Effects of intraduodenal fatty acids on appetite, antropyloroduodenal motility, and plasma CCK and GLP-1 in humans vary with their chain length

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Feltrin, Kate L., Tanya J. Little, James H. Meyer, Michael Horowitz, Andre J. P. M. Smout, Judith Wishart, Amelia N. Pilichiewicz, Thomas Rades, Ian M. Chapman, and Christine Feinle-Bisset. Effects of intraduodenal fatty acids on appetite, antropyloroduodenal motility, and plasma CCK and GLP-1 in humans vary with their chain length. Am J Physiol Regul Integr Comp Physiol 287: R524–R533, 2004. First published May 27, 2004; 10.1152/ajpregu.00039.2004.—The gastrointestinal effects of intraluminal fats may be critically dependent on the chain length of fatty acids released during lipolysis. We postulated that intraduodenal administration of lauric acid (12 carbon atoms; C12) would suppress appetite, modulate antropyloroduodenal pressure waves (PWs), and stimulate the release of cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1) more than an identical dose of decanoic acid (10 carbon atoms; C10). Eight healthy males (19–47 yr old) were studied on three occasions in a double-blind, randomized fashion. Appetite perceptions, antropyloroduodenal PWs, and plasma CCK and GLP-1 concentrations were measured during a 90-min intraduodenal infusion of 1) C12, 2) C10, or 3) control (rate: 2 ml/min, 0.375 kcal/min for C12/C10). Energy intake at a buffet meal, immediately after completion of the infusion, was also quantified. C12, but not C10, suppressed appetite perceptions (P < 0.001) and energy intake (control: 4.604 ± 464 kJ, C10: 4.109 ± 588 kJ, and C12: 1.747 ± 632 kJ; P < 0.001, C12 vs. control/C10). C12, but not C10, also induced nausea (P < 0.0001). C12 stimulated basal pyloric pressures and isolated pyloric PWs and suppressed antral and duodenal PWs compared with control (P < 0.05 for all). C10 transiently stimulated isolated pyloric PWs (P < 0.001) and had no effect on antral PWs but markedly stimulated duodenal PWs (P = 0.004). C12 and C10 increased plasma CCK (P < 0.001), but the effect of C12 was substantially greater (P = 0.001); C12 stimulated GLP-1 (P < 0.05), whereas C10 did not. In conclusion, there are major differences in the effects of intraduodenal C12 and C10, administered at 0.375 kcal/min, on appetite, energy intake, antropyloroduodenal PWs, and gut hormone release in humans.

A comparison of oleic acid (C18), but not C8, reduced energy intake (25). In humans, it has been reported that oleic acid (C18), but not C8, reduces energy intake (41); however, the effects of C12 and C10 have not been reported. In humans, it has been reported that oleic acid (C18), but not C8, reduces energy intake (41); however, the effects of C12 and C10 have not been reported. In humans, fatty acids with ≥12 carbon atoms are transported from the gut predominantly in lymphatic chylomicrons, a transport process that triggers a variety of gut signals including satiety (45) and the slowing of gastric emptying (54). In rats, intraluminally perfused lauric acid (C12) inhibits energy intake, whereas isoenergetic amounts of decanoic (C10) or octanoic (C8) acid do not (46). In humans, it has been reported that oleic acid (C18), but not C8, reduces energy intake (41); however, the effects of C12 and C10 have not been compared. In humans, fatty acids with ≥12 carbon atoms also empty from the stomach more slowly than fatty acids with <12 carbon atoms (27), and intragastric infusion of C12 relaxes the fundus and reduces the amplitude of antral contractions more than C10 (44). Gastrointestinal peptides, including CCK and GLP-1, mediate, at least in part, the effects of fat on energy intake (4, 15, 21, 39) and gastrointestinal motility (2, 16, 18, 57); hence, the effects of fatty acid chain length on gastrointestinal hormone secretion are clearly of interest. A study in humans suggests that CCK is stimulated by intraduodenal infusion of C12 but not C10 (44). However, the control solution in that study (containing the nonionic surfactant Tween 80 to solubilize C10 and C12) also raised plasma CCK concentrations (44), confounding interpretation of the data. Thus it is still uncertain whether C10, if infused alone as a sodium salt, might release smaller, but detectable, amounts of CCK. There is no information as to how fatty acid chain length may affect the release of GLP-1.

The aims of this study were to evaluate in humans the hypotheses that 1) intraduodenally administered C12 and C10 have different effects on appetite-related sensations and energy intake; 2) C12, but not C10, releases CCK (44) and GLP-1; and

NUTRIENTS CONTROL APPETITE AND ENERGY INTAKE through two principal mechanisms arising from the stomach and small intestine: 1) inhibition of gastric emptying, which activates stretch receptors through an increase in gastric distension (1, 3); and 2) triggering of neural (29, 53) and humoral (21, 36, 42) signals as a result of the interaction of nutrients with the small intestine (38, 42). Although gastric and intestinal signals in-
3) effects on pressure patterns in the antrum, pylorus, and duodenum differ between C12 and C10.

MATERIALS AND METHODS

The Royal Adelaide Hospital Research Ethics Committee approved the study protocol, and all subjects provided written, informed consent before their inclusion into the study.

