an electronic VAS (EVAS) anchored at the low end with the most negative or lowest intensity feelings (eg, extremely unpleasant, not at all), and with opposing terms at the high end (eg, extremely pleasant, very high, extreme) (14). Volunteers were asked to indicate on a line which place on the scale best reflected their feelings at that moment. Measurements were taken every 30 min during the test day and every 15 min during infusion of the emulsion.

### Food intake

Each test day, an ad libitum lunch was served to measure food intake. Each lunch was offered in excess and consisted of 15 equal slices of brown bread with mature cheese (48% fat). Each sandwich was cut in different-sized pieces, so the subject would not be able to assess the number of sandwiches eaten. During the ad libitum lunch, the subjects were not allowed to watch television, listen to the radio, or read books because this could have influenced the amount eaten.

### Liquid meal

As breakfast, subjects received a fat-free Slim-Fast Optima drink (Slim-Fast Optima, Slim-Fast Foods, West Palm Beach, FL;
325 mL, 145 kcal). These are vitamin- and mineral-fortified meal replacement products used by consumers primarily to aid in weight loss and/or to prevent weight gain.

**Emulsions**

The emulsions consisted of 10% oil in water. As an emulsifier, 2.5% K-caseinate was used. Very small amounts of xanthan gum (0.1%) and guar gum (0.1%) were used as a stabilizer. Sodium chloride was added to obtain iso-osmotic solutions (0.8% NaCl). See Table 1 for the fatty acid composition of the emulsions. The total infused volume was 60 mL, which contained 6 g fat. The caloric load was 54 kcal. The pH of the emulsions ranged from 6.7 to 6.8.

**Hormone assays**

Blood samples were drawn at regular intervals throughout the test day. After collection, the blood was kept on ice. PYY (total) and CCK were measured by sensitive and specific radioimmunoassays, as described previously (15, 16).

**Statistical analysis**

The results are presented as least-squares means ± SEM unless otherwise specified. Satiety VAS scores were expressed as percentages of the maximal score (0 cm = 0% and 6.4 cm = 100%) and as incremental cumulative areas under the curve (AUCs). The incremental AUC was calculated, using the trapezoid rule, with the starting point of the test day (0 min) or of the infusion period (105 min) as a covariate.

All variables were analyzed by using analysis of variance with subjects as blocks and treatment as factor. Differences between the treatment groups and control group were established by using Dunnett’s least-significant difference test (2-tailed). A P value of 0.05 was considered significant. When a significant time × treatment interaction was present, differences at individual time points are reported.

**RESULTS**

**Satiety**

Because the results of all satiety parameters were similar and consistent, we only showed scores for fullness and hunger. Mean VAS scores for hunger are given in Figure 1 and for fullness in Figure 2. Fasting scores for hunger and fullness did not differ between the 4 treatments. Consumption of the liquid meal led to a decrease in hunger feelings and an increase in fullness in all 4 treatments.

The AUCs for hunger for the 105–240-min period for both canola and safflower differed significantly from the control (Figure 1). Over the test day (0–240 min), both canola and safflower resulted in lower AUCs for hunger than did the control. The AUCs for fullness for the 105–240-min period for both canola and safflower differed significantly from the control (Figure 2).

**Food intake**

Food intake did not differ significantly between treatment groups (control: 180 g; shea oil: 173 g; canola oil: 167 g; and safflower oil: 189 g; SE = 10).

**Peptide YY**

Plasma PYY concentrations are shown in Figure 4. Baseline plasma PYY concentrations did not differ between study days. The liquid meal induced a slight increase in PYY concentrations
in all 4 treatments. The start of the infusion induced an increase in plasma PYY concentrations after all fat emulsions compared with the control. For the 105–240-min and 0–240-min periods, the AUCs (Figure 4) for all fat emulsions increased significantly compared with the control.

DISCUSSION

We showed that the degree of fatty acid saturation of triacylglycerols infused into the ileum affects satiety levels and the release of CCK, but not food intake or the release of PYY. Intraduodenal infusion of fat reduces hunger and increases satiety (17), and physicochemical properties of fat (eg, chain length) affect its satiating potency (8, 9). Evidence exists that the degree of fatty acid saturation affects the satiating effect of fat (10), but this has not been shown consistently (9, 11, 12). Whereas it has been shown that ileal infusion of fat reduced hunger and food intake (2), the effect of differences in fatty acid saturation on ileal fat–induced satiety has never been tested. In the present study, triacylglycerols with unsaturated fatty acids reduced hunger and increased fullness significantly compared with the saline control infusion, whereas shea oil, which consists of saturated fatty acids, did not. This clearly indicates that ileal brake–mediated satiety is most potently stimulated by oils containing high amounts of unsaturated fatty acids.

The anorectic effect of intestinal fat when infused in the duodenum and in the ileum has been shown repeatedly and consistently (2, 9, 18). French et al (9) observed an effect of fatty acid saturation on food intake after duodenal fat infusion. We observed that the different fat emulsions affected satiety, but did not affect food intake. In the studies by French et al (9) and Welch et al (2), the ad libitum meal was provided already during the infusion. In our study, the interval between the end of the infusion and the start of the ad libitum lunch was 75 min. Rolls et al (19) showed that the time interval between a caloric load and an ad libitum meal affects meal intake. We hypothesize that the longer interval between infusion and lunch (75 min) may have affected the results and may explain the lack of effect of the treatments (fat infusion) on food intake, and a significant effect on food intake may have been present if we had measured food intake at an earlier point in the
significant. Data are means ± SEMs; n = 15.

