Effects of load, and duration, of duodenal lipid on antropyloroduodenal motility, plasma CCK and PYY, and energy intake in healthy men

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Ingestion of fat triggers a number of gastrointestinal responses (6–8, 12, 25) as a result of the interaction of lipolytic products (principally fatty acids) with small intestinal receptors (9, 10, 23, 24). Studies in animals indicate that fat-induced small intestinal feedback on both gastric emptying, intestinal transit, and appetite is dependent on both the length (17, 30, 31), and region (19, 30, 31), of small intestine exposed to fat. The effects of exposing different lengths, or regions, of the small intestine to fat on gastric emptying, antropyloroduodenal (APD) motility, gut hormone release, or appetite have not been evaluated directly in humans (14, 35, 38); however, the effects of different loads (g or kcal/min; see Refs. 2 and 8) and durations (5) of small intestinal lipid infusions on some of these parameters have been explored, indirectly assessing the effects of length, or region, of exposure. It seems reasonable to assume that, with greater lipid loads, a greater length of small intestine would be exposed to lipid. For example, in both dogs (18) and rats (29), ingestion of meals with an increasing fat content results in an increasing load of triglyceride emptying from the stomach into the duodenum, associated with an increasing spread of both lipolysis and absorption of fat to, and along, the ileum. This caudal spread of digestion and absorption results from exceeding the digestive capacity of lipase, as well as the absorptive capacities for lipolytic products per centimeter of gut (29, 31).

Much less is known about how the duration of small intestinal fat exposure might affect gastrointestinal responses. Theoretically, this could have two important effects, i.e., 1) depending on the rate of intestinal transit, the duration of infusion could affect the distribution of lipolytic products along the gut, and 2) at any given rate of infusion, the duration could affect the total amount of fat digested and absorbed. Duodenal infusion of a high lipid load (which exceeds proximal gut lipolytic and absorptive capacities) for a prolonged period would ultimately result in the release of lipolytic products in both the proximal, and distal, small intestine, i.e., a long length of contact, whereas a shorter infusion at the same rate would limit spread of contact initially to the proximal small intestine, and then, after cessation of the infusion (as the bolus of fat and lipase moves distally), contact in the distal, but no longer the proximal, small intestine. By contrast, the duration of infusion would not be expected to affect the distribution along the gut of a low lipid load, because, irrespective of the time period over which the fat was infused, it would be digested and absorbed efficiently within the proximal small intestine. The effects of the duration of exposure of the small intestine to lipid on the simulation of CCK and peptide YY (PYY) have also not been assessed in humans. It would, however, be anticipated that the patterns of release of these hormones would differ in response to changes in the load, and duration, of lipid infusions, because these affect the distributions of lipolytic products along the gut, and CCK-secreting cells are located in the proximal small intestine (3), whereas those that release PYY are located more distally (1).
responses in healthy subjects. We, accordingly, quantified the effects of “low” (1.33 kcal/min, total: 67 kcal) and “high” (4 kcal/min, total: 200 kcal) lipid loads, infused intraduodenally for 50 min. The responses to these 50-min infusions were compared with those observed in response to infusions of lipid (1.33 kcal/min, total: 200 kcal) and saline (control), given over 150 min. Fat empties initially from the human stomach at rates that vary with the fat content of the meal and the physical state of fat within the stomach, so that 1.33 kcal/min is a moderate rate typical of a meal containing 15–20 g of oil, whereas 4 kcal/min is a rapid rate typical of a meal containing 60 g of fat emulsified in a relatively large volume of water, as in a cream soup or milkshake (26, 28). Because transit through the jejunum is known to take 60 min or less (39), we postulated that infusion of the 4 kcal/min load for 50 min would result in the release of lipolytic products in the jejunum between 0 and 50 min, followed by further release of lipolytic products in the ileum between 50 and 150 min, whereas, by contrast, with the 1.33 kcal/min loads, whether given for 50 or 150 min, lipolytic products would be confined to the jejunum. If so, the plasma time courses of CCK and PYY release would be anticipated to differ fundamentally after the low- and high-fat loads.

SUBJECTS AND METHODS

Subjects

Eleven healthy male subjects (age: 25 ± 2 yr; body mass index: 23.4 ± 0.7 kg/m²) participated in the study. All subjects were unrestrained eaters (scoring <12 on the Eating Restriction questionnaire (36)), had no history of gastrointestinal disease, and were not taking medication known to affect gastrointestinal motility or appetite. No subject smoked or habitually consumed >20 g alcohol/day. The study protocol, which conformed to the standards set by the Declaration of Helsinki, was approved by the Royal Adelaide Hospital Research Ethics Committee, and all subjects provided written, informed, consent before inclusion.

Protocol

Each subject was studied on four occasions, separated by 3–10 days, on which they received, in randomized, double-blind fashion, an intraduodenal infusion of lipid emulsion at 1) 1.33 kcal/min for 50 min (“1.33/50”), 2) 4 kcal/min for 50 min (“4/50”), 3) 1.33 kcal/min for 150 min (“1.33/150”), or 4) intraduodenal isotonic saline (“control”) for 150 min. APD pressures, plasma CCK and PYY concentrations, appetite, and energy intake were evaluated.

Subjects attended the Department of Medicine at 0830 h after an overnight fast (14 h for solids, 12 h for liquids) and were intubated with a 16-channel manometric catheter (Dentsleeve, Adelaide, Australia). The catheter was inserted through an anesthetized nostril and allowed to pass through the stomach and into the duodenum by peristalsis (11). Six side holes (channels 1–6) were positioned in the antrum, a 4.5-cm sleeve sensor (channel 7) with two side holes (channels 8 and 9) on the back of the sleeve was positioned across the pylorus, and seven side holes (channels 10–16) were positioned in the duodenum. All side holes were spaced at 1.5-cm intervals. An additional channel, used for intraduodenal infusions, was located 11.75 cm distal to the end of the sleeve sensor (i.e., ~14.5 cm from the pylorus). The correct positioning of the catheter, so that the sleeve sensor straddled the pylorus, was maintained by continuous measurement of the transmucosal potential difference (TMPD) between the most distal antral (channel 6; approximately ~40 mV), and the most proximal duodenal (channel 10; ~0 mV), channel (11). For this purpose, an intravenous cannula filled with sterile saline was placed subcutaneously in the left forearm and was used as a reference electrode (11). All manometric channels were perfused with degassed, distilled water, except for the two TMPD channels, which were perfused with degassed 0.9% saline (11). An intravenous cannula was also placed in a right forearm vein for blood sampling.