Subjects

A total of 12 subjects was studied: 4 subjects in a nonrandomized pilot study and 8 subjects in the randomized study using the protocol described below. This subject number was based on “crude” power calculations derived from our previous studies (13, 14). The healthy, male volunteers had a mean age of 24 ± 4 yr (range 19–47 yr) and were of normal body weight (body mass index = 22.0 ± 1.6 kg/m²). All subjects were unrestrained eaters [scoring <12 on the eating restraint part (factor 1) of the Three-Factor Eating questionnaire (58)], had no gastrointestinal disease or symptoms, and were not taking any medication known to affect gastrointestinal motility or appetite. No subject smoked or habitually consumed >20 g alcohol/day.

Preparation and Doses of Fatty Acids

Fatty acid solutions were prepared using 5.3 g of commercially available food grade saturated free fatty acids, lauric (C12:0) and decanoic (C10:0) acid (Sigma-Aldrich; Milwaukee, WI). C12 was dissolved in 0.89 g NaOH (Sigma-Aldrich; St. Louis, MO) and distilled water to a total volume of 250 ml. C12 was maintained in solution by heating it to 37°C, with a resulting pH of 8.2. C10 was also dissolved in NaOH (1.21 g), and the pH of the C10 and control solution by heating it to 37°C, with a resulting pH of 8.2. C10 was also dissolved in NaOH (1.21 g), and the pH of the C10 and control solution by heating it to 37°C, with a resulting pH of 8.2. C10 was also dissolved in NaOH (1.21 g), and the pH of the C10 and control

Protocol

In a pilot study, we started with an infusion rate of 1.5 kcal/min, comparable to a well-tolerated dose of intraduodenal triglycerides used in previous studies by ourselves (6) and by others (17, 42). However, during the C12 infusion, the first subject tested reported severe nausea and only tolerated the infusion for 30 min. A second subject, who again reported severe nausea, tolerated the infusion for 60 min. Rates of 1 kcal/min (n = 1) and, subsequently, 0.75 kcal/min (n = 1) of C12 were also only tolerated for some 60 min due to severe nausea and vomiting. After these initial observations, we chose a dose of 0.375 kcal/min because C12 had been reported to empty from the human stomach at this rate (27) and because C12 had been perfused into human duodenum at 0.36 kcal/min without adverse effects (40).

Each subject was studied on three occasions, separated by 3–10 days, to evaluate, in a double-blind, randomized fashion, the effects of intraduodenal infusions of 1) C12, 2) C10, or 3) control solution for 90 min on appetite perceptions, energy intake, APD pressures, and plasma CCK and GLP-1 concentrations.

Subjects attended the laboratory at 0830 after fasting from 2200 the previous night from both solids and liquids. They were intubated with a 17-channel manometric catheter (Dentsleeve; Adelaide, South Australia, Australia) that was inserted through an anesthetized nostril and allowed to pass through the stomach and into the duodenum by peristalsis (7). The manometric catheter, which consisted of 16 side holes spaced at 1.5-cm intervals, measures pressures in the antrum, pylorus, and duodenum. Six side holes (channels 1–6) were positioned in the antrum, a 4.5-cm sleeve sensor (channel 7), with two channels present on the back of the sleeve (channels 8 and 9), designed to measure pressure waves occurring over the entire pyloric region, were positioned across the pylorus, and seven channels were positioned in the duodenum (channels 10–16). An additional channel, used for intraduodenal infusions, was positioned 11.75 cm distal to the end of the sleeve sensor. The correct positioning of the catheter, so the sleeve sensor straddled the pylorus, was maintained by a continuous measurement of the transmucosal potential difference (TMPD) between the most distal antral channel (channel 6; approximately −40 mV) and the most proximal duodenal channel (channel 10; −0.0 mV) (22). For this purpose, an intravenous cannula filled with sterile saline was placed subcutaneously in the left forearm and used as a reference electrode (7). All manometric channels were perfused with degassed, distilled water except for the two TMPD channels, which were perfused with degassed 0.9% saline at 0.15 ml/min (7). An intravenous cannula was also placed into a right forearm vein for blood sampling to measure plasma CCK and GLP-1 concentrations.

Once the catheter was positioned correctly, fasting motility was monitored until the occurrence of phase III of the interdigestive migrating motor complex (MMC) (7). Immediately after the cessation of MMC activity [at time 0, phase III activity (i.e., the most proximal duodenal channel (channel 10; −0.0 mV) (22). For this purpose, an intravenous cannula filled with sterile saline was placed subcutaneously in the left forearm and used as a reference electrode (7). All manometric channels were perfused with degassed, distilled water except for the two TMPD channels, which were perfused with degassed 0.9% saline at 0.15 ml/min (7). An intravenous cannula was also placed into a right forearm vein for blood sampling to measure plasma CCK and GLP-1 concentrations.

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Measurements

Appetite sensations and energy intake. Subjective sensations of appetite, including hunger, fullness, desire to eat, prospective consumption (“how much food do you think you could eat if you were given a meal now?”), were assessed using a validated VAS as described (51). Sensations of nausea and bloating were also assessed. Each VAS evaluated a sensation on a 100-mm horizontal line, where 0 represented “sensation is not felt at all” and 100 represented “sensation is felt the greatest.” Subjects were asked to place a vertical stroke on the 100-mm line in relation to what they were feeling at that particular point in time.

