Load-dependent effects of duodenal lipid on antropyloroduodenal motility, plasma CCK and PYY, and energy intake in healthy men

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The presence of fat in the small intestine is associated with a number of changes in gastrointestinal function, including modulation of antropyloroduodenal (APD) motility, specifically suppression of antral (22) and duodenal (2) pressures and stimulation of isolated pyloric pressure waves (IPPWs) and basal pyloric pressure (14, 22), resulting in the modulation of gastric emptying (22) and gastrointestinal hormone secretion (30), including CCK from the proximal (9), and peptide YY (PYY) from the distal (1), small intestine. Small intestinal fat also has the capacity to suppress appetite and energy intake (13).

The effects of fat on gastrointestinal function and energy intake are dependent on fat digestion (6, 17, 18, 41). On contact with sensors in the small intestine, lipolytic products, especially fatty acids, stimulate gut hormone secretion, modulate gastrointestinal motility, and suppress energy intake (17, 20, 28). These responses depend on the load of lipid delivered to the small intestine (19, 38), as well as the region (35, 36, 44), and length (26, 35, 36) of small intestine contacted. The rate of postprandial gastric emptying of lipid is highly variable, ranging from ~0.4 to 4.1 kcal/min in normal human subjects (15, 25, 29, 33). Much of this variability is accounted for by how thoroughly water-insoluble fat is dispersed in meal contents along the antropyloric region. There is very little information as to how triglycerides may evoke the above responses during digestion after entering the small intestine over this range of caloric loads.

A recent study in our laboratory suggested that suppression of energy intake may relate to changes in APD motility, particularly that of the pylorus (8). In healthy subjects intravenous infusion of CCK (1.8 pmol·kg⁻¹·min⁻¹) markedly stimulated IPPWs and basal pyloric pressures, which was associated with suppression of energy intake; while glucagon-like peptide-1 (GLP-1) in the dose used (0.9 pmol·kg⁻¹·min⁻¹) did not stimulate pyloric pressures or suppress energy intake (8). The association between nutrient-induced changes in APD motility and energy intake has not been assessed.

The first aim of this study was therefore to evaluate the load-dependent effects of intraduodenal lipid on APD motility, plasma CCK and PYY, appetite, and energy intake. For this purpose, we infused Intralipid (the most commonly used triglyceride emulsion in physiological studies of this kind) into the duodenum at rates of 0.25, 1.5, and 4 kcal/min and compared the effects with those of a 0.9% saline control infusion. The doses selected thus encompassed the range of emptying rates of gastric emptying of dietary fat hitherto reported in humans (15, 25, 29, 33). The second aim was to determine potential relationships between the effects of intraduodenal lipid on appetite and energy intake and those on APD motility and plasma CCK and PYY.

MATERIALS AND METHODS

Subjects

Sixteen healthy males (mean age: 31 ± 3 yr; body mass index: 23.8 ± 0.5 kg/m²) participated in this study. All subjects were unrestrained eaters with a score of ≥12 on the Eating Restraint component (Factor 1) of the Three Factor Eating Questionnaire (42), had no history of gastrointestinal disease, were not taking medication known to affect gastrointestinal motility, mood, or appetite, and had a stable body weight for at least 6 mo prior to the study (within 5% of their average weight). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
the screening weight). No subject was a smoker or habitually consumed more than 20 g of alcohol per 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 each subject provided written, informed consent prior to their inclusion. The number of subjects was determined by power calculations based on our previous study in which the difference in energy intake was 199 ± 18 kcal (mean ± SE) (17). We calculated that \( n = 15 \) subjects would allow us to detect a 14% difference in energy intake with a power of >80%.

**Protocol**

Each subject was studied on four occasions, each separated by 3–7 days, on which they received in randomized, double-blind fashion an intraduodenal infusion of a lipid emulsion (IL; 10%; Intralipid, 300 mOsmol/kg, 1.1 kcal/ml; Fresenius Medical Care Australia, Smithfield, NSW, Australia), at \( J(t) \) 0.25 kcal/min (IL0.25), \( 2 \) 1.5 kcal/min (IL1.5), \( 3 \) 4 kcal/min (IL4), or \( 4 \) isotonic saline (control), each for 50 min. Intralipid was 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 over 50 min was 200 ml in all study conditions, i.e., time, and volume were fixed and energy delivery varied. Effects on APD motility, plasma CCK and PYY, appetite, and subsequent energy intake were quantified. These rates were chosen to encompass loads lower than the rate of normal gastric emptying (0.25 kcal/min), as well as loads reflecting the lower-to-intermediate (1.5 kcal/min) and higher (4 kcal/min) rates observed during the postprandial time course of gastric emptying (29, 33). Intralipid, which consists predominantly of long-chain triacylglycerides extracted from soy beans, was selected as it has been used in the majority of studies that have evaluated the effects of lipid on gastrointestinal function and appetite (2, 12, 13, 16, 23). Both the primary investigators (A. N. Pilchiewicz and P. Papadopoulos) and the subjects were blinded to the study treatments. The infusions were prepared by one of the other investigators who was not involved in the data analysis, and furthermore, the infusion apparatus was covered at all times during the study.

