Dose-related effects of lauric acid on antropyloroduodenal motility, gastrointestinal hormone release, appetite, and energy intake in healthy men

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We recently reported that intraduodenal infusion of lauric acid (C12) (0.375 kcal/min, 106 mM) stimulates isolated pyloric pressure waves (IPWs), inhibits antral and duodenal pressure waves (PWs), stimulates release of cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1), and suppresses energy intake and that these effects are much greater than those seen in response to isocarbox decanoic acid (C10) infusion. Administration of C12 was, however, associated with nausea, confounding interpretation of the results. The aim of this study was to evaluate the effects of different intraduodenal doses of C12 on antropyloroduodenal (APD) motility, plasma CCK and GLP-1 concentrations, appetite, and energy intake. Thirteen healthy males were studied on 4 days in double-blind, randomized fashion. APD pressures, plasma CCK and GLP-1 concentrations, and appetite perceptions were measured during 90-min ID infusion of C12 at 0.1 (14 mM), 0.2 (28 mM), or 0.4 (56 mM) kcal/min or saline (control; rate 4 ml/min). Energy intake was determined at a buffet meal immediately following infusion. C12 dose-dependently stimulated IPWs, decreased antral and duodenal motility, and stimulated secretion of CCK and GLP-1 (r > 0.4, P < 0.05 for all). C12 (0.4 kcal/min) suppressed energy intake compared with control, C12 (0.1 kcal/min), and C12 (0.2 kcal/min) (P < 0.05). These effects were observed in the absence of nausea. In conclusion, intraduodenal C12 dose-dependently modulated APD motility and gastrointestinal hormone release in healthy male subjects, whereas effects on energy intake were only apparent with the highest dose infused (0.4 kcal/min), possibly because only at this dose was modulation of APD motility and gastrointestinal hormone secretion sufficient for a suppressant effect on energy intake.

free fatty acid; cholecystokinin; glucagon-like peptide-1; pyloric motility

STUDIES UTILIZING PHARMACOLOGICAL AGENTS, such as tetrahydro-lipstatin, to inhibit fat digestion have provided evidence that the effects of fat on gastric emptying, gastrointestinal motility, gastrointestinal hormone secretion, and appetite are dependent on the presence of free fatty acids in the small intestine (4, 10, 11, 22, 27, 29, 33). The effects of free fatty acids on gastrointestinal function, including motility, hormone release, and energy intake (12, 16, 22, 23), also are dependent on their acyl chain length. Hunt and Knox (16) were the first to demonstrate that fatty acids with a chain length of 12 and more carbon atoms empty from the stomach much more slowly than fatty acids containing 10 or fewer carbon atoms.

In a recent study from our laboratory (12), intraduodenal administration of lauric acid, a fatty acid with 12 carbon atoms (C12), at a rate of 0.375 kcal/min and a concentration of 106 mM, was shown to stimulate pyloric motility and suppress antral and duodenal motility in healthy subjects much more than decanoic acid, a fatty acid with 10 carbon atoms (C10). Intraduodenal C12 also stimulated the release of cholecystokinin (CCK) (12, 23) and glucagon-like peptide-1 (GLP-1), whereas C10, in the dose evaluated, stimulated CCK, albeit to a lesser extent than C12, and had no effect on plasma concentrations of GLP-1 (12). Intraduodenal infusion of C18, but not C8, has been shown to inhibit energy intake in humans (22), and we recently reported (12) an inhibitory effect of intraduodenal infusion of C12, but not C10, on appetite and energy intake in healthy subjects. In this latter study (12), infusion of C12 potently attenuated ratings of hunger and desire to eat and suppressed energy intake at a subsequent meal. However, in some subjects C12 also induced nausea, and the suppression of energy intake was greater in those subjects (3,516 kJ) compared with those that did not experience nausea (1,801 kJ), confounding interpretation of the observations (12). It is also possible, albeit less likely, that the observed effects on gastrointestinal motility and hormone release may also have been attributable to nausea. Therefore, it remains unclear whether the modulation of antropyloroduodenal (APD) motility, gastrointestinal hormone secretion, appetite, and energy intake during intraduodenal infusion of C12 represents a physiological effect of lauric acid or is secondary to the induction of nausea.