Once the manometric catheter was positioned correctly, fasting motility was monitored until the occurrence of phase III of the interdigestive migrating motor complex (MMC; see Ref. 10). Immediately after cessation of phase III [at time (t) = –10 min], a baseline blood sample was taken, and a visual analog scale (VAS) questionnaire, assessing appetite-related sensations and gastrointestinal symptoms (32), was completed. At time (t) = 0 min, during phase I of the MMC, the duodenal infusion of lipid or saline was commenced. APD pressures were monitored throughout the infusion period; VASs were completed every 15 min from time (t) = 0–150 min, and blood samples were taken every 15 min from t = 0–30 min and every 30 min from t = 30–150 min. At time (t) = 150 min, the infusion was terminated, and the subject was extubated immediately and then offered a cold buffet-style meal (10). Each subject was given 30 min (i.e., t = 150–180 min) to consume the meal and instructed to eat until comfortably full. After the meal, the intravenous cannula was removed, and the subject was allowed to leave the laboratory.

Intraduodenal Infusions

The intraduodenal lipid emulsion (10% Intralipid, 300 mOsmol/kg, 1.1 kcal/ml; Baxter Healthcare, Old Toongabbie, NSW, Australia) was administered as: 1) 1.33 kcal/min for 50 min, 2) 4 kcal/min for 50 min, and 3) 1.33 kcal/min for 150 min. Intralipid, a lipid emulsion consisting predominantly of long-chain triglycerides extracted from soybeans, was selected since it has been used in the majority of studies evaluating the effects of lipid on gastrointestinal function and appetite (2, 5, 6, 8, 12). On the remaining study day, isotonic saline was given for 150 min. Conditions 1 and 2 were designed to assess the effects of lipid load, resulting in total energy deliveries of 67 and 200 kcal, respectively, over 50 min. Conditions 2 and 3 allowed evaluation of the effects of identical intraduodenal lipid loads (200 kcal), given over different time periods (50 vs. 150 min). In conditions 1 and 2, the intraduodenal lipid infusion was followed by an intraduodenal saline infusion between 50 and 150 min so that the infusion duration was identical in all four study conditions. Lipid solutions were diluted with isotonic saline to achieve the specific loads, and all infusions were administered at a rate of 4 ml/min, so that the total volume infused in all study conditions was 600 ml. Both the primary investigator (Plichtiewicz) and the subjects were blinded to the treatments on each study day; the infusions were prepared by one of the other investigators who was not involved in the data analysis; furthermore, the infusion apparatus was covered at all times during the study.

Measurements

APD pressures. Manometric pressures were digitized and recorded on a computer-based system running commercially available software [Flexisoft, version 3, G. S. Hebbard, Royal Melbourne Hospital, Melbourne, Australia, written in Labview 3.1.1 (National Instruments)], and stored for subsequent analysis. APD pressures were analyzed for the 1) number and amplitude of antral pressure waves (PWs), 2) basal pyloric pressure and number and amplitude of isolated pyloric pressure waves (PPWs), 3) number and amplitude of duodenal PWs, and 4) number of pressure wave sequences (PWSSs) in the APD region. Phasic PWs in the antrum and IPPWs were defined by an amplitude of ≥10 mmHg, with a minimum interval between peaks of 15 s (34). Basal pyloric pressure (“tone”) was calculated for each minute by subtracting the mean basal pressure (excluding phasic pressures) recorded at the most distal antral side hole from the mean basal pressure recorded at the sleeve (13), using custom-written software (MAD, C. H. Malbert, Institut National de la Recherché Agronomique, Rennes, France). Phasic duodenal PWs were defined.

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by an amplitude of $\geq 10$ mmHg, with a minimum interval of 3 s between peaks (34). PWs were defined as two or more temporally related PWs with onsets within $\pm 5$ s (in the antrum), or $\pm 3$ s (in the duodenum), of each other (34). PWs were characterized according to the distance traveled, i.e., over 2 (1.5 to $<3$ cm), 3 (3 to $<4.5$ cm), 4 (4.5 to $<6$ cm), . . . , 15 (21 to $<22.5$ cm) channels, and expressed as the total number of waves, using custom-written software (Gastrointestinal Motility Unit, University Hospital Utrecht, Utrecht, Netherlands), modified to our requirements.

**Plasma hormone concentrations.** Venous blood samples (10 ml) were collected in ice-chilled EDTA tubes containing 400 kallikrein inhibitor units aprotinin (Trasylo; Bayer Australia, Pymble, Australia) per milliliter blood. Plasma was separated by centrifugation (3,200 revolutions/min, 15 min, 4°C) within 30 min of collection and stored at $-70^\circ$C until assayed.

Plasma CCK concentrations (pmol/l) were determined after ethanol extraction using a previously described radioimmunoassay (RIA; see Ref. 22). A commercially available antibody (C258, Lot 105H4852; Sigma Chemical, St. Louis, MO) raised in rabbits against the synthetic sulfated CCK-8 was employed. This antibody binds to all CCK peptides containing the sulfated tyrosine residue in position 7, shows a 26% cross-reactivity with unsulfated CCK-8, <2% cross-reactivity with human gastrin (0.2% with gastrin I and 1% with Big gastrin), and does not bind to structurally unrelated peptides. The intra-assay coefficient of variation (CV) was 9% and the inter-assay CV was 27%, with a sensitivity of 2.5 pmol/l.