Subjects attended the laboratory in the Discipline of Medicine at 0830 after an overnight fast (14 h for solids, 12 h for liquids). A baseline blood sample was taken, and simultaneously subjects were comfortably full (20). The intravenous cannula was then removed, and the subject was allowed to leave the laboratory.

**Measurements**

**APD pressures.** APD pressures were recorded and digitized using a computer-based system running commercially available software [Flexisoft Version 3; Oakfield Instruments, 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 \( 1 \) number and amplitude of antral and duodenal pressure waves (PPWs), \( 2 \) number and amplitude of IPPWs, \( 3 \) basal pyloric pressure, and \( 4 \) number and length of pressure wave sequences (PWSs) as described previously (38).

**Plasma CCK and PYY.** Ten-milliliter venous blood samples were collected in ice-chilled EDTA-tubes containing 400 KIU aprotinin (Trasylol; Bayer, Pymble, Australia) per milliliter of blood. Plasma was separated by centrifugation (3,200 rpm, 15 min, 4°C) within 30 min of collection and then stored at −70°C until assayed.

Plasma CCK concentrations (pmol/l) were determined after ethanol extraction by using an adaptation of a previously described radioimmunoassay (39). A commercially available antibody (cat. no. C258, lot no. 105H4852; Sigma, 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 cross-reactivity of 26% 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%, the interassay CV was 15%, and the limit of detection was 2.5 pmol/l.

Plasma PYY concentrations (pmol/l) were measured by radioimmunoassay as described previously (38). 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) was employed. This antiserum shows <0.001% cross-reactivity with human pancreatic polypeptide and sulfated CCK-8, and 0.0025% cross-reactivity with human neuropeptide Y. The intra-assay CV was 12.3%, the interassay CV was 16.6%, and the limit of detection was 4 pmol/l.

**Appetite perceptions and energy intake.** Perceptions of hunger and fullness were rated on validated VAS (37). Nausea and bloating were also assessed. Each VAS consisted of an unnumbered 100-mm horizontal line for each sensation. The subject placed a vertical mark along the line indicating the strength of the sensation at a particular time point, i.e., a vertical mark placed on zero indicated the sensation was not felt and on 100 that the sensation was extremely strong.

Energy intake was assessed by measuring consumption at the buffet-style meal. The items of the buffet meal were: 4 slices (125 g) each of white and whole meal bread, 100 g sliced chicken, 100 g sliced ham, 4 slices of cheese, 100 g cucumber, 100 g lettuce, 100 g tomato, 140 g fruit salad, 150 g chocolate custard, 200 g strawberry yoghurt, 28 g margarine, 28 g mayonnaise, an apple, a banana, 500 ml orange juice, 600 ml iced coffee and 600 ml water (20). The amount (g) and energy (kcal) consumed and the macronutrient distribution (%energy from fat, carbohydrate, and protein) were evaluated using commercially available software (Foodworks Version 3.01; Xyris Software, Highgate Hill, QLD, Australia) (20).

**Data and Statistical Analyses**

Baseline values (0) were calculated as the mean values obtained between \( t = −10 \) and 0 min for the number and amplitude of IPPWs and antral and duodenal PWs, basal pyloric pressures, and PWSS, and at \( t = −10 \) and 0 min for plasma hormone concentrations and VAS scores. The numbers of antral and duodenal PWs were expressed as total numbers, whereas amplitudes and motility indices (MIs) were expressed as mean values, over the 50-min infusion (i.e., \( t = 0 \)–50 min). Antral and duodenal MIs were derived using the equation, MI =
LIPID LOADS AND GUT MOTILITY, HORMONES, AND APPETITE

natural logarithm \([\text{sum of amplitudes} \times \text{number of phasic PWs}] + 1\). Basal pyloric pressures and the number and amplitude of IPPWs were expressed as means of 10-min periods over the 50-min infusion period. APD PWs were expressed as the total number of PWs spanning over two (1.5–3 cm), three (3–4.5 cm), . . . , 15 (21–22.5 cm) channels during the 50-min infusion period. All motility and VAS data were expressed as changes from baseline, while plasma CCK and PYY were expressed as absolute values.