The mechanisms by which C12 inhibits subsequent energy intake are unclear. There is some evidence that the effects of C12 are dependent on the release of CCK (19); for example, the inhibitory effects of C12 on gastric emptying and the perception of intragastric volume are attenuated by the CCK receptor antagonist loxiglumide (19). The effects of fatty acids also appear to involve the activation of vagal afferents, either directly or via CCK (8, 18). The effects of C12 on energy intake may also be mediated through the actions of GLP-1 (12), and possibly other peptides, and by the changes in gastrointestinal motility, perhaps particularly the stimulation of...
pyloric motility (37). In animals, the effects of small intestinal C12 on gastric emptying and energy intake may be influenced by both the concentration and/or energy load (20, 25), although the energy load may be relatively more important (20). It is, therefore, possible that either the concentration and/or the energy load of C12 used in our previous study (12) may have contributed to the observed effects of C12 on appetite, energy intake, and nausea by modulating gastrointestinal motility and hormone secretion. The concentration of the C12 solution (106 mM) employed in our previous study was based on that which had been infused intragastrically (100 mM) in humans without inducing nausea (23). The observation that infusion of C10 at a slightly higher (123 mM) concentration was not associated with adverse side effects also argues against the concept that the concentration of the C12 solution was responsible for the observed nausea. However, under physiological conditions, i.e., after ingestion of a meal, fatty acids are present within the small intestine at much lower concentrations, ranging from ~25 to 65 mM (1, 3, 30). Therefore, infusion of C12 at these concentrations may have more physiological effects on gastrointestinal function and energy intake. Likewise, it is possible that the energy load of C12 delivered to the small intestine may play a role in mediating the observed effects on APD motility, gastrointestinal hormone release, perceptions of appetite, and energy intake. The data of Hunt and Knox (16) suggest that C12 empties from the stomach at rates ranging from ~0.1 to 0.4 kcal/min; however, the load dependency has not been investigated.

We have now evaluated the effects of increasing intraduodenal doses of C12, given over a range of concentrations and energy loads, on APD motility, plasma CCK and GLP-1 concentrations, appetite, and energy intake, to test the hypothesis that C12 would dose-dependently stimulate phasic and tonic pyloric motility, suppress antral and duodenal pressures, and stimulate the release of CCK and GLP-1 in the absence of nausea and that these effects of C12 would be associated with a dose-dependent suppression of energy intake.

MATERIALS AND METHODS

Subjects

Thirteen healthy males were included in the study; the number of subjects was based on power calculations derived from a previous study (6). We calculated that with 13 subjects we would observe a 10% decrease in energy intake at α = 0.05, with a power of 80%. Subjects had a mean age of 23.4 ± 1.7 yr (range: 19–30 yr) and were required to have a normal body weight for their height (mean BMI: 23.6 ± 0.5 kg/m²). Subjects were unrestrained eaters (scoring <12 on the eating restraint section (Factor 1) of the Three-Factor Eating Questionnaire (35)), had no gastrointestinal diseases or symptoms, and were not taking medication known to affect gastrointestinal motility or appetite. Consumption of >20 g of alcohol or smoking >10 cigarettes/day also represented exclusion criteria. The study protocol was approved by the Royal Adelaide Hospital Research Ethics Committee. All subjects provided informed written consent before their enrollment in the study.

Study Design

Each subject was studied on four occasions, separated by 3–10 days, in a double-blind, randomized fashion to evaluate the effects of 90-min intraduodenal infusions of lauric acid (C12) at 0.1, 0.2, or 0.4 kcal/min or control (isotonic saline) on APD pressures, appetite, energy intake, and plasma CCK and GLP-1 concentrations. The infusion pump and tubing were covered with a sheet to ensure that both the subject and the investigator (T. J. Little) who analyzed the data were blinded to the nature of the infusion.

Protocol

Subjects attended the laboratory at 8:30 AM after fasting from both solids and liquids from 10:00 PM the previous night and were intubated, via an anesthetized nostril, with a 17-channel manometric catheter (Dentsleeve, Adelaide, Australia). The catheter was allowed to pass through the stomach and into the duodenum by peristalsis (7) and contained 16 side holes, spaced at 1.5-cm intervals, to measure pressures within the APD region. Six side holes (channels 1–6) were positioned in the antrum, a 4.5-cm sleeve sensor (channel 7) with two channels (channels 8 and 9) on the back of the sleeve was positioned across the pylorus, and seven channels (channels 10–16) were positioned in the duodenum. The correct positioning of the catheter, with the sleeve sensor straddling the pylorus, was maintained as described previously by measuring the transmucosal potential difference (TMPD) (14). The manometric channels were perfused with degassed, distilled water, except for the TMPD channels, which were perfused with degassed 0.9% saline, at 0.15 ml/min (14). An additional channel, used for intraduodenal infusion of the C12 and control solutions, was located 14.5 cm distal to the pylorus. An intravenous cannula was placed into the right antecubital vein for blood sampling for the subsequent determination of plasma CCK and GLP-1 concentrations.