Energy intake from the buffet meal [energy consumption (in kJ) and macronutrient distribution (in % and g energy)] was analyzed using commercially available software (Food works 3.0, Xyris Software; Highgate Hill, Queensland, Australia) (38). The time taken to finish the meal (in min) was also evaluated.

APD pressures. Manometric pressures were digitized, recorded on a computer-based system (PowerMac 7100/75, Apple Computers; Cupertino, CA) running commercially available software (HAD, Associate Prof. GS Hebbard, 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 isolated pyloric pressure waves (IPPWs) and pressure waves (PWs) in the antrum and duodenum and 2) number and length of PW sequences...
Table 1. Composition of the buffet meal

<table>
<thead>
<tr>
<th>Food Items</th>
<th>Amount Served, g</th>
<th>Energy content, kJ</th>
<th>Fat, g</th>
<th>Carbohydrate, g</th>
<th>Protein, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wholemeal bread, 4 slices</td>
<td>125</td>
<td>1,304</td>
<td>3.6</td>
<td>50.0</td>
<td>12.6</td>
</tr>
<tr>
<td>White bread, 4 slices</td>
<td>125</td>
<td>1,295</td>
<td>2.9</td>
<td>56.4</td>
<td>11.8</td>
</tr>
<tr>
<td>Ham, sliced</td>
<td>100</td>
<td>453</td>
<td>3.6</td>
<td>0</td>
<td>18.8</td>
</tr>
<tr>
<td>Chicken, sliced</td>
<td>100</td>
<td>677</td>
<td>7.0</td>
<td>0</td>
<td>24.6</td>
</tr>
<tr>
<td>Cheese, sliced</td>
<td>85</td>
<td>1,436</td>
<td>28.3</td>
<td>0.9</td>
<td>21.9</td>
</tr>
<tr>
<td>Tomato, sliced</td>
<td>100</td>
<td>56</td>
<td>0.1</td>
<td>1.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Lettuce</td>
<td>100</td>
<td>27</td>
<td>0</td>
<td>0.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Cucumber, sliced</td>
<td>100</td>
<td>44</td>
<td>0.1</td>
<td>1.9</td>
<td>0.5</td>
</tr>
<tr>
<td>Strawberry yogurt</td>
<td>200</td>
<td>966</td>
<td>6.2</td>
<td>33.8</td>
<td>9.4</td>
</tr>
<tr>
<td>Fruit salad</td>
<td>140</td>
<td>345</td>
<td>0.1</td>
<td>19.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Chocolate custard</td>
<td>150</td>
<td>662</td>
<td>5.3</td>
<td>22.7</td>
<td>4.8</td>
</tr>
<tr>
<td>Apple</td>
<td>170</td>
<td>359</td>
<td>0.2</td>
<td>21.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Banana</td>
<td>190</td>
<td>680</td>
<td>0.2</td>
<td>37.8</td>
<td>3.2</td>
</tr>
<tr>
<td>Orange juice, unsweetened</td>
<td>500</td>
<td>800</td>
<td>5.0</td>
<td>42.5</td>
<td>5.0</td>
</tr>
<tr>
<td>Iced coffee</td>
<td>600</td>
<td>1,788</td>
<td>10.2</td>
<td>61.8</td>
<td>21.0</td>
</tr>
<tr>
<td>Water</td>
<td>600</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Margarine</td>
<td>20</td>
<td>609</td>
<td>16.4</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Mayonnaise</td>
<td>20</td>
<td>310</td>
<td>6.5</td>
<td>4.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Total</td>
<td>3,425</td>
<td>11,808</td>
<td>95.7</td>
<td>354.6</td>
<td>136.9</td>
</tr>
</tbody>
</table>

*Sunblest, Tiptop, Australia; aDeli leg ham, Woolworths, Australia; aVirginian chicken, Woolworths, Australia; aCoon Tasty Cheese slices, Australian Cooperative Foods Ltd., Australia; aYoplait, National Foods Ltd., Australia; aGoulburn Valley, SPC, Ardmona Operations Ltd., Australia; aYogo, National Foods Ltd., Australia; aDaily Juice Company, Australia; aFarmers Union, Balemar Pty. Ltd., Australia; aFlora, Unilever Australasia, Australia; aKraft, Kraft Foods Ltd., Australia.

(PWSs) in the duodenum using custom-written software (Gastrointes-
tinal Motility Unit; Utrecht, The Netherlands) (56) modified to our requirements. Basal pyloric pressure (“tone”) was also 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 (23) using custom-written soft-
ware (MAD, Prof. Charles Malbert, Institut National de la Recherche Agronomique, Rennes, France). Phasic PWSs in the antrum and IPPWs were defined by an amplitude ≥10 mmHg, with a minimum interval of 15 s between peaks. Phasic duodenal PWSs were defined by an amplitude ≥10 mmHg, with a minimum interval of 3 s between peaks. Duodenal pressures were regarded as related between channels if their velocities between adjacent side holes were between 9 and 160 mm/s and thus defined as duodenal PWSs (56). Duodenal PWSs were characterized according to the distances travelled, i.e., over at least two (1.5 to <3 cm), three (3 to <4.5 cm), four (4.5 to <6 cm), five (6 to <7.5 cm), six (7.5 to <9 cm), and seven (9 to <10.5 cm) channels and expressed as total number of waves. Antral PWSs were not analyzed because these were infrequent.