Number, amplitude, and MI of antral and duodenal PWs and the parameters measured from the buffet meal [energy intake (kcal), amount eaten (g), macronutrient distribution (%)] were analyzed by one-way ANOVA. 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 PWs was analyzed by repeated-measures ANOVA, with number and length (cm) as factors. Post hoc paired comparisons, corrected for multiple comparisons by Bonferroni’s correction, were performed if ANOVAs revealed significant effects. Correlations corrected for repeated measures were determined for 1) the total number of antral and duodenal PWs and APD PWs, mean amplitude and MI of antral and duodenal PWs, areas under the curve (AUCs; calculated using the trapezoidal rule) for number and amplitude of IPPWs, basal pyloric pressures, plasma CCK and PYY, and appetite perception scores between \(t = 0\) and 50 min, as well as energy intake and amount eaten at the buffet meal, with the ln (natural logarithm)–transformed loads of lipid administered and 2) appetite perceptions and energy intake (and amount eaten) with APD motility and plasma CCK and PYY, for both AUCs and values at \(t = 50\) min, using the method described by Bland and Altman (5). When correlations between the above variables were found, multiple regression analysis was performed to establish determinants of energy and food intake. Statistical significance was accepted at \(P < 0.05\), and data are presented as means ± SE.

RESULTS

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

APD Pressures

Antral PWs. IL0.25, IL1.5, and IL4 decreased the number of antral PWs when compared with control (\(P < 0.05\) for all), with no significant differences between the lipid treatments (Table 2). IL1.5 and IL4 decreased the amplitude and MI of antral PWs when compared with control and IL0.25 (\(P < 0.05\) for all); there was no difference between control and IL0.25 or between IL1.5 and IL4 (Table 2). There was an inverse relationship between the number (\(r = -0.69, P < 0.05\)), amplitude (\(r = -0.81, P < 0.05\)), and MI (\(r = -0.74, P = 0.05\)) of antral PWs with the load of lipid administered.

Pyloric pressures. Basal pressures. There was a treatment \(\times\) time interaction for basal pyloric pressures (\(P < 0.001\)) (Fig. 1A). IL1.5 (\(P < 0.05\)) and IL4 (\(P < 0.001\)) stimulated basal pyloric pressure between \(t = 10\) and 50 min when compared with control. IL1.5 stimulated basal pyloric pressure between \(t = 20\) and 40 min (\(P < 0.05\)) and IL4 between \(t = 10\) and 50 min (\(P < 0.01\)) when compared with IL0.25. IL4 stimulated basal pyloric pressure between \(t = 20\) and 50 min when compared with IL1.5 (\(P < 0.01\)). There was no difference between control and IL0.25. By the end of the infusion (i.e., \(t = 40\)–50 min) basal pyloric pressures had returned to levels close to baseline for control; IL0.25 and IL1.5, however, remained elevated for IL4 (\(P < 0.01\)). There was a direct relationship between the AUC for basal pyloric pressure with the load of lipid administered (\(r = 0.82, P < 0.05\)).

Phasic pressures. There was a treatment \(\times\) time interaction for the number of IPPWs (\(P < 0.001\)) (Fig. 1B). IL0.25, IL1.5, and IL4 increased the number of IPPWs when compared with control; IL0.25 between \(t = 0\) and 20 min (\(P < 0.001\)), and IL1.5 (\(P < 0.01\)) and IL4 (\(P < 0.001\)) between \(t = 0\) and 50 min. IL1.5 and IL4 increased the number of IPPWs when compared with IL0.25. IL1.5 between \(t = 20\) and 50 min (\(P < 0.01\)), and IL4 between \(t = 0\) and 50 min (\(P < 0.05\)). IL4 increased the number of IPPWs when compared with IL1.5 between \(t = 20\) and 50 min (\(P < 0.05\)). After \(t = 20\) min, the number of IPPWs began to slightly decline for IL1.5 and IL4, however, during IL0.25 the numbers were not sustained and fell to values similar to control. There was a treatment \(\times\) time interaction for the amplitude of IPPWs (\(P < 0.05\)) (Fig. 1C). IL0.25, IL1.5, and IL4 increased the amplitude of IPPWs when compared with control; IL0.25 between \(t = 10\) and 30 min (\(P < 0.05\)), and IL1.5 (\(P < 0.05\)) and IL4 (\(P < 0.01\)) between \(t = 10\) and 50 min. IL4 increased the amplitude of IPPWs between \(t = 20\) and 50 min (\(P < 0.05\)) when compared with IL0.25, and between \(t = 30\) and 40 min when compared with IL1.5. There was no difference between IL0.25 and IL1.5. There was a direct relationship between the AUC for the number (\(r = 0.80, P < 0.001\)) and amplitude (\(r = 0.68, P < 0.05\)) of IPPWs with the load of lipid administered.