Once the catheter was positioned correctly, fasting motility was observed until the occurrence of phase III of the interdigestive migrating motor complex (MMC) (7). Immediately after the end of phase III, during a period of motor quiescence (i.e., at t = −15 min), a baseline venous blood sample was taken, and the subject completed a visual analog scale questionnaire (VAS) for the assessment of appetite-related sensations, as well as nausea and bloating. At t = 0 min, intraduodenal infusion of C12 commenced at a rate of 4 ml/min for 90 min (i.e., from t = 0–90 min). APD pressures were recorded throughout the infusion; blood samples were collected and VAS was completed every 15 min. At t = 90 min, the infusion was terminated and the nasoduodenal catheter removed. The subjects were then offered a standardized, cold buffet-style meal and allowed 30 min (from t = 90–120 min) to consume as much food as they wished until they felt comfortably full. The type of food as well as the macronutrient composition and energy content of the meal was described in detail previously (12). Briefly, the meal consisted of 100 g each of white and wholemeal bread, 100 g of deli ham, 100 g of deli chicken, four cheese slices, 100 g of sliced tomato, 100 g of sliced cucumber and 100 g of lettuce, 200 g of yogurt, 150 g of custard, 150 g of fruit salad, 500 g of orange juice, 600 g of iced coffee, 600 g of water, one apple, one banana, 20 g of margarine, and 20 g of mayonnaise, i.e., the total quantity of food was in excess of what the subject would be expected to consume. Further blood samples were collected and VAS completed by the subjects after the meal (at t = 120 and t = 150 min), the intravenous cannula was then removed and the subjects were allowed to leave the laboratory.

Preparation and Doses of C12 Solutions

Fatty acid solutions were designed to deliver J) 0.1 kcal/min [C12 (0.1)]; concentration, 14 mmol; total energy in 90 min, 9 kcal (37.5 kJ)], 2) 0.2 kcal/min [C12 (0.2)]; concentration, 28 mmol; total energy, 18 kcal (75 kJ)], or 3) 0.4 kcal/min [C12 (0.4)]; concentration, 56 mmol; total energy, 36 kcal (150 kJ)]. The 0.1 and 0.2 kcal/min loads were selected to encompass the range for gastric emptying of fatty acids reported in the study by Hunt and Knox (16). The 0.4 kcal/min load, albeit at a lower concentration, was selected on the basis of our previous study (12) in which C12 was administered intraduodenally at 0.375 kcal/min (106 mmol) to healthy subjects and shown to potentially suppress energy intake. The concentrations of the solutions were
within the range of fatty acid concentrations observed in the small intestine after triglyceride digestion (1, 3, 30).

Solutions were prepared using the commercially available food grade saturated fatty acid, lauric acid (C12:0) (Sigma-Aldrich, Milwaukee, WI). C12 in the amount of 1.13, 2.26, or 4.52 g was dissolved with 0.18, 0.36, or 0.75 g of NaOH (Sigma-Aldrich), respectively, in 0.9% saline to a total volume of 400 ml, with a resulting pH of 8.4. All solutions were infused at 37°C. The pH of the control solution (0.9% saline) was adjusted to 8.4 by the addition of NaOH. All solutions were prepared on the morning of the study and were infused at a rate of 4 ml/min so that the total volume infused in 90 min was 360 ml.

**Antropyloroduodenal Pressures**

Manometric pressures were digitized and recorded on a computer-based system (PowerMac 7100/75; Apple Computers, Cupertino, CA) running commercially available software (HAD, Assoc. Prof. G. S. Hebbard, Royal Melbourne Hospital, Melbourne, Australia) written in LabView 3.1.1. (National Instruments) and were stored for subsequent analysis. APD pressures were analyzed for 1) number and amplitude of antral pressure waves (PWs), 2) basal pyloric pressure (tone), 3) number and amplitude of isolated pyloric pressure waves (IPPWs), 4) number and amplitude of duodenal PWs, and 5) number and length of pressure wave sequences involving the antrum, pylorus, and duodenum, using custom-written software (Gastrointestinal Motility Unit, University Hospital Utrecht, Utrecht, The Netherlands) (32) tailored to our requirements. Basal pyloric pressure was determined by subtracting the mean basal pressure recorded at the most distal antral side hole from the mean basal pressure recorded at the sleeve, using custom-written software (MAD, Prof. Charles Malbert, Institut National de la Recherche Agronomique, Rennes, France) (15). Phasic PWs in the antrum and pylorus were defined by pressure increases that lasted 1–20 s and had an amplitude of >10 mmHg, with a minimum interval of 15 s between peaks. Phasic PWs in the duodenum were defined as those having an amplitude of >10 mmHg, with a minimal interval of 3 s between peaks. APD pressure wave sequences (APD PWs) were defined as two or more temporally related PWs with onsets within ±5 s (in the antrum) or ±3 s (in the duodenum) of each other (32).