Plasma PYY concentrations (pmol/l) were measured by RIA using an antiserum (kindly donated by Dr. B. Otto, Medizinische Klinik, Klinikum Innenstadt, University of Munich, Munich, Germany) raised in rabbits against human PYY(1–36) (Sigma-Aldrich, St. Louis, MO). This antiserum showed $<0.001$% cross-reactivity with human pancreatic polypeptide and sulfated CCK-8 and 0.002% cross-reactivity with human neuropeptide Y. Tracer (purchased from Prosearch International, Malvern, VIC, Australia) was prepared by radiolabeling synthetic human PYY(1–36) (Auspep; Parkville, VIC, Australia) using the lactoperoxidase method. Monoioido-tyrosine-PYY was separated from free iodine-125, diiodo-PYY, and unlabeled PYY by reverse-phase HPLC (Phenomenon Jupiter C4 300A 5u column cat. no. 005R-4167E-O 250 $\times$ 4.6 mm). An elution gradient from 27 to 40% acetonitrile in triethylamine phosphoric acid (pH 3.0) yielded four peaks of labeled PYY of which peak 3 demonstrated the highest specific binding and was used in the assays. Standards (1.6–50 fmol/tube) or samples (200 μl plasma) were incubated in assay buffer [0.05 M phosphate containing 0.5% BSA and 0.02% azide (pH 7.4)] with 100 μl antiserum at a final dilution of 1:10,000 for 20–24 h at 4°C, 100 μl iodinated PYY (10,000 cpm) was then added, and the incubation continued for another 20–24 h. Separation of the antibody-bound tracer from free tracer was achieved by the addition of 200 μl dextran-coated charcoal containing gelatin (0.015 g gelatin, 0.09 g dextran, 0.15 g charcoal/30 ml assay buffer), was incubated at 4°C for 20 min, and then was centrifuged at 4°C for 25 min. Radioactivity of the bound fraction was determined by counting the supernatants in a gamma counter. The intra-assay CV was 16.6%, and the minimum detectable concentration was 4 pmol/l.

**Appetite perceptions and energy intake.** Appetite perceptions [desire to eat, hunger, fullness, prospective consumption ("How much would you eat if given a meal now?")] were rated on validated VAS (32). Nausea and bloating were also assessed. Energy intake was quantified from a cold buffet-style meal, the composition of which has been described previously (10). The amount of food offered was in excess of what the subject was expected to consume. Energy (kcal) and amount (g) consumed and macronutrient distribution (%energy from fat, carbohydrate, and protein) were analyzed using commercially available software (Foodworks version 3.01, Xyris Software Australia, Highgate Hill, QLD, Australia; see Ref. 10).

**Data and statistical analyses.** Baseline (“0”) values were calculated as the means of values obtained between $t = -10$–0 min for the number and amplitude of IPPWs and antral and duodenal PWs, basal pyloric pressure, and PWSs and at $t = -10$ and 0 min for plasma hormone concentrations and VAS scores. Antral and duodenal PWs and PWSs were analyzed in three periods, i.e., from $t = 0$ to 50 min (the duration of the intraduodenal lipid infusion in conditions 1 and 2), $t = 50$–100 min, and $t = 100$–150 min (to evaluate the effect of the more prolonged infusion in condition 3). Antral and duodenal motility indexes (MI) were derived using the following equation: natural logarithm(sum of amplitudes $\times$ no. of phasic PWs) + 1] (4). Basal pyloric pressures and the number and amplitude of IPPWs were expressed as the mean of 10 min periods over the 150-min infusion period. PWSs were expressed as the total number of waves traveling over 2 (1.5 to $<3$ cm), 3 (3 to $<4.5$ cm), 4 (4.5 to $<6$ cm), . . . , 15 (21 to $<22.5$ cm) channels. Mean values for plasma CCK and PYY concentrations and VAS scores were calculated at each time point from $t = 0$ to 150 min. All motility and VAS data were expressed as changes from baseline, whereas plasma CCK and PYY were expressed as absolute values.

**Basal pyloric pressure, the number and amplitude of IPPWs, plasma concentrations of CCK and PYY, and VAS scores** were analyzed by repeated-measures ANOVA, with time and treatment as factors. The total number of PWSs was analyzed by repeated-measures ANOVA, between $t = 0$ and 50 min, $t = 50$ and 100 min, and $t = 100$ and 150 min, with number and length (cm) as factors. The number, amplitude, and MI of antral and duodenal PWs, between $t = 0$ and 50 min, $t = 50$ and 100 min, and $t = 100$ and 150 min, and energy intake were analyzed by one-way ANOVA. Statistical significance was accepted at $P < 0.05$, and data are presented as means $\pm$ SE.

**RESULTS**

All subjects tolerated the studies well. Baseline values for motility and VAS data are given in Table 1. There were no differences between the four experimental conditions.