Duodenal PWs. IL1.5 and IL4 decreased the number, amplitude, and MI of duodenal PWs when compared with control and IL0.25 (\(P < 0.05\) for all), with no differences between control and IL0.25, or between IL1.5 and IL4 (Table 2). There was a direct relationship between the AUC for the number and amplitude of duodenal PWs with the load of lipid administered (\(r = 0.93, P < 0.05\)).

Table 1. Baseline values for antral and duodenal pressure waves (PWs), basal pyloric pressure, number, and amplitude of isolated pyloric PWs (IPPPWs), hunger, fullness, nausea, and bloating, i.e., before commencement of intraduodenal lipid (IL) infusions of 10% Intralipid or saline

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>IL0.25</th>
<th>IL1.5</th>
<th>IL4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antral PWs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>6±1</td>
<td>6±1</td>
<td>6±1</td>
<td>6±1</td>
</tr>
<tr>
<td>Amplitude, mmHg</td>
<td>7±2</td>
<td>7±2</td>
<td>7±2</td>
<td>7±2</td>
</tr>
<tr>
<td>MI, mmHg</td>
<td>2±1</td>
<td>2±1</td>
<td>2±1</td>
<td>2±1</td>
</tr>
<tr>
<td>Duodenal PWs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>29±6</td>
<td>38±9</td>
<td>35±7</td>
<td>29±6</td>
</tr>
<tr>
<td>Amplitude, mmHg</td>
<td>17±2</td>
<td>17±2</td>
<td>18±2</td>
<td>16±1</td>
</tr>
<tr>
<td>MI, mmHg</td>
<td>5±0</td>
<td>6±0</td>
<td>6±0</td>
<td>5±0</td>
</tr>
<tr>
<td>Pyloric Pressures</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal, mmHg</td>
<td>48±7</td>
<td>52±7</td>
<td>54±7</td>
<td>51±7</td>
</tr>
<tr>
<td>No. of IPPWs</td>
<td>10±4</td>
<td>7±2</td>
<td>7±2</td>
<td>9±3</td>
</tr>
<tr>
<td>Amplitude of IPPWs, mmHg</td>
<td>7±2</td>
<td>9±3</td>
<td>6±2</td>
<td>8±3</td>
</tr>
<tr>
<td>VAS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hunger, mm</td>
<td>4±1</td>
<td>4±1</td>
<td>4±1</td>
<td>4±1</td>
</tr>
<tr>
<td>Fullness, mm</td>
<td>48±7</td>
<td>52±7</td>
<td>54±7</td>
<td>51±7</td>
</tr>
<tr>
<td>Nausea, mm</td>
<td>10±4</td>
<td>7±2</td>
<td>7±2</td>
<td>9±3</td>
</tr>
<tr>
<td>Bloating, mm</td>
<td>7±2</td>
<td>9±3</td>
<td>6±2</td>
<td>8±3</td>
</tr>
</tbody>
</table>

Data are means ± SE; \(n = 16\). IL0.25, 0.25 kcal/min; IL1.5, 1.5 kcal/min; IL4, 4 kcal/min; MI, motility indices; VAS, visual analogue scale.
was an inverse relationship between the number \( r = -0.79, P < 0.0001 \), amplitude \( r = -0.64, P < 0.001 \), and MI \( r = -0.67, P < 0.05 \) of duodenal PWs with the load of lipid administered.

PWs. Only PWs that spanned 2–6 channels (1.5–9 cm) were analyzed statistically, as PWs spanning over 7–15 channels were infrequent (no/50 min; control, 6 ± 3; IL0.25, 3 ± 2; IL1.5, 2 ± 1; IL4, 1 ± 1).