**Plasma CCK and GLP-1 Concentrations**

Venous blood samples (10 ml) were collected into ice-chilled EDTA-treated tubes containing 400 KIU aprotinin per milliliter of blood (Trasylo1; Bayer Australia, Pymble, Australia). Plasma was separated by centrifugation at 3,200 rpm for 15 min at 4°C within 30 min of collection and stored at −70°C until assayed.

Plasma CCK concentrations (pmol/l) were determined after ethanol extraction by using an established radioimmunoassay (21). A commercially available antibody raised in rabbits against synthetic sulfated CCK-8 was employed (C258, lot no. 105H4852; Sigma Chemical, St. Louis, MO). This antibody binds to all CCK analogs with the sulfated tyrosine residue in position 7, has 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%, and the interassay CV was 27%. The assay has a sensitivity of 2.5 pmol/l.

Plasma GLP-1 concentrations (pmol/l) were measured by radioimmunoassay (36). The antibody supplied by Prof. S. R. Bloom (Hammersmith Hospital, London, UK) did not cross-react with glucagon, gastric inhibitory peptide (GIP), or any other gut or pancreatic peptides. The antibody has been shown using chromatography to measure intact GLP-1,2–36, amide, and it is likely that this antibody also binds the degraded form of GLP-1,0–36, amide. The intra-assay CV was 17%, and the interassay CV was 18%. The assay has a sensitivity of 1.5 pmol/l.

**Appetite Sensations and Energy Intake**

Ratings of appetite, including hunger, fullness, desire to eat, and prospective consumption ("how much food do you think you could eat right now?") were measured using validated VAS (28). Nausea and bloating also were assessed. Each VAS evaluated a sensation on a 100-mm horizontal line, where 0 mm represented "sensation not felt at all" and 100 mm represented "sensation felt the greatest." Subjects were asked to indicate how they were feeling at that particular time by placing a vertical mark on the 100-mm line. Other perceptions, such as anxiety and drowsiness, also were assessed to distract from the main purpose of the questionnaire, but they were not evaluated.

Energy intake (kJ) and the amount of food consumed in total (g), as well as from solid (g) and liquid (g) meal components, and the macronutrient distribution (%energy from carbohydrate, fat, and protein) were analyzed using commercially available software (Foodworks 3.01; Xyris Software, Highgate Hill, Queensland, Australia) (12).

**Data and Statistical Analyses**

The number and amplitude of antral and duodenal PWs were used to calculate motility indexes (MI) using the following equation: MI (mmHg × number) = natural logarithm {[sum of amplitudes × number of contractions (PWs)] + 1} (5). For number, amplitude, and MI of antral and duodenal PWs, number and amplitude of IPPWs, basal pyloric pressures, and number of APD PWs, baseline values (0) were calculated as the mean of values obtained between t = −15 and 10 min. For VAS and plasma CCK and GLP-1 concentrations, baseline values (0) were calculated as the mean of values obtained at t = −15 and t = 0 min. The number and amplitude of IPPWs and basal pyloric pressures are expressed as mean values over 15-min periods during the 90-min infusion period (i.e., 0–15, 15–30, 30–45, 45–60, 60–75, 75–90 min), whereas the number, amplitude, and MI of antral and duodenal PWs are expressed as mean values for the entire 90-min infusion period. APD PWs are expressed as the total number of PWs traveling over 2 (i.e., 1.5 to <3 cm), 3 (i.e., 3 to <4.5 cm), ..., 15 channels (i.e., 21 to <22.5 cm) during the 90-min infusion period. All data are expressed as changes from baseline values.