Satiety regulates meal frequency and food intake during a next meal, in which learned habits also play a role (1). In the studies by French et al (9) and Welch et al (2, 18), food intake was measured during the infusion; therefore, the variable studied was satiation. During our study, we studied the effects between meals and focused on satiety.

Entry of fat into the small intestine induces the release of CCK (1). Beardshall et al (20) found that ingestion of a meal high in monounsaturated fat resulted in significantly higher plasma CCK concentrations than did a saturated fat meal. In our study, both canola oil and safflower oil significantly increased plasma CCK concentrations compared with the saline infusion, whereas shea oil had no significant effect. Release of CCK after ileal fat infusion was not anticipated. However, the release of CCK in response to the ileal fat infusions has been reported previously (21). In general, the secretion of CCK is thought to be confined to the duodenum and jejunum. Sjölund et al (22) and others (23) have shown the presence of CCK-secreting I cells in the (terminal) ileum. However, the number of CCK cells was smaller in the ileum than in the duodenum or jejunum (22). In a previous study (5), we compared the secretion of CCK after isocaloric infusion of fat into the duodenum and ileum. We found that both infusions increased the secretion of CCK, although CCK secretion was more potently stimulated after duodenal compared with ileal fat infusion.

We excluded the possibility that incorrect positioning of the catheter affected CCK release. Both during the positioning of the catheter and on each test day at the start and end of the procedure, the position of the catheter was checked fluoroscopically to ascertain placement of the tip of the catheter in the ileum. A full 22-h period was used for positioning of the catheter. Subjects were routinely instructed to assist passage and aboral progression of the catheter at home. Furthermore, in 3 cases, contrast fluid was used to ascertain the position of the catheter. After infusion of the contrast fluid, the contrast appeared in the colon within a few minutes.

Triacylglycerols are hydrolyzed in the proximal gastrointestinal tract to fatty acids and monoaoylglycerols (24). Hydrolysis is necessary to induce the effects of fat on gastrointestinal function, hormone release, and satiety (25, 26). Fatty acids are incorporated in micelles and transported to the surface of epithelial cells (24), where sensing and subsequent absorption of fatty acids occur (27). As the ease of micelle formation increases with the degree of fat unsaturation (28), unsaturated fatty acids are more readily available for sensing and absorption, which leads to increased satiety and hormone release (27). Jones et al (29) showed that significantly more stearic acid (saturated) than oleic acid (unsaturated) was found in stool after oral ingestion of labeled fatty acids. This was probably due to an increased ability of saturated fatty acids to interact with calcium, which resulted in the formation of insoluble calcium-fatty acid soaps and increased fecal excretion (30). Both mechanisms (micelle formation, calcium binding) may be involved in the differences in satiety and CCK release observed in our study. However, recent studies showed that the metabolism of saturated and unsaturated fats occurs roughly at the same rate, and differences in plasma peaks are caused more by the sn-structure than by the degree of saturation or unsaturation.

In the present study, all fat emulsions induced a significant increase in PYY secretion compared with the saline infusion, with no differences between the fat emulsions. With respect to the gastrointestinal peptides, both CCK and PYY are considered satiety signals. Infusion of CCK reduces food intake during a meal (17). The reduction in hunger observed after fat infusion can be abolished by a CCK receptor antagonist (17), which points to a role for CCK in the physiologic regulation of eating behavior. Batterham et al (31, 32) showed that infusion of PYY reduced food intake in both animals and humans. Whether PYY is involved in the physiologic regulation of food intake is still debatable (33). In animals, this debate has been resolved. Whereas many studies failed to confirm the results of Batterham et al (33), adequate habituation and acclimatization of animals proved to be essential in these studies, and PYY is considered to be a physiologic satiety signal (34).

We previously performed a study in human subjects to examine whether the satiating effect of ileal brake activation was mediated...
by PYY. At plasma PYY concentrations that were similar after intravenous PYY infusion and after ileal fat infusion, the effects on satiety after the intestinal fat infusion were much more pronounced (35). We concluded that the satiating effect of the ileal brake is not solely mediated by PYY. From the results of the current study, and from previous studies (36), we hypothesize that a role for CCK in ileal brake–mediated satiety should be considered. However, other peptides may also be involved. Release of glucagon-like peptide-1, a gut peptide released from L cells that predominate in the distal small intestine and colon has been shown to increase after a monounsaturated fat–rich meal compared with a saturated fat–rich meal (37), and a reduction in both hunger and food intake was shown after glucagon-like peptide-1 infusion (1). In animals, Kalogeris et al (38) showed that, compared with saturated fatty acids, unsaturated fatty acids significantly increased the lymphatic outflow of apolipoprotein A-IV—a chylomycin particle that has been shown to reduce food intake in rats (39). In conclusion, when infused into the ileum, triacylglycerol with unsaturated fatty acids increases satiety compared with saline, whereas triacylglycerol with saturated fatty acids does not.

The authors' responsibilities were as follows—JM: participated in the design of the experiment, the data collection, data analysis, and writing of the manuscript; EAR: assisted with the data collection and analysis; EH: participated in the design of the experiment, data collection, and data analysis; HPFP: participated in the design of the experiment and assisted with the data analysis and the drafting of the manuscript; and AAMM: participated in the design of the experiment, guidance during all aspect of the experimental protocol, data analysis, and writing of the manuscript. All of the authors participated in a critical review and in the final approval of the manuscript. None of the authors had a personal or financial conflict of interest.

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