**Table 1. Motility indexes of antral and duodenal pressure waves, basal pyloric pressure, number of IPPWs, plasma CCK and PYY concentrations, and nausea at baseline before intraduodenal infusions of 10% Intralipid or saline commenced**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Control (Saline)</th>
<th>Intralipid</th>
<th>Intralipid</th>
<th>Intralipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.33 kcal/50 min</td>
<td>3.7 $\pm$ 0.7</td>
<td>2.3 $\pm$ 0.6</td>
<td>3.4 $\pm$ 0.8</td>
<td>4.3 $\pm$ 0.8</td>
</tr>
<tr>
<td>1.33 kcal/150 min</td>
<td>7.0 $\pm$ 0.9</td>
<td>5.8 $\pm$ 0.6</td>
<td>6.2 $\pm$ 0.7</td>
<td>6.3 $\pm$ 0.5</td>
</tr>
<tr>
<td>4 kcal/50 min</td>
<td>0.4 $\pm$ 0.5</td>
<td>0.0 $\pm$ 0.7</td>
<td>2.6 $\pm$ 3.0</td>
<td>0.0 $\pm$ 0.5</td>
</tr>
<tr>
<td><strong>Pyloric Pressure</strong></td>
<td><strong>Basal, mmHg</strong></td>
<td><strong>IPPWs, no.</strong></td>
<td><strong>Plasma Hormones</strong></td>
<td><strong>Nausea, mm</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.3 $\pm$ 0.6</td>
<td>2.0 $\pm$ 0.8</td>
<td>4.1 $\pm$ 1.8</td>
<td>1.1 $\pm$ 0.6</td>
<td>8.1 $\pm$ 2.6</td>
</tr>
<tr>
<td>7.5 $\pm$ 1.3</td>
<td>8.7 $\pm$ 1.4</td>
<td>9.8 $\pm$ 1.3</td>
<td>8.0 $\pm$ 1.0</td>
<td></td>
</tr>
<tr>
<td>8.1 $\pm$ 2.7</td>
<td>6.0 $\pm$ 2.5</td>
<td>6.6 $\pm$ 2.3</td>
<td>6.7 $\pm$ 2.6</td>
<td></td>
</tr>
</tbody>
</table>

Data are means $\pm$ SE; $n = 11$. PWs, pressure waves; IPPWs, isolated pyloric pressure waves; CCK, cholecystokinin; PYY, peptide YY. Intraduodenal infusions consisted of either 10% Intralipid at 1.33 kcal/min for 50 min followed by saline for 100 min, 1.33 kcal for 150 min, 4 kcal for 50 min followed by saline for 100 min or saline (control) for 150 min.
**APD Pressures**

*Antral pressures.* **PERIOD 1: 0–50 MIN.** There was no effect of treatment on the number, or amplitude, of antral PWs (data not shown). There was, however, an effect of treatment on the MI of antral PWs ($P < 0.05$; Fig. 1A); 4/50 ($P < 0.01$) decreased and 1.33/50 ($P = 0.07$) tended to decrease the MI when compared with saline, with no difference between saline and 1.33/150 or between 1.33/50, 1.33/150, and 4/50.

**PERIOD 2: 50–100 MIN.** There was no effect of treatment on the number of antral PWs (data not shown). There was, however, a significant effect of treatment on the amplitude of antral PWs ($P < 0.05$; saline: $14.7 \pm 7.6$ mmHg, 1.33/50: $16.0 \pm 5.9$ mmHg, 1.33/150: $0.2 \pm 5.2$ mmHg, 4/50: $-5.9 \pm 4.9$ mmHg (the negative value reflects the correction for baseline)); 4/50 decreased the amplitude when compared with saline and 1.33/50 ($P < 0.05$ for both). There was a trend for 1.33/150 to decrease the amplitude when compared with saline ($P = 0.09$) and 1.33/50 ($P = 0.07$). There was no difference between 1.33/150 and 4/50. There was also an effect of treatment on the MI of antral PWs ($P < 0.05$; Fig. 1B); 1.33/150 and 4/50 decreased the MI when compared with saline and 1.33/50 ($P < 0.05$ for all), with no difference between saline and 1.33/50, or between 1.33/150 and 4/50.

**PERIOD 3: 100–150 MIN.** There was an effect of treatment on the number of antral PWs ($P < 0.01$; saline: $61.6 \pm 12.9$, 1.33/50: $31.5 \pm 10.2$, 1.33/150: $2.1 \pm 0.5$, 4/50: $28.7 \pm 12.7$); 1.33/50 ($P < 0.05$), 1.33/150 ($P < 0.001$), and 4/50 ($P < 0.05$) decreased the number of antral PWs when compared with saline; 1.33/150 decreased the number of antral PWs when compared with 1.33/50 and 4/50 ($P < 0.05$ for both), with no difference between 1.33/50 and 4/50. There was an effect of treatment on the amplitude of antral PWs ($P < 0.05$; saline: $21.1 \pm 5.4$ mmHg, 1.33/50: $29.9 \pm 9.3$ mmHg, 1.33/150: $4.0 \pm 5.5$ mmHg, 4/50: $-4.9 \pm 7.8$ mmHg (the negative value reflects the correction for baseline)); 4/50 decreased the amplitude when compared with saline ($P < 0.05$) and 1.33/50 ($P < 0.01$); 1.33/150 decreased the amplitude when compared with 1.33/50 ($P < 0.05$), whereas there was no difference between 1.33/150 and 4/50. There was an effect of treatment on the MI for antral PWs ($P < 0.05$; Fig. 1C); 1.33/150 ($P < 0.01$) and 4/50 ($P < 0.05$) decreased the MI when compared with saline; 1.33/150 decreased the MI when compared with 1.33/50 ($P < 0.05$), with no differences between 1.33/50 and saline or 4/50, or between 1.33/150 and 4/50.

*Pyloric pressures. Basal pressure (tone).* There was a treatment by time interaction for basal pyloric pressure ($P < 0.01$; Fig. 2A); 1.33/50, 1.33/150, and 4/50 increased basal pyloric pressure when compared with saline: 1.33/50 between 0 and 20 min, 1.33/150 between 0 and 20 min and 120 and 140 min, and 4/50 between 20 and 40 min ($P < 0.05$ for all); 4/50 increased basal pressure when compared with 1.33/50 between 20 and 60 min and 1.33/150 between 20 and 40 min ($P < 0.01$ for both); 1.33/150 increased basal pressure when compared with 1.33/50 between 60 and 70 min and 120 and 150 min ($P < 0.05$), whereas there was no difference between 1.33/150 and 4/50 between 40 and 150 min.