The number and amplitude of IPPWs, basal pyloric pressures, VAS scores, and plasma hormone concentrations were analyzed using repeated-measures ANOVA with time (t = 0–15, 15–30,..., 75–90 min for IPPWs and basal pyloric pressures; t = 0, 15, 30,..., 90 min for VAS scores and plasma hormone concentrations) and treatment as factors. The number of APD PWs was analyzed using repeated-measures ANOVA with length of propagation (1.5 to <3, 3 to <4.5, ..., 21 to <22.5 cm) and treatment as factors. One-way ANOVA was used to analyze the effects of treatment on the number, amplitude, and MI of antral and duodenal PWs, energy intake (kJ), macronutrient distribution, and the total (g), as well as the solid (g) and liquid (g), amount of food consumed at the buffet meal. Post hoc paired comparisons, corrected for multiple comparisons using Bonferroni’s correction, were performed if ANOVAs revealed significant effects. Dose-response relationships were determined using linear associations between the dose of C12 administered (i.e., 0, 0.1, 0.2, or 0.4 kcal/min) and the mean values over 90 min of the number, amplitude, and MI of antral and duodenal PWs, number of IPPWs, basal pyloric pressure and energy intake, as well as plasma CCK and GLP-1 concentrations at 90 min, by calculating correlation coefficients adjusted for repeated measures (2). Statistical significance was accepted at P < 0.05, and data are presented as means ± SE.

**RESULTS**

All subjects completed the four randomized study days, and the study protocol was tolerated well by these subjects. The
Table 1. Total number and amplitude of antral and duodenal pressure waves during 90-min intraduodenal infusions of lauric acid or control

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Antral Pressure Waves</th>
<th>Duodenal Pressure Waves</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Amplitude, mmHg</td>
</tr>
<tr>
<td>Control</td>
<td>91.1±19.5</td>
<td>38.7±6.4</td>
</tr>
<tr>
<td>C12 (0.1)</td>
<td>127.4±69.6</td>
<td>39.6±6.6</td>
</tr>
<tr>
<td>C12 (0.2)</td>
<td>26.5±13.0</td>
<td>21.2±3.5†‡</td>
</tr>
<tr>
<td>C12 (0.4)</td>
<td>5.5±6.3</td>
<td>19.2±3.3†‡</td>
</tr>
</tbody>
</table>

Data are means ± SE (n = 13). Lauric acid (C12) was infused at 0.1, 0.2, and 0.4 kcal/min. *P < 0.5 vs. control. †P < 0.5 vs. C12 (0.1). ‡P < 0.05 vs. C12 (0.2).

Antropyloroduodenal Pressures

Antral pressures. There was a trend for an effect of treatment on the number of antral PWs (P = 0.08). C12 (0.2) and C12 (0.4) appeared to decrease the number of antral PWs compared with control and C12 (0.1). There was a significant effect of treatment on the amplitude of antral PWs (P < 0.01). C12 (0.4) reduced the amplitude compared with control and C12 (0.1) (P < 0.01 for both). C12 (0.2) reduced the amplitude compared with control and C12 (0.1) (P < 0.01 for both) (Table 1). There also was a significant effect of treatment on the MI of antral PWs (P < 0.001) (Fig. 1). C12 (0.4) decreased the MI compared with both control and C12 (0.1) (P < 0.01 for both). C12 (0.2) significantly decreased the MI compared with control (P < 0.01). C12 (0.1) appeared to reduce the MI of antral PWs compared with control; however, this was not significant (P = 0.2). There were negative correlations between the dose of C12 administered and the number, amplitude, and MI of antral PWs such that the greater the dose of C12, the lower the number (r = −0.3, P < 0.05), amplitude (r = −0.4, P < 0.01), and MI of antral PWs (r = −0.5, P < 0.001).

Pyloric pressures. Basal pyloric pressure (tone). There was no significant effect of treatment or time on basal pyloric pressure, although the mean values for C12 (0.4) were higher compared with control, C12 (0.1), and C12 (0.2) (Fig. 2A). There was no correlation between the dose of C12 administered and basal pyloric pressure.

Phasic pressures. There was a significant treatment × time interaction for the number of IPPWs (P < 0.01) (Fig. 2B). C12 (0.4) increased the number between 0 and 60 min compared
Duodenal pressures. There was a significant effect of treatment on the number of duodenal PWs (P < 0.01) (Table 1). C12 (0.4) decreased the number of duodenal PWs compared with control, C12 (0.1), and C12 (0.2) (P < 0.05 for all). C12 (0.2) decreased the number compared with C12 (0.1) (P < 0.05). There was no significant effect of treatment on the amplitude of duodenal PWs. There was a significant effect of treatment on the MI of duodenal PWs (P < 0.05) (Table 1). C12 (0.4) decreased the MI compared with both control and C12 (0.1) (P < 0.05 for both). There was no difference between C12 (0.1) or C12 (0.2) and control. There was an inverse relationship between the number and MI, but not the amplitude, of duodenal PWs and the dose of C12 administered such that the greater the dose of C12, the lower the number (r = −0.5, P < 0.001) and MI of duodenal PWs (r = −0.4, P < 0.01).