Plasma CCK and GLP-1 Concentrations

Venous blood samples (10 ml) were collected in ice-chilled EDTA-
treated tubes containing 400 kIU aprotinin (Trasylol, Bayer Australia) per milliliter of blood. Plasma was separated by centrifugation (3,200 rpm, 15 min, 4°C) within 30 min of collection and stored at −70°C until assayed.

Plasma CCK concentrations (in pmol/l) were determined after ethanol extraction using a previously described radioimmunoassay (39). A commercially available antibody (C258, Lot 105H4852, Sigma Chemical) 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 and <2% cross-reactivity with human gastrin, and does not bind to structurally unrelated peptides. The intra-assay coefficient of variation (CV) was 9% and the interassay CV was 2.5 pmol/l. The sensitiv-
ity of the assay relates to its reliable detection limit.

Plasma GLP-1 concentrations (in pmol/l) were measured by radio-
immunoassay using an adaptation (60) of a previously published method (50). Antibody, supplied by Prof. S. R. Bloom (Hammersmith Hospital, London, UK), did not cross-react with glucagon, gastric inhibitory peptide, or other gut or pancreatic peptides and has been demonstrated by chromatography to measure intact GLP-1(7–30) amide. It is likely that this antibody also reacts with the degraded form of GLP-1(9–36) amide. Intra-assay CV was 17% and interassay CV was 18%, with a sensitivity of 1.5 pmol/l.

Statistical Analysis

Baseline (“0”) values were calculated as the mean of values obtained at t = −15 and 0 min for VAS scores and plasma hormone concentrations and between t = −15 to 0 min for basal pyloric pressures, number, and amplitude of IPPWs, antral and duodenal PWSs, and total number of duodenal PWSs. Basal pyloric pressures and the number and amplitude of IPPWs were expressed as means over 15-min segments during the 90-min infusion period. Numbers and amplitudes of antral and duodenal PWSs were expressed as total number and mean values, respectively, for the 90-min infusion period. Duodenal PWSs were expressed as the total number of waves travelling over two, three, four, five, six, and seven channels during the 90-min infusion period. All data, except for plasma CCK and GLP-1 concentrations, were expressed as changes from baseline.

VAS scores, basal pyloric pressures, number and amplitude of IPPWs, and plasma hormone concentrations were analyzed by repeated-measures mixed model analysis of covariance (ANCOVA), with baseline as the covariate and time (0–90 min, at 15-min inter-
vals) and treatment as within-subject factors. One-way ANOVA was used to assess the effect of treatment on energy intake, time taken to complete eating, total numbers and mean amplitudes of antral and duodenal PWSs, and total number of duodenal PWSs. A logarithmic transformation was applied to nausea scores, antral amplitudes, and duodenal PWSs travelling 7.5 and 9 cm to better address the under-
lying assumptions of the models used (as these variables lacked normal distribution). If signi-
cance or near signi-
fi

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associations were assessed between 1) the desire to eat and 2) nausea with plasma CCK, plasma GLP-1, the sum of plasma CCK and GLP-1 (calculated by adding plasma CCK and GLP-1 concentrations at each time point), basal pyloric pressure, and number and amplitude of IPPWs, by calculating correlation coefficients adjusted for repeated measures (5). Statistical analysis was performed using SAS (SAS Institute; Cary, NC). Statistical significance was accepted at \( P < 0.05 \), and data are presented as means \( \pm \) SE.

RESULTS

The subjects, with the exception of one, tolerated all experimental conditions well. The subject, who was able to tolerate the C12 infusion for only 30 min due to severe nausea (and was dismissed at that time), completed the two other infusions successfully. All available data were included in the analyses.

APPETITE PERCEPTIONS (VAS SCORES)

There was a significant effect of treatment on scores for the desire to eat (Fig. 1A), hunger (Fig. 1B), and prospective consumption (data not shown) \( (P < 0.001 \) for all). During the infusion of C12, scores for desire to eat, hunger, and prospective consumption were lower compared with control \( (P < 0.001 \) for all) and C10 \( (P < 0.001 \) for all), whereas no differences were found between C10 and control [not significant (NS)]. There was a small, but significant, increase in fullness (Fig. 1C) over the infusion period with all three treatments (time effect: \( P = 0.034 \)), without any differences between them (treatment effect: NS).

There was a significant effect of treatment on nausea (Fig. 1D; \( P < 0.001 \)). Infusion of C12 increased nausea scores compared with control \( (P < 0.001 \) and C10 \( (P < 0.001 \). Five of the eight subjects reported nausea, whereas the other three subjects did not experience nausea. There was no difference in nausea scores between C10 and control (NS). There was a significant effect of treatment on bloating \( (P < 0.001 \); data not shown). Infusion of C12 increased the scores for bloating compared with both C10 \( (P < 0.001 \) and control \( (P < 0.001 \). C10 also increased scores for bloating compared with control \( (P = 0.013 \).

After ingestion of the meal, scores for the desire to eat (Fig. 1A), hunger (Fig. 1B), and prospective consumption (data not shown) decreased significantly (time effects: \( P < 0.05 \), but there were no differences between treatments at 120 or 150 min (treatment effects: NS). Scores for fullness increased after meal ingestion (Fig. 1C; time effect: \( P = 0.001 \) ), and at 120 and 150 min fullness was less after C12 than C10 \( (P = 0.042 \) ) or control \( (P = 0.012 \) ), with no difference between C10 and control (NS; treatment effect: \( P = 0.030 \)).