*Phasic pressures.* There was a treatment by time interaction for the number of IPPWs ($P < 0.001$; Fig. 2B); 1.33/50, 1.33/150, and 4/50 increased the number of IPPWs when compared with saline: 1.33/50 between 0 and 60 min and 1.33/150 and 4/50 between 0 and 70 min ($P < 0.05$ for all). There was a trend for 1.33/150 to increase IPPWs between 90 and 120 min ($P = 0.08$) when compared with saline; 4/50 increased the number of IPPWs more than 1.33/50 between 30 and 70 min and 1.33/150 between 30 and 50 min ($P < 0.05$ for both). There was no difference between 1.33/50 and 1.33/150 between 0 and 60 min; however, between 70 and 110 min the number of IPPWs was greater for 1.33/150 when compared with 1.33/50 ($P < 0.05$). There was no difference between 1.33/150 and 4/50 after 70 min, although the mean values for 1.33/150 were higher. There was no effect of treatment on the amplitude of IPPWs (data not shown).

*Duodenal pressures.* **PERIOD 1: 0–50 MIN.** There was a trend for an effect of treatment on the number of duodenal PWs ($P = 0.08$; saline: $204.2 \pm 23.7$, 1.33/50: $172.4 \pm 34.3$, 1.33/150: $203.7 \pm 35.1$, and 4/50: $110.5 \pm 34.3$); the number of duodenal PWs was less with 4/50 when compared with saline and 1.33/150 ($P < 0.05$ for both), with no difference between saline, 1.33/50, and 1.33/150. There was no effect of treatment on the amplitude of duodenal PWs (data not shown). There was an effect of treatment on the MI of duodenal PWs ($P = 0.05$; Fig. 3A); 4/50 decreased the MI when compared with saline, 1.33/50, and 1.33/150 ($P < 0.05$ for all), with no difference between saline, 1.33/50, and 1.33/150.

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**Fig. 1.** Motility index (MI) for antral pressure waves (PWs) between 0 and 50 min (A), 50 and 100 min (B), and 100 and 150 min (C) during intraduodenal infusion of 10% Intralipid at 1.33 kcal/min for 50 min (1.33/50), 1.33 kcal/min for 150 min (1.33/150), and 4 kcal/min for 50 min (4/50) or saline for 150 min. A: 1.33/50 and 4/50 decreased the MI when compared with saline. Treatment effect: $P < 0.05$; *$P < 0.07$ vs. saline; **$P < 0.01$ vs. saline and 1.33/50. Treatment effect: $P < 0.05$; *$P < 0.01$ vs. saline and 1.33/50. B: 1.33/150 and 4/50 decreased the MI when compared with saline and 1.33/50. Treatment effect: $P < 0.05$; *$P < 0.07$ vs. saline; **$P < 0.01$ vs. saline and 1.33/50. Treatment effect: $P < 0.05$; *$P < 0.01$ vs. saline and 1.33/50. C: 1.33/150 and 4/50 decreased the MI when compared with saline; 1.33/150 decreased the MI compared with 1.33/50. Treatment effect: $P < 0.05$ vs. saline (*) and vs. 1.33/50 (#). Data are means ± SE (n = 11 subjects).
PERIOD 2: 50–100 MIN. There was no effect of treatment on the number, or amplitude, of duodenal PWs (data not shown). There was, however, an effect of treatment on the MI of duodenal PWs (P < 0.05; Fig. 3B); the MI during 1.33/50 was greater when compared with saline, 1.33/150, and 4/50 (P < 0.05 for all), whereas there was no difference between saline, 1.33/150, and 4/50.

PERIOD 3: 100–150 MIN. There was an effect of treatment on the number of duodenal PWs (P < 0.01; saline: 276.5 ± 21.3, 1.33/50: 288.7 ± 45.4, 1.33/150: 167.0 ± 27.0, and 4/50: 349.9 ± 33.5); the number of duodenal PWs was less for 1.33/150 when compared with saline (P < 0.05), 1.33/50 (P < 0.05), and 4/50 (P < 0.001), with no difference between saline, 1.33/50, and 4/50. There was no effect of treatment on the amplitude of duodenal PWs (data not shown), but there was a trend for an effect of treatment on the MI of duodenal PWs (P = 0.07; Fig 3C); the MI during 1.33/50 (P = 0.07) and 4/50 (P < 0.01) was greater when compared with 1.33/150, with no difference between saline, 1.33/50, and 4/50.

PWSs. Only PWSs that traveled over 2–6 channels (i.e., 1.5–9 cm) were analyzed statistically, since PWSs traveling over 7–15 channels were infrequent (0–50 min: saline, 3 ± 0; 1.33/50, 2 ± 0; 1.33/150, 2 ± 0; and 4/50, 5 ± 1; 50–100 min: saline, 6 ± 0; 1.33/50, 6 ± 1; 1.33/150, 3 ± 0; and 4/50, 4 ± 1; 100–150 min: saline, 6 ± 0; 1.33/50, 3 ± 0; 1.33/150, 3 ± 0; and 4/50, 7 ± 1).

PERIOD 1: 0–50 MIN. There was a treatment by length interaction for the number of PWSs (P < 0.05; Fig. 4A); both 1.33/50 and 1.33/150 decreased the number of PWSs that traveled over two and three channels when compared with saline (P < 0.05 for all); 4/50 decreased the number of PWSs that traveled over two, three, and four channels when compared with saline (P < 0.05), and the number of PWSs that traveled over two channels when compared with 1.33/50 and 1.33/150 (P < 0.01). There was no difference between 1.33/50 and 1.33/150.

PERIOD 2: 50–100 MIN. There was a treatment by length interaction for the number of PWSs (P < 0.05; Fig. 4B); 4/50 decreased the number of waves that traveled over two channels when compared with saline (P < 0.05). The number of waves that traveled over two and three channels was greater during 1.33/50 when compared with 1.33/150 and 4/50 (P < 0.05 for both), whereas there was no difference between 1.33/150 and 4/50.

PERIOD 3: 100–150 MIN. There was a treatment by length interaction for the number of PWSs (P < 0.05; Fig. 4C); 1.33/150 decreased the number of waves that traveled over two channels when compared with saline, 1.33/50, and 4/50 (P < 0.01 for all).