There was a treatment\(^\#\)length interaction for the number of PWs spanning 2 (1.5–3 cm), 3 (3–4.5 cm), 4 (4.5–6 cm), 5 (6–7.5 cm), and 6 (7.5–9 cm) channels \( P < 0.001 \) (Fig. 2). IL0.25 decreased the number of PW that spanned over 2 channels \( P < 0.01 \), IL1.5 over 2 and 3 channels \( P < 0.001 \), and IL4 over 2–6 channels \( P < 0.05 \), when compared with control. IL1.5 decreased the number of PWs spanning over 2–4 channels \( P < 0.05 \), and IL4 over 2–5 channels \( P < 0.05 \), when compared with IL0.25. IL4 decreased the number of PWs spanning 2 channels compared with IL1.5 \( P < 0.05 \). There was an inverse relationship between the total number of PWs with the load of lipid administered \( r = -0.80, P < 0.001 \).

Plasma Hormone Concentrations

CCK. There was a treatment \(\times\) time interaction for plasma CCK concentrations \( P < 0.001 \) (Fig. 3A). Plasma CCK rapidly increased during all lipid infusions after which levels plateaued for IL1.5 and IL4 and decreased to baseline values by \( t = 30 \) min for IL0.25. IL0.25, IL1.5, and IL4 increased plasma CCK when compared with control: IL0.25 between \( t = 10 \) and 20 min \( P < 0.05 \), and IL1.5 \( P < 0.01 \) and IL4 \( P < 0.001 \) between \( t = 10 \) and 50 min. IL1.5 \( P < 0.05 \) and IL4 \( P < 0.01 \) increased plasma CCK between \( t = 10 \) and 50 min when compared with IL0.25, and IL4 between \( t = 20 \) and 50 min when compared with IL1.5 \( P < 0.01 \). There was a direct relationship between the AUC for plasma CCK concentrations with the load of lipid \( r = 0.96, P < 0.001 \).

PYY. There was a treatment \(\times\) time interaction for plasma PYY concentrations \( P < 0.001 \) (Fig. 3B). There was an ongoing rise in PYY in response to IL1.5 and IL4. IL1.5 and IL4 increased plasma PYY when compared with control and IL0.25: IL1.5 between \( t = 10 \) and 50 min \( P < 0.05 \), when both) and IL4 between \( t = 20 \) and 50 min \( P < 0.001 \), when both). IL4 increased plasma PYY when compared with IL1.5 between \( t = 30 \) and 50 min \( P < 0.01 \). There was no difference between control and IL0.25. There was a direct relationship between the AUC for plasma PYY concentrations and the load of lipid \( r = 0.91, P < 0.001 \).

Appetite Perceptions and Energy Intake

Appetite perceptions. There was a treatment \(\times\) time interaction for hunger \( P < 0.001 \) (Fig. 4A). Hunger was less during IL0.25 between \( t = 30 \) and 50 min \( P < 0.05 \) and during IL1.5 and IL4 between \( t = 20 \) and 50 min \( P < 0.05 \) for both) when compared with control during which hunger increased progressively throughout the infusion period. IL4 decreased hunger at \( t = 40 \) and 50 min when compared with IL0.25, and at \( t = 50 \) min when compared with IL1.5 \( P < 0.05 \) for both). There was no difference between IL0.25 and IL1.5. There was no effect of treatment on scores of fullness (data not shown). There was a treatment \(\times\) time interaction for nausea \( P = 0.05 \) (Fig. 4B). Although scores were very low, IL4 increased nausea between \( t = 30 \) and 50 min when compared with control, IL0.25, and IL1.5 \( P < 0.01 \) for all). During IL1.5, nausea was increased at \( t = 50 \) min when compared with IL0.25 \( P < 0.05 \). There were no differences between control and IL0.25 or IL1.5. There was no effect of treatment on scores of bloating (data not shown). There were no significant relationships between hunger or nausea with the load of lipid.

Energy intake. There was an effect of treatment on the energy (kcal) and amount (g) (Table 2) consumed at the buffet meal \( P < 0.05 \). IL4 reduced the energy and amount of food consumed when compared with control \( P < 0.01 \) and IL0.25 \( P < 0.05 \). There were no differences between control, IL0.25 and IL1.5 or between IL1.5 and IL4. There was no difference in the macronutrient distribution between the four experimental

Table 2. Mean values for antral and duodenal PWs during intraduodenal infusion of 10% Intralipid and saline, and energy intake, amount eaten, and %macronutrient distribution at the buffet meal