Scores for both nausea (Fig. 1D) and bloating (data not shown) diminished rapidly in the nauseated subjects after termination of the C12 infusion at 90 min; by 120 min, the mean score for nausea was close to baseline, and there were no significant differences between treatments at 120 and 150 min (NS).

ENERGY INTAKE

There was a significant effect of treatment on energy intake \( (P = 0.001 \); Fig. 2). Infusion of C12 dramatically decreased energy intake compared with control \( (P < 0.001 \) and C10 \( (P < 0.001 \), whereas there was no difference between C10 and control infusion (NS). The decrease in energy intake after the C12 infusion was evident in all subjects; however, the magnitude of the reduction was greater in those subjects who reported nausea (\( \sim 3,840 \) kJ) compared with those that did not experience nausea (\( \sim 1,801 \) kJ) (because of the small subject...
Energy content, kJ 4,604

Table 2. Energy intake from the buffet meal and macronutrient distribution in response to 90-min intraduodenal infusions of decanoic acid, lauric acid, and control

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>C10</th>
<th>C12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy content, kJ</td>
<td>4,604 ±464</td>
<td>4,109 ±589</td>
<td>1,747 ±633*</td>
</tr>
<tr>
<td>Fat</td>
<td>39.1 ±4.4</td>
<td>35.8 ±6.1</td>
<td>13.1 ±4.7*</td>
</tr>
<tr>
<td>%</td>
<td>33.1 ±1.7</td>
<td>34.0 ±3.0</td>
<td>29.2 ±4.5</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>134.0 ±13.6</td>
<td>121.5 ±16.5</td>
<td>60.1 ±17.7*</td>
</tr>
<tr>
<td>g</td>
<td>49.4 ±1.6</td>
<td>50.3 ±4.5</td>
<td>42.3 ±11.1</td>
</tr>
<tr>
<td>%</td>
<td>34.4 ±1.6</td>
<td>34.0 ±3.0</td>
<td>29.2 ±4.5</td>
</tr>
<tr>
<td>Protein</td>
<td>54.2 ±5.8</td>
<td>47.8 ±7.5</td>
<td>19.1 ±8.4*</td>
</tr>
<tr>
<td>g</td>
<td>63.4 ±7.6</td>
<td>53.0 ±9.8</td>
<td>18.3 ±8.4</td>
</tr>
<tr>
<td>%</td>
<td>31.8 ±7.6</td>
<td>31.0 ±9.8</td>
<td>18.3 ±8.4</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 8. C10, decanoic acid; C12, lauric acid. *P < 0.001, C12 vs. C10/control.

Fig. 2. Energy intake (in kJ) at a buffet meal in response to 90-min duodenal infusions of C12, C10, and control. C12 dramatically reduced energy intake, whereas there was no difference between C10 and control. Treatment effect, P = 0.001; *C12 vs. control/C10, P < 0.001; C10 vs. control, NS. Data are means ± SE; n = 8.

numbers in each “subgroup,” these data were not subjected to formal statistical analysis.

There was also a significant effect of treatment on the macronutrient distribution of the food consumed (Table 2; P < 0.001 for all). The infusion of C12 reduced the amount of energy (in g) ingested from fat, carbohydrate, and protein compared with control (fat: P < 0.001; carbohydrate: P < 0.001; protein: P < 0.001) and C10 infusion (fat: P = 0.001; carbohydrate: P = 0.002; protein: P = 0.003), whereas there was no difference between C10 and control.

The time at which eating from the buffet meal was completed tended to be less after C12 infusion (13 ± 3.5 min) compared with C10 (20.6 ± 2.6 min) or control (21.8 ± 1.8 min); however, this did not reach statistical significance (NS).

Antropyloroduodenal Pressures

Visual inspection of the pressure traces suggested that intraduodenal infusion of C12 resulted in a typical “fed” motor pattern in which antral and duodenal PWs were inhibited and pyloric pressures stimulated. A duodenal phase III episode occurred in two subjects: in one subject during the control and in the other subject during the C10 infusion. There were no evident differences in motor patterns between nauseated and non-nauseated subjects during the C12 infusion.

Antral pressures. There was a significant effect of treatment on the number of antral PWs (P = 0.004; Fig. 3A). The infusion of C12 decreased the number of antral PWs compared with control (P = 0.002) and C10 (P = 0.003), whereas there was no difference between C10 and control (NS). There was a trend for the amplitude of antral pressures to differ between study conditions (treatment effect: P = 0.080), and the amplitude tended to be lower during the C12 compared with both the C10 and control infusions (Fig. 3B).

Fig. 3. Number (A) and amplitude (B) of antral pressure waves (PWs) during 90-min duodenal infusions of C12, C10, and control. A: C12 decreased the number of antral PWs compared with C10 and control. Treatment effect, P = 0.004; *C12 vs. control/C10. P < 0.003; C10 vs. control, NS; B: there was a trend for the amplitude of antral PWs to be less during C12 than both control and C10. Treatment effect, P = 0.080. Data are means ± SE; n = 8.

with control (P = 0.002) and C10 (P = 0.003), whereas there was no difference between C10 and control (NS). There was a trend for the amplitude of antral pressures to differ between study conditions (treatment effect: P = 0.080), and the amplitude tended to be lower during the C12 compared with both the C10 and control infusions (Fig. 3B).