### Duodenal motility index

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Saline</th>
<th>1.33 kcal/50 min</th>
<th>1.33 kcal/150 min</th>
<th>4 kcal/50 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–50</td>
<td></td>
<td>276.5 ± 21.3</td>
<td>288.7 ± 45.4</td>
<td>167.0 ± 27.0</td>
</tr>
<tr>
<td>50–100</td>
<td></td>
<td>288.7 ± 45.4</td>
<td>167.0 ± 27.0</td>
<td>349.9 ± 33.5</td>
</tr>
<tr>
<td>100–150</td>
<td></td>
<td>349.9 ± 33.5</td>
<td>167.0 ± 27.0</td>
<td>288.7 ± 45.4</td>
</tr>
</tbody>
</table>

Data are means ± SE (n = 11).
Plasma Hormone Concentrations

**CCK.** There was a treatment by time interaction for plasma CCK concentrations \((P < 0.001; \text{Fig. 5A})\). Plasma CCK concentrations reached a maximum at 15 min during all lipid treatments \((1.33/50: 9.3 \pm 1.1 \text{ pmol/l}, 1.33/150: 9.2 \pm 1.1 \text{ pmol/l}, \text{and } 4/50: 11.8 \pm 1.4 \text{ pmol/l})\). During 1.33/50 and 1.33/150, plasma CCK declined after 15 min, returning to baseline values for 1.33/50 by 90 min, whereas for 1.33/150 levels gradually decreased but were still higher than baseline at 150 min \((P < 0.01)\). During 4/50, CCK concentrations plateaued between 15 and 60 min and thereafter progressively decreased to be just above baseline at 90 min; 1.33/50, 1.33/150, and 4/50 increased plasma CCK when compared with saline during the first 60 min of the infusion \((P < 0.01 \text{ for all})\) and the effect of 4/50 was greater when compared with 1.33/50 and 1.33/150 \((P < 0.01 \text{ for both})\), with no difference between 1.33/50 and 1.33/150. During 1.33/150, plasma CCK concentrations were greater when compared with saline, 1.33/50, and 4/50 between 90 and 150 min \((P < 0.05 \text{ for all})\), with no difference between saline, 1.33/50, and 4/50.

**PYY.** There was a treatment by time interaction for plasma PYY concentrations \((P < 0.001; \text{Fig. 5B})\). With both 1.33/50 and 1.33/150, PYY concentrations reached a maximum \((1.33/50: 17.3 \pm 1.4 \text{ pmol/l}, 1.33/150: 18.7 \pm 2.2 \text{ pmol/l})\) at 30 min, after which levels for 1.33/50 gradually declined; whereas for 1.33/150, levels remained constant until 150 min. In response to 4/50, there was a marked stimulation of PYY concentrations with a peak at 60 min \((36.7 \pm 3.8 \text{ pmol/l})\) and a decline after that time, although levels were still higher than baseline at 150 min \((P < 0.001; 1.33/50, 1.33/150, \text{and } 4/50 \text{ increased plasma PYY when compared with saline over the entire infusion period } (P < 0.05 \text{ for all})\), and the effect of 4/50 was greater...
when compared with 1.33/50 between 30 and 150 min and with 1.33/150 between 30 and 120 min. There was no difference between 1.33/50 and 1.33/150 during the first 90 min of infusion, whereas PYY was higher during 1.33/150 between 120 and 150 min. 

Appetite Perceptions and Energy Intake

Although there was no effect of treatment on appetite perceptions, mean scores for prospective consumption were less during 4/50 when compared with the other infusions between 0 and 105 min (P = 0.28; Fig. 6A). There was an effect of treatment on scores for nausea (P < 0.05; Fig. 6B); although mean scores were low, they were greater during 4/50 when compared with saline, 1.33/50, and 1.33/150 (P < 0.05 for all) between 0 and 60 min, with no difference between saline, 1.33/50, and 1.33/150. There was no effect of treatment on energy intake, the amount eaten, or macronutrient distribution of food eaten at the buffet meal; however, mean energy intake was lower after 1.33/150 (P = 0.3; Table 2).

DISCUSSION

As anticipated from previous studies (2, 8, 20), APD motor and hormone responses to the intraduodenal infusions between 0 and 50 min were dependent on the rate of lipid infusions, i.e., over the first 50 min, responses to 1.33/50 were comparable to those to 1.33/150, but less than those to the 4/50 infusion. Thus, the inhibition of duodenal PWs and APD PWs and the stimulation of basal pyloric pressures, IPPWs, plasma CCK and PYY, and the induction of mild nausea were greater during the 4 kcal/min infusion than either of the 1.33 kcal/min infusions; in turn, the 1.33 kcal/min infusion, over a prolonged period, evoked higher basal pyloric pressures, more IPPWs, greater suppression of antral PWs, and greater plasma CCK and PYY responses than saline (control).

Such dose Responsiveness reflects two phenomena as follows: 1) the concentration of the stimulating nutrients (in this case, fatty acids and monoglycerides) exceeds thresholds for detection by sensors located along the gut. For example, as the load (kcal/min) of triglyceride entering the duodenum increases, so do the rates of release and thus luminal concentrations of lipolytic products (27), and 2) with further increases in the rate of duodenal entry of triglycerides, hydrolysis takes longer to complete, and consequently lipolytic products are distributed further downstream. Also, mainly because of the slow rates of aqueous diffusion of poorly soluble lipolytic products, absorption is also slow; hence, an increasing length of gut is required to complete the absorptive process as duodenal loads increase (18, 29, 31). For example, Lin et al. (18) reported that a 15-g fat emulsion emptied initially from the canine stomach at 0.9 kcal/min, but only 7% of this duodenal load reached the ileum (i.e., was not absorbed proximally); in contrast, a 60-g fat emulsion emptied initially at 2 kcal/min,
Table 2. Energy content, weight, and macronutrient distribution (% energy derived from fat, CHO, or protein) of food consumed at a buffet meal after intraduodenal infusions of 10% Intralipid or saline