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>IL0.25</th>
<th>IL1.5</th>
<th>IL4</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antral PWs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No./50 min</td>
<td>51±12</td>
<td>29±8*</td>
<td>9±3*</td>
<td>10±5*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Amplitude, mmHg</td>
<td>22±5</td>
<td>26±9</td>
<td>4±2*#</td>
<td>8±3*#</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MI, mmHg</td>
<td>4±1</td>
<td>3±1</td>
<td>1±1*#</td>
<td>1±1*#</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Duodenal PWs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No./50 min</td>
<td>329±32</td>
<td>317±32</td>
<td>207±34*#</td>
<td>133±21*#</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Amplitude, mmHg</td>
<td>13±2</td>
<td>16±3</td>
<td>5±2*#</td>
<td>7±2*#</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MI, mmHg</td>
<td>5±0</td>
<td>5±1</td>
<td>3±1*#</td>
<td>3±0*#</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Food Intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy, kcal</td>
<td>1,289±62</td>
<td>1,282±44</td>
<td>1,235±71</td>
<td>1,139±65*#</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Amount, g</td>
<td>1,425±101</td>
<td>1,371±95</td>
<td>1,327±111</td>
<td>1,231±109*#</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>% kcal from fat</td>
<td>35±1</td>
<td>34±2</td>
<td>35±1</td>
<td>35±1</td>
<td>NS</td>
</tr>
<tr>
<td>% kcal from CHO</td>
<td>43±1</td>
<td>42±2</td>
<td>41±2</td>
<td>43±1</td>
<td>NS</td>
</tr>
<tr>
<td>% kcal from protein</td>
<td>23±1</td>
<td>24±1</td>
<td>23±1</td>
<td>23±1</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are means ± SE; \( n = 16 \). Data were analyzed using one-way ANOVA. Significant differences: * from control, # from IL0.25. CHO, carbohydrate; NS, not significant.
conditions (Table 2). There was an inverse relationship between the energy ($r = -0.63, P < 0.05$) and amount ($r = -0.61, P < 0.05$) consumed at the buffet meal with the load of lipid administered.

**Relations of APD Motility and Plasma CCK and PYY With Appetite Perceptions and Energy Intake**

**Relationships between APD motility with appetite perceptions, energy intake, and amount eaten.** There were no significant relationships between antral and duodenal PWs, and the AUC for basal pyloric pressure, IPPWs, or PWSs with energy intake. There were direct relationships between the amount eaten with the number ($r = 0.42, P < 0.05$) and amplitude ($r = 0.54, P < 0.05$) of antral PWs and an inverse relationship with the number of IPPWs ($r = -0.56, P < 0.05$). There were direct relationships between the number ($r = 0.57, P < 0.05$) and MI ($r = 0.55, P < 0.05$) of duodenal PWs and PWSs ($r = 0.58, P < 0.05$) with scores for hunger. There were no relationships between antral and duodenal PWs, basal pyloric pressure, IPPWs, or PWSs with scores for fullness, nausea, or bloating.

**Relationships between plasma CCK and PYY with appetite perceptions, energy intake, and amount eaten.** There were inverse relationships between energy intake with AUC ($r = -0.63, P < 0.01$) and the values at $t = 50$ min ($r = -0.61, P < 0.05$) for plasma CCK and AUC ($r = -0.50, P < 0.05$) and the values at $t = 50$ min ($r = -0.62, P < 0.05$) for plasma PYY. There were inverse relationships between the amount eaten with AUC ($r = -0.60, P < 0.001$) and values at $t = 50$ min ($r = -0.58, P < 0.01$) for CCK and AUC ($r = -0.52, P < 0.01$) and values at $t = 50$ min ($r = -0.62, P < 0.01$) for plasma PYY. There was an inverse relationship between hunger ($r = -0.49, P < 0.05$) and a direct relationship between nausea ($r = 0.55, P < 0.05$) scores with plasma CCK. There were no correlations between appetite perceptions with PYY concentrations.

**Relationships between appetite perceptions with energy intake.** There was a direct relationship between hunger scores with energy intake ($r = 0.51, P = 0.07$) and the amount eaten ($r = 0.51, P < 0.05$). There were no significant relationships
between fullness, nausea, or bloating scores with either energy intake or the amount eaten at the buffet meal.

**Relationships between APD motility with plasma CCK and PYY.** There were inverse relationships between the number ($r = 0.64$, $P < 0.05$) and amplitude ($r = 0.64$, $P < 0.05$) of antral PWs, the number ($r = 0.70$, $P < 0.001$) and amplitude ($r = 0.54$, $P < 0.001$) of duodenal PWs, and the number of APD PWSs ($r = 0.72$, $P < 0.001$), and direct relationships between the number ($r = 0.82$, $P < 0.001$) and amplitude ($r = 0.54$, $P < 0.001$) of IPPWs with the AUC for plasma CCK. There were inverse relationships between the number ($r = 0.61$, $P < 0.001$) and amplitude ($r = 0.59$, $P < 0.05$) of antral PWs, the number ($r = 0.68$, $P < 0.001$) of duodenal PWs and the number of APD PWSs ($r = 0.57$, $P < 0.001$), and direct relationships between the number ($r = 0.80$, $P < 0.01$) and amplitude ($r = 0.68$, $P < 0.05$) of IPPWs with the AUC for plasma PYY.