Pyloric pressures. PHASIC PRESSURES. A treatment × time interaction approached statistical significance for the number of IPPWs (P = 0.062; Fig. 4A). The infusion of C12 increased the number of IPPWs between 15–30 and 30–45 min (P = 0.046, P = 0.015, respectively) compared with C10 and among 0–15, 15–30, and 30–45 min (P < 0.001, P = 0.019, and P = 0.02, respectively) compared with control. C10 increased the number of IPPWs compared with control between 0 and 15 min (P = 0.001).

There was a significant treatment × time interaction for the amplitude of IPPWs (P = 0.008; Fig. 4B). The infusion of C12 increased the amplitude of IPPWs compared with control between 0–15 and 15–30 min (P = 0.015 and P = 0.011). C12 decreased the amplitude of IPPWs between 75 and 90 min compared with C10 (P = 0.025) and between 60 and 75 min compared with control (P = 0.010). There were no significant differences between C10 and control throughout the infusion period (NS).

BASAL PRESSURE (“TONE”). There was a significant effect of treatment on basal pyloric pressure (P < 0.001; Fig. 4C). The infusion of C12 increased basal pyloric pressure compared with both C10 (P = 0.002) and control (P < 0.001). There was also a significant difference between C10 and control (P = 0.029).

Duodenal pressures. PRESSURE WAVES. There was a significant effect of treatment on the number of duodenal PWs (P < 0.001; Fig. 5A). The infusion of C12 decreased the number of duodenal PWs compared with both control (P = 0.002) and C10 (P < 0.001). In contrast, C10 increased the number of
duodenal PWs compared with control ($P = 0.004$). There were no differences in the amplitude of duodenal PWs between treatments (NS; Fig. 5B).

Pressure wave sequences. There was a significant effect of treatment on the number of duodenal PWs travelling over two (i.e., 1.5 to <3 cm; $P < 0.001$), three (i.e., 3 to <4.5 cm; $P = 0.023$), six (i.e., 7.5 to <9 cm; $P = 0.007$), and seven (i.e., 9 to <10.5 cm; $P = 0.021$) channels (Fig. 6). The infusion of C12 decreased the number of waves that travelled over two channels compared with control ($P = 0.017$) and C10 ($P < 0.001$), whereas C10 increased the number of waves compared with control ($P = 0.002$). The infusion of C12 decreased the number of waves that travelled over three channels compared with C10 ($P = 0.007$), whereas there was no difference between C12 or C10 and control (NS). Both C12 ($P = 0.002$) and C10 ($P = 0.043$) decreased the number of waves that travelled over six channels compared with control. There was no difference between the C12 and C10 infusions (NS). C12 infusion also decreased the number of waves that travelled over nine channels compared with both control ($P = 0.021$) and
C10 ($P = 0.010$). There was no difference between C10 and control (NS).

**Plasma CCK and GLP-1 Concentrations**

Baseline plasma CCK concentrations did not differ among study days. There was a significant effect of treatment on plasma CCK concentrations ($P < 0.001$; Fig. 7A). The infusion of C12 increased plasma CCK compared with control ($P < 0.001$) and C10 ($P < 0.001$), where C12 produced a rapid rise in plasma CCK within 15 min, which then plateaued. However, C10 also increased plasma CCK concentrations, with a peak at 15 min, compared with control ($P < 0.001$).

Baseline plasma GLP-1 concentrations did not differ among study days. There was a significant effect of time interaction for plasma GLP-1 concentrations ($P < 0.001$; Fig. 7B). The infusion of C12 increased plasma GLP-1 from 45 to 90 min compared with C10 ($P < 0.001$) and from 30 to 90 min compared with control ($P < 0.05$). There was no difference between C10 and control (NS).

During infusion of C12, the increases in plasma concentrations of CCK and GLP-1 were of similar magnitudes in nauseated and non-nauseated subjects.

After meal ingestion, plasma CCK concentrations increased during the control and C10 infusions, and at $t = 120$ and 150 min there were no longer any differences between treatments (NS). In contrast, there was still a significant effect of treatment on plasma GLP-1 concentrations ($P = 0.013$), which were higher after the C12 infusion compared with both control ($P = 0.007$) and C10 ($P = 0.012$), whereas there was no difference between C10 and control (NS).

**Relationships Between the Desire to Eat and Nausea with Plasma Hormone Concentrations and Pyloric Pressures**

Negative correlations were found between scores for the desire to eat and plasma CCK ($r = -0.45$, $P < 0.001$), plasma GLP-1 ($r = -0.38$, $P < 0.001$), and the sum of CCK and GLP-1 ($r = -0.45$, $P < 0.001$). There was also a negative correlation between the desire to eat and basal pyloric pressure ($r = -0.26$, $P = 0.003$). No significant correlations were found between the desire to eat and either the number or amplitude of IPPWs. There was a strong correlation between the score for the desire to eat at 90 min and subsequent energy intake ($r = 0.83$, $P < 0.001$).

There were correlations between scores for nausea and plasma CCK ($r = 0.52$, $P < 0.001$), plasma GLP-1 ($r = 0.45$, $P < 0.001$), and the sum of CCK and GLP-1 ($r = 0.52$, $P < 0.001$). Correlations were also evident between nausea and the number of IPPWs ($r = 0.32$, $P < 0.001$) and basal pyloric pressure ($r = 0.40$, $P < 0.001$). A negative correlation was observed between the score for nausea at 90 min and subsequent energy intake ($r = -0.65$, $P = 0.007$).