<table>
<thead>
<tr>
<th>Intralipid</th>
<th>Control (Saline)</th>
<th>1.33 kcal/50 min</th>
<th>1.33 kcal/150 min</th>
<th>4 kcal/50 min</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kcal</td>
<td>1,346±90</td>
<td>1,346±109</td>
<td>1,242±84</td>
<td>1,358±132</td>
<td>0.3</td>
</tr>
<tr>
<td>Weight, g</td>
<td>1,433±111</td>
<td>1,467±141</td>
<td>1,340±87</td>
<td>1,489±128</td>
<td>0.2</td>
</tr>
<tr>
<td>%kJ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>From fat</td>
<td>29±2</td>
<td>30±2</td>
<td>32±2</td>
<td>29±2</td>
<td>0.2</td>
</tr>
<tr>
<td>From CHO</td>
<td>50±3</td>
<td>50±3</td>
<td>47±3</td>
<td>50±3</td>
<td>0.2</td>
</tr>
<tr>
<td>From protein</td>
<td>20±1</td>
<td>21±1</td>
<td>21±2</td>
<td>20±1</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 11. CHO, carbohydrate. Intraduodenal infusions consisted of either 10% Intralipid at 1.33 kcal/min for 50 min followed by saline for 100 min, 1.33 kcal for 150 min, 4 kcal for 50 min followed by saline for 100 min or saline (control) for 150 min.

with 30% of this load reaching the ileum (18). Some responses to luminal nutrients, including the inhibition of gastric emptying of aqueous contents (17) and energy intake (31), increase with the length of gut contacted; other nutrient-driven responses appear to be uniquely mediated by ileal mechanisms (14, 19, 35).

In the present study, we postulated that 1) intestinal transit would carry the 4/50 bolus into the ileum after the first hour and that this transit would displace lipolytic products from >30% of the infused load from the jejunum to the ileum where digestion and absorption would continue over the 100 min after cessation of the fat infusion, 2) a correspondingly prolonged stimulation of ileal neural reflexes and/or release of ileal peptides would sustain a feedback inhibition of upper gastrointestinal pressures, but 3) suppression of energy intake may not be apparent, since this is driven maximally by combined feedback from both proximal and distal gut sensors (30, 31), and is less when lipolytic products are confined to the ileum (30, 38). Our observations suggested that these predictions were correct; after the 4/50 load (as opposed to the 1.33/50 infusion), continuing feedback from the ileum is attested to by sustained alterations in upper gastrointestinal pressures and continuing release of PYY, yet CCK had returned to baseline by 90 min and there was no suppression of energy intake at 150 min. Although we did not directly measure the distribution of lipolytic products along the gut, the simplest explanation for the persisting alterations in motility and changing ratios of CCK-to-PYY in the plasma beyond 90 min after the 4/50 bolus is the persistence of lipolytic products in the ileum from 90 to 150 min.

Arguably the most striking, and novel, observation was the effect of altering the rate, and duration, of duodenal infusion of 200 kcal of fat on the time course of plasma CCK and PYY secretion. During the 4/50 infusion, CCK rose rapidly, peaked at 15 min, and then remained elevated for a further 45 min at a concentration that was about two times the level sustained throughout the 1.33/150 infusion. Soon after cessation of the 4/50 infusion at 50 min, CCK fell to baseline values. In contrast to the rapid fall of CCK, plasma concentrations of PYY peaked at 60 min and remained elevated between 50 and 150 min after cessation of the 4/50 infusion. Although PYY fell progressively after the 4/50 infusion was stopped at 50 min, plasma PYY levels remained significantly higher between 50 and 120 min when compared with the relatively constant level evident between 30 and 150 min during the 1.33/150 infusion. By contrast, after cessation at 50 min of the lower, 1.33/50, lipid load, both CCK and PYY fell rapidly to levels indistinguishable from control values, presumably because lipolysis of fat was completed, and thus lipolytic products were removed from the gastrointestinal lumen. The elevation of PYY between 50 and 150 min after the 4/50 infusion was stopped is presumably attributable to the presence of lipolytic products from this infusion in the gastrointestinal lumen, as a result of continuing lipolysis in the ileum or ileocolon as the 50 min bolus of fat and lipase moved along the distal small intestine. If so, the rapid decline in plasma CCK after cessation of the 4/50 infusion reflected not the disappearance of lipolytic products from the gastrointestinal lumen but rather their movement distally, out of the CCK-bearing area of the jejunum (3) into the PYY-rich area of the ileocolon (1). By contrast, the nearly steady plasma concentrations of both CCK and PYY sustained by the infusion of 1.33/150 must have reflected the continuing presence of lipolytic products in the CCK-bearing area of the proximal small intestine.

Although the explanation put forth in the preceding paragraph adequately explains the time courses for the plasma CCK and PYY responses, it is at odds with a canine study (15). In dogs, as in humans, CCK is stored in jejunal (3), whereas PYY is stored in ileocolonic (37), mucosa. In the study by Lin and Chey (15), duodenally infused oleic acid was either confined to the proximal small intestine, infused into the distal small intestine, or simultaneously infused into both the proximal and distal small intestine in dogs. When oleic acid infusion was confined to the proximal small intestine, PYY rose slowly (peaking at 90 min), but substantially. On the other hand, PYY concentrations were some 20% higher when the oleic acid was in direct contact with the distal bowel mucosa (whether infused distally or both proximally and distally), and the release of PYY by fat confined to the proximal small intestine was shown to be mediated, at least in part, by CCK (16). The observations described thus far are not in conflict with the scenario suggested in the preceding paragraph. However, in contrast with the present study, in dogs, infusion of oleic acid induced similar rises in plasma CCK, whether confined to the proximal or distal small intestine (15). Thus we speculate that the mechanism(s) for fat-induced CCK release in the dog may differ substantially from that in humans.