**Predictors of Energy Intake**

Multiple regression analysis of the combined data for CCK and PYY identified CCK concentrations at $t = 50$ min as an independent predictor of energy intake ($\beta = -128.148$, $P < 0.05$) but not the amount eaten. For the combined data of the number and amplitude of antral PWs and the number of IPPWs, multiple regression analysis identified the AUC for the number of IPPWs as an independent predictor of the amount eaten ($\beta = -0.467$, $P < 0.01$).

**DISCUSSION**

This study has demonstrated for the first time that duodenal lipid loads as low as 0.25 kcal/min are able to stimulate IPPWs and CCK release and suppress antral PWs, APD PWSs, and hunger in healthy subjects, while higher loads were required for the stimulation of basal pyloric pressure, PYY release, and suppression of duodenal pressures and energy intake. The...
effects of duodenal lipid on energy intake/amount of food eaten were related to the release of CCK and stimulation of IPPWs.

**Effect of Duodenal Lipid Load on APD Motility**

We evaluated the effects of intraduodenal lipid at loads that encompass rates below, similar to, and above the normal range of gastric emptying and demonstrated a load-dependent suppression of antral and duodenal motility and stimulation of pyloric motility. Duodenal lipid loads between 1 and 4 kcal/min are known to stimulate pyloric pressures and suppress antral and duodenal PWs (22, 23, 38). We have now demonstrated that lipid loads below the normal range of gastric emptying (0.25 kcal/min) also have the capacity to stimulate IPPWs and suppress antral PWs and APD PWSs. At higher loads (IL1.5 and IL4), hydrolysis takes longer to complete, resulting, as shown in previous studies (27, 34, 36), in the digestion process and lipolytic products being distributed further downstream where more chemosensors are contacted, inducing a greater inhibitory response on gastric emptying as reflected in the observed effects on APD motility with the larger lipid loads. This concept is supported by a study in dogs, which demonstrated that the magnitude of feedback inhibition of gastric emptying is dependent on the length of the gut exposed to lipid (26).

Our data also confirm the findings of a recent study from our laboratory (38) of regional differences between antrum, pylorus, and duodenum in relation to the load of lipid required to stimulate a response. In the present study, while the lowest load (0.25 kcal/min) was sufficient to suppress antral (IL0.25) and APD PWSs and temporarily stimulate IPPWs, higher loads (≥1.5 kcal/min) were required to stimulate basal pyloric pressure and suppress duodenal PWs, suggesting that the mechanisms that regulate duodenal pressures are less sensitive to lipid than those of the antrum and pylorus.

**Effect of Duodenal Lipid Load on Plasma CCK and PYY**

It has been established that there is a load-dependent stimulation of CCK (16, 38) and PYY (38) in response to increasing small intestinal lipid loads. In the present study, the lowest load (0.25 kcal/min) stimulated CCK release during the first 20 min of infusion but did not stimulate PYY. The CCK response to 0.25 kcal/min, however, was not sustained, presumably because the lipolytic products of this small load would have been rapidly digested and absorbed. This may also account for the lack of an increase in PYY during the 0.25 kcal/min infusion, as lipolytic products would not have reached the distal part of the small intestine to directly stimulate PYY secretion. While CCK is known to indirectly stimulate PYY, our data suggest that a certain amount/concentration of CCK is required, confirming previous findings of a lack of PYY release during perfusion of free fatty acids into the first 45 cm of canine small intestine (3). With the higher loads (1.5 kcal/min and 4 kcal/min) plasma PYY increased more slowly and progressively than CCK in that, while plasma CCK peaked within 20 min and then plateaued, plasma PYY continued to rise until the cessation of the infusion. These results are consistent with the release of CCK from I cells located in the proximal small intestine (9) and the release of PYY from L cells located in the distal small intestine (1). The substantial increase in CCK during IL1.5 and IL4, but not IL0.25, within 10 min of the infusion is, therefore, likely to have contributed to the initial rise in PYY. In contrast, toward the end of infusion, particularly with the highest load (4 kcal/min), lipid would have most likely reached the more distal parts of the small intestine (although it should be recognized that this was not measured in our study), stimulating a greater release of PYY by direct nutrient contact with PYY releasing cells (1).