**DISCUSSION**

This study established that there are major differences in the effects of C12 and C10 fatty acids infused into the duodenum at a dose of 0.375 kcal/min on appetite, APD motility, and gastrointestinal hormones in healthy males. Compared with an isocaloric dose of C10 and control, C12 reduced appetite and subsequent energy intake at a buffet meal, increased basal pyloric pressure and IPPWs, decreased antral and duodenal pressure waves, shortened the length of propagation of duodenal PWs, and increased plasma CCK and GLP-1 concentrations. Whereas C10 stimulated CCK release, albeit less than C12, no effect on GLP-1 was evident.

A striking observation was that the small amount of C12 (total amount of energy delivered: ~141 kJ) markedly attenuated appetite-related sensations and subsequent energy intake: C12 reduced energy intake by ~2,857 kJ compared with the control infusion. In a previous study in humans (41), intraduodenal infusion of C18:1 for 90 min (providing ~288 kJ) reduced energy intake by ~1,580 kJ. Accordingly, intraduodenal infusion of C12 may be a more potent suppressant of energy intake in humans than isenergetic amounts of C18:1, as is the case in rats (46). However, the unexpected occurrence of nausea in our study (discussed below) during the infusion of C12 may affect this interpretation. In our previous studies in which triglyceride emulsions were infused directly into the duodenum at a rate of ~2.8 kcal/min [corresponding to observed rates of gastric emptying (8)], the gastrointestinal responses (including stimulation of pyloric motility and plasma CCK) were maximal within ~30–45 min of the start of the infusions (13, 22, 23). However, in the present study, similar effects were evident during C12 infusion at a much lower dose (0.375 kcal/min) with an onset at ~15 min (i.e., 15–30 min earlier than that observed during previous triglyceride infusions, suggesting that only very small amounts of fatty acid are required to bring about gastrointestinal effects). It is possible that the gastrointestinal tract is more sensitive to the action of...
lauric acid, as opposed to longer-chain fatty acids. Although lauric acid is contained in a variety of foods, including coconut milk (up to ~50%), butter (~5–8%) and beef, it, at least in most cases, does not represent a major component of our total energy intake: on the basis of compositions of stored bodily fatty acids, it is estimated that C12 represents ≥6% of dietary fatty acids. Breast milk, which contains up to 20% of lauric acid, is only part of the human diet in early life.

Previous studies have determined that fatty acids with ≥12 carbon atoms slow gastric emptying (27), induce proximal gastric relaxation, and reduce antral PWs (44) to a greater extent than fatty acids with <12 carbon atoms, but this is the first study to evaluate effects on pyloric and duodenal pressures. The intraduodenal infusion of C12 caused a substantial increase in basal pyloric pressure and the number and amplitude of IPPWs and decreased the number of antral and duodenal PWs as well as the number of PWSs that travelled over two, three, six, and seven channels. The motor patterns observed during C12 infusion are known to be associated with a slowing of gastric emptying (22, 33), which is thought to play a role in reducing energy intake (24). C10 also stimulated IPPWs; however, this effect was confined to the first 15 min of the infusion and subsequently disappeared. In contrast to C12, C10 increased the number of PWSs that travelled over two channels. These differences in motor patterns induced by C10 and C12, particularly the effects on basal pyloric pressure and IPPWs (33), may underlie their differential rates of gastric emptying (27).

It has been suggested that fatty acids with a chain length of ≥12 carbon atoms increase plasma CCK concentrations, whereas fatty acids with a chain length of ≤11 carbon atoms do not (41, 44). For example, in humans, duodenal infusion of C18, but not C8 (41), and gastric infusion of C12, but neither C11 nor C10 (44), were reported to stimulate CCK secretion. In the latter study (44), the authors concluded that C10 had no effect because C10 was associated with a rise in plasma CCK concentration that did not differ from that of the control solution (Tween 80). In contrast, our data establish that C10, when infused as a sodium salt without the confounding effects of a dispersing agent, stimulated plasma CCK (although the effect of C10 was substantially less than that of C12), consistent with earlier studies in both animals (47) and humans (28, 40). The magnitude of the rise in plasma CCK during the C12 infusion (~12 pmol/l in the first 15 min) also demonstrates the greater potency of free fatty acids compared with duodenal triglyceride infusion, which, at doses of ~2.8 kcal/min, results in plasma concentrations of ~6 pmol/l (13). Our study is the first to evaluate the effects of fatty acids of different chain lengths on GLP-1 secretion; in contrast to CCK, C12 but not C10 increased plasma GLP-1 concentrations. It is noteworthy that CCK was released almost immediately after the start of the C10 and C12 infusions, whereas there was a 30-min delay until plasma GLP-1 concentrations rose, indicating a discordance between CCK and GLP-1 release, which may relate to the release of these peptides by localized contact with fatty acids at a more distal ileal, site for GLP-1 (10) as opposed to a more proximal, jejunal, site for CCK (55).