The much higher CCK release between 15 and 60 min in response to the 4 kcal/min, compared with 1.33 kcal/min, infusion might reflect a more rapid release and thus higher luminal concentrations of lipolytic products in the proximal small intestine (27), that is, a concentration-dependent release of CCK by fatty acids. Alternatively, it may reflect the much longer length of jejunal exposure to lipolytic products in the CCK-bearing region. If the latter were correct, the modest, but significant, release of PYY by the 1.33 kcal/min loads must have resulted from indirect, probably CCK-mediated, stimulation of PYY release (16), rather than release by direct contact, because the maximal CCK-bearing areas of the small intestine are proximal to the maximal PYY bearing areas, with minimal overlap.

A second interesting observation in the present study was the difference in the effect of lipid on antral and duodenal PWs...
during the first 50 min of infusion. The suppression of antral PWs was similar for all lipid infusions; however, there was no suppressive effect of either 1.33 kcal/min infusion on duodenal PWs when compared with saline. This demonstrates that the 1.33 kcal/min load may be sufficient to have a maximal inhibitory effect on the antrum but did not reach the threshold needed for suppression in the duodenum. To date, only one study has demonstrated load-related effects of lipid on intestinal motility, in which the number of duodenal PWSs was reported to decrease as the duodenal load of lipid increased (2). However, in this study, only relatively low loads of lipid were used (0.25–1.5 kcal/min), and the effects of lipid on pressures in the antrum and pylorus were not evaluated. In contrast, proximal gastric relaxation in response to duodenal lipid (Intralipid as used in the current study) at 1, 2, and 3 kcal/min, as measured by an electronic barostat, was found not to be load-dependent, since maximal relaxation occurred at the lowest load of 1 kcal/min (8). These observations taken together indicate that there are regional differences in the upper gastrointestinal tract in relation to the nutrient load required for a maximal inhibitory effect. It is also interesting that the inhibitory effect of the 4/50 infusion on antral PWs persisted to some extent after this infusion was stopped, particularly between 50 and 100 min and were still present between 100 and 150 min. Similarly, antral PWs continued to be strongly inhibited for the entire 150 min during the 1.33/150 infusion. The sustained inhibition of antral PWs after the 4/50 or the 1.33/150 infusion might be explained by persisting elevation of PYY, although it should be recognized that Raybould et al. (33) have demonstrated in rats that PYY does not play a role in the inhibition of gastric emptying (not exactly the same as inhibition of antral or duodenal motility) after either duodenal or ileal infusion of lipid. By contrast, the time courses of both basal pyloric pressures and IPPWs correspond closely to the time courses of plasma CCK observed after the three lipid infusions if it is assumed that the pylorus responded similarly to plasma CCK concentrations of 6 pmol/l or above.

Appetite scores and energy intake were not affected by any of the lipid infusions, although there was a significant, albeit slight, increase in nausea and a tendency for reduced scores for prospective consumption during 4/50 compared with the other infusions. The lack of effect of the 4/50 infusion on energy intake is likely to be accounted for by the experimental paradigm in which the meal was consumed 100 min after cessation of the lipid infusion, at a time when plasma concentrations of CCK and the number of IPPWs had declined to baseline, concentrations of PYY had waned, and unabsorbed lipolytic products had diminished and probably contacted only a portion of distal bowel mucosa. For example, Castiglione et al. (5) assessed the effect of 20% Intralipid on energy intake over different durations of infusion [15 min (30 kcal), 45 min (90 kcal), or 90 min (180 kcal)] in healthy subjects and found a reduction in energy intake only for the 45- and 90-min infusions. In contrast, in our study, the 1.33/50 (67 kcal) and 4/50 (200 kcal) lipid infusions were discontinued 100 min before the evaluation of energy intake, so that only the 1.33/150 (200 kcal) infusion, which ceased immediately before the meal, was comparable to the 90-min infusion in the study by Castiglione et al. (5). Moreover, we found no reduction in energy intake after the 150-min infusion. This may reflect the higher rate of infusion (2 kcal/min) used by Castiglione et al. (5) over a period of time sufficient to permit caudal movement of digesting fat and lipase, thereby exposing lipolytic products to a sufficient length of bowel to induce an effect on energy intake (5, 31). In contrast, in our study, lipolysis and absorption were probably completed over a shorter length of small intestine during a slower lipid infusion of 1.33/150. Higher loads of intraduodenal lipid (2–2.9 kcal/min) have been shown to reduce hunger, increase fullness, and suppress subsequent energy intake, even after shorter infusion periods in healthy young (9), old (7, 21), and obese (6) subjects.

Suppression of energy intake by small intestinal fat is likely mediated by combinations of neural responses to luminal nutrients and nutrient-stimulated release of anorexogenic peptides, including CCK, PYY, and glucagon-like peptide-1. We recently observed that energy intake was not suppressed by increasing loads of duodenally infused lauric acid until the rate of fatty acid entry was high enough to sustain a plasma level of CCK ~7.5 pmol/l (4 pmol/l above basal; see Ref. 20), substantially greater than the CCK concentration at 150 min after the sustained 1.33/150 infusion. Thus it is probable that we did not observe a reduction in energy intake after any of our infusions because the potent effects of the short-duration, but high-dose, 4/50 infusion had disappeared by 150 min, whereas the sustained 1.33/150 infusion was an impotent dose.

In summary, our study establishes that there are regional differences in the regulation of APD motility and the release of CCK and PYY in response to small intestinal fat, as demonstrated by altering the load, and duration, of intraduodenal lipid infusion. Although sustained exposure of the small intestine to lipid was associated with sustained suppression of antral PWs, there was an attenuation in the stimulation of pyloric pressures and plasma CCK, which may account for the absence of a significant effect on energy intake.

GRANTS

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REFERENCES