**Effect of Duodenal Lipid Load on Energy Intake**

This is the first study to assess the effect of very low and relatively high loads of lipid on energy intake. A similar study was conducted in which 20% Intralipid was infused at a constant rate (2 kcal/min) over different durations, and a reduction in energy intake was observed after 45-min (90 kcal) and 90-min (180 kcal), but not 15-min (30 kcal), infusions (12). In the present study, only IL4 (200 kcal) reduced energy intake. Taken together these observations indicate that loads >1.5 kcal/min for 50 min (75 kcal) but <2 kcal/min for 45 min (90 kcal) (12) represent the minimum required to reduce energy intake, raising the question as to whether load, rate, and/or duration of small intestinal lipid is responsible for the reduction in energy intake. We have reported (38) that duodenal infusion of 10% Intralipid at 1.33 kcal/min for 150 min, which also yields 200 kcal, is insufficient to reduce energy intake in healthy subjects. During this slower infusion, lipolysis and absorption may well have been completed over a shorter length of small intestine, which may account for the absence of any effect of loads at 0.25 kcal/min and 1.5 kcal/min on energy intake in the present study. As the load of 1.5 kcal/min is considered similar to average gastric emptying, the data may also suggest that if the duration of infusion was longer, plasma PYY may have continued to rise (to a specific threshold) to have an effect on energy intake. It is important to recognize that unlike duodenal infusions, gastric emptying of fat is not a steady process but rather is usually more rapid early after a meal, slowing later, and presumably predominantly pulsatile rather than continuous (32), a pattern that is likely to result in a different distribution of free fatty acids along the gut.

**Relations Between Plasma CCK and PYY and APD Motility With Energy Intake**

It is well documented that gastrointestinal hormones, particularly CCK and PYY, mediate, at least in part, the modulation of gastrointestinal motility by lipid (8, 21, 24, 40), and the present study has demonstrated positive correlations between pyloric pressures and negative correlations between antral and duodenal PWs and APD PWSs with CCK and PYY. Interestingly, only concentrations of CCK and PYY, but not APD motility, were significantly correlated with energy intake. However, there were correlations between the amount of food eaten at the buffet meal with motility, suggesting that different mechanisms may be responsible for the regulation of food intake vs. energy intake. Previous studies have suggested that the stimulation of the pylorus may have a critical involvement in the suppression of energy intake (8, 45); however, these studies did not directly assess the effects of nutrient-induced stimulation of pyloric pressures. Intravenous infusions of CCK and PYY have been shown to decrease hunger perceptions and suppress energy intake in humans (4, 8), although it is important to recognize that the effects of exogenous PYY relate...
specifically to PYY(3-36) (4). Nevertheless, in the present study the substantial increase in plasma CCK and PYY, as well as pyloric pressures, which were all significantly greater during IL4 infusion compared with the other lipid infusions, may have contributed to the decrease in energy intake in that the sum of the various parameters may have reached a critical threshold to result in the decrease in energy intake. There was a slight increase in nausea during IL4 before the buffet meal; however, mean scores were very low, and no subject actually reported nausea. Moreover, there was no correlation between nausea scores with energy intake.

Perspectives and Significance

This study establishes that intraduodenal lipid at loads that encompass rates below, similar to, and above normal gastric emptying, suppress antral and duodenal PWs, APD PWSs, appetite, and energy intake, and stimulate basal pyloric pressure, IPPWs, and plasma CCK and PYY, in a load-dependent manner. While loads as low as 0.25 kcal/min modulate antral and pyloric pressures and transiently increase plasma CCK, higher loads (1.5 and 4 kcal/min) have greater effects and are required for stimulation of PYY, and only the highest load suppresses energy intake. Presumably, at the higher loads, lipid was distributed further along the small intestine, stimulating more chemosensors and thus providing greater feedback inhibition/stimulation.

Our observations may have implications for our understanding of conditions associated with altered gastrointestinal function in response to consumption of high-caloric diets (7, 11) such as in obesity. The few studies that have compared gastric motility and hormone release between obese and healthy lean subjects have produced inconsistent observations (31, 43). Individuals habitually consuming high-caloric diets may well be less sensitive to nutrient stimuli, and therefore, it is reasonable to assume that higher loads may be required to induce similar responses to those demonstrated in the present study. It would, accordingly, be of interest to compare the thresholds required for the stimulation or suppression of gastric motility, hormone release, appetite, and energy intake in lean and obese subjects.

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