In humans, intravenous infusions of both CCK (4, 18, 37, 39) and GLP-1 (2, 15, 21) inhibit energy intake and modulate gastrointestinal motility, consistent with the effects produced by C12. Our present observations do not establish that CCK and/or GLP-1 mediate the effects on energy intake and motility (and it still should be recognized that plasma hormone concentrations probably do not precisely reflect events at the sites of release) but favor the concept that the effects of C12 may reflect an interaction between CCK and GLP-1 and possibly other gut peptides. That the two peptides acted together on appetite and other parameters is strongly suggested by the time courses of some of our observations (Figs. 1 and 6). For example, after C12, the desire to eat decreased progressively over the entire 90 min of infusion, whereas nausea increased progressively; however, plasma CCK levels were nearly constant after 15 min, whereas the later rise in GLP-1 may have added to the CCK signal to produce the observed effects. In contrast, our observations during the C10 infusion suggest that the magnitude of CCK secretion in the absence of GLP-1 was insufficient to sustain the effect on motility or induce changes in appetite perceptions and energy intake. Possible interactions between CCK and GLP-1, therefore, deserve further evaluation; recent studies have emphasized the importance of the interaction between signals arising from the gut, e.g., gastrointestinal distension with duodenal nutrients (11) or intravenous CCK (31).

In our study, five of eight subjects experienced nausea during the C12 infusion. This might potentially have resulted from a transitory cytotoxic effect of fatty acids (34). Cytotoxicity increases with fatty acid concentration, chain length, and degree of unsaturation, so that C12 is half as cytotoxic as C18:1 at similar concentrations, and the cytotoxicities of isomeric concentrations of C10 and C12 are similar (59). Because Matzinger et al. (41) infused 280 mM C18:1 (i.e., some 2.6 times the concentration we used) intraduodenally without observing nausea, it is unlikely that the 106 mM C12 infused in our study produced nausea as a result of cytotoxicity. Furthermore, the concentration of C10 was slightly greater than that of C12 (123 vs. 106 mM). The differential effects of C12 and C10 on the secretion of GLP-1 and CCK may be important. Intravenous administration of both GLP-1 and CCK, albeit in pharmacological doses, can induce nausea (9, 48). Our dose of C12 resulted in high levels of both GLP-1 (40% higher than after the ingestion of ~4,528 kJ from the buffet meal after the control infusion) and CCK (comparable to postprandial levels after the control infusion), which may support this concept. Although we observed correlations between CCK and GLP-1 with nausea, it is important to recognize that correlations cannot be taken to infer any cause-effect relationships. Moreover, Nolan et al. (49) recently reported the absence of an association between the perception of “sickness” and plasma CCK. Alternatively, the pronounced nausea during C12, but not C18:1 (41), may reflect the unique effect of C12 on pancreatic bicarbonate secretion. Luminal C12 differs from C10, C16, C18:0, C18:1, and C18:2 by stimulating more than twice as much secretion of pancreatic bicarbonate as these other fatty acids (47), a vasoactive intestinal peptide-, secretin- or neurotensin-like effect.

It should be recognized that the several effects of C12 on gastroduodenal motility that differed from those evoked by C10 are not characteristic of nausea. It is well established from animal studies that vomiting (presumably preceded by nausea) inhibits gastric contractions and produces retrograde duodenal peristalsis (35). In contrast, C12 did not abolish but did shorten the length of antegrade peristalsis in the duodenum. Increases
in pyloric tone and IPPWs stimulated by C12 (vs. C10) have not been described during nausea but are typical of the physiological effects of intraduodenal fat (7, 13, 22). Furthermore, subjects who experienced no nausea exhibited similar pressure patterns. Thus it is unlikely that nausea accounted for the differing pressure patterns during C12 vs. C10. In addition, although it is clear that C12-induced nausea substantially contributed to inhibition of energy intake, and our data indicate a correlation between nausea at 90 min and energy intake, C12 also inhibited energy intake in the absence of nausea.

Although the mechanisms underlying the discrepant effects of C10 and C12 on appetite and gastrointestinal function are incompletely defined, differences in the postsorptive processing of the two fatty acids are likely to be important. In rats, fatty acids with chain lengths of ≤11 carbon atoms are transported from the absorptive cell directly into the systemic circulation via the portal vein (32), whereas fatty acids with chain lengths of ≥12 carbon atoms are absorbed into the lymphatic circulation as chylomicrons (43). When chylomicron transport was blocked in rats through the use of luminal l-pluronic acid, fat-induced suppression of energy intake (45), inhibition of gastric emptying and release of CCK (54), and stimulation of vagal discharge by intraluminal oleic acid (53) were blocked. Moreover, results from a recent study in rats suggest that chylomicrons, or chylomicron derivatives, activate CCK-A receptors on vagal afferent nerve fibers by releasing CCK (19). The close relationship between the transport of lymphatic chylomicrons and signaling pathways evident in these experiments may, therefore, explain why C12 has a greater effect on appetite, APD PWs, and plasma hormone concentrations than C10. However, it must be recognized that a small percentage of fatty acids with ≥12 carbon atoms are transported via the portal vein, whereas a small percentage of fatty acids with ≤10 carbon atoms are transported through the lymphatic circulation (43), potentially explaining some of the effects of C10 observed in our study.

In conclusion, our study demonstrated marked differences in the effects of isoeenergetic doses of C12 and C10 administered intraduodenally. C12, but not C10, inhibited appetite and energy intake and increased plasma GLP-1 concentrations, and C12 modulated APD motility patterns and increased plasma CCK concentrations to a greater extent than C10.

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