APD sequences. There was a significant effect of treatment on the number of PWSs traveling over two (i.e., 1.5 to <3 cm), three (i.e., 3 to <4.5 cm), four (i.e., 4.5 to <6 cm), five (i.e., 6 to <7.5 cm), six (i.e., 7.5 to <9 cm), and seven channels (i.e., 9 to <10.5 cm) (P < 0.001) (Fig. 4). C12 (0.4) decreased the number of PWSs traveling over two, three, four, and five channels compared with control, C12 (0.1), and C12 (0.2), decreased the number of PWSs traveling over six channels compared with control, and decreased the number of PWSs traveling over seven channels compared with C12 (0.1) (P < 0.05 for all). C12 (0.2) decreased the number of PWSs traveling over two channels compared with C12 (0.1) (P < 0.001). C12 (0.1) increased the number of PWSs traveling over 2, 3, 4, and 5 channels compared with control (P < 0.01). Infusion of C12 (0.2) decreased the number of PWSs traveling over 6 channels compared with control, and decreased the number of PWSs traveling over 7 channels compared with C12 (0.1). Infusion of C12 (0.2) decreased the number of PWSs traveling over 2 channels compared with C12 (0.1). Infusion of C12 (0.1) increased the number of PWSs traveling over 2, 3, and 4 channels compared with control (P < 0.01). *P < 0.05 vs. control. #P < 0.05 vs. C12 (0.1). αP < 0.05, C12 (0.2). Data are means ± SE (n = 13).

**Plasma CCK and GLP-1 Concentrations**

Baseline plasma CCK concentrations did not differ between study days [control: 4.0 ± 0.4 pmol/l, C12 (0.1): 3.9 ± 0.3 pmol/l, C12 (0.2): 3.8 ± 0.3 pmol/l, C12 (0.4): 3.9 ± 0.3 pmol/l]. There was a significant treatment × time interaction for plasma CCK concentrations (P < 0.001) (Fig. 5A). Plasma concentrations of CCK peaked at ~15 min. C12 (0.4) increased plasma CCK concentrations between 15 and 90 min compared with control and C12 (0.1) and between 30 and 90 min compared with C12 (0.2) (P < 0.01 for all). C12 (0.2) increased plasma CCK concentrations between 15 and 90 min compared with control and at t = 15, 30, 60, and 90 min compared with C12 (0.1) (P < 0.05 for all). C12 (0.1) increased plasma CCK concentrations at t = 15 min and from 45 to 90 min compared with control (P < 0.05 for all). There was a positive correlation between the dose of C12 administered and the plasma concentrations of CCK at 90 min such that the greater the dose of C12, the greater the concentration of CCK at 90 min (r = 0.7, P < 0.001).

Baseline plasma GLP-1 levels were slightly variable over study days [control: 13.9 ± 1.8 pmol/l, C12 (0.1): 17.6 ± 2.9 pmol/l, C12 (0.2): 15.1 ± 2.1 pmol/l, C12 (0.4): 17.6 ± 2.9 pmol/l]. There was a significant treatment × time interaction for plasma GLP-1 concentrations (P < 0.001) (Fig. 5B). Plasma concentrations of GLP-1 peaked at ~15 min. C12 (0.4) increased plasma GLP-1 concentrations between 15 and 90 min compared with control and C12 (0.1) and between 30 and 90 min compared with C12 (0.2) (P < 0.01 for all). C12 (0.2) increased plasma GLP-1 concentrations between 15 and 90 min compared with control and at t = 15, 30, 60, and 90 min compared with C12 (0.1) (P < 0.05 for all). C12 (0.1) increased plasma GLP-1 concentrations at t = 15 min and from 45 to 90 min compared with control (P < 0.05 for all). There was a positive correlation between the dose of C12 administered and the plasma concentrations of GLP-1 at 90 min such that the greater the dose of C12, the greater the concentration of GLP-1 at 90 min (r = 0.7, P < 0.001).
increased plasma GLP-1 concentrations at $t = 30, 60$, and $90$ min compared with control and at $t = 30$ min compared with C12 (0.1) ($P < 0.05$ for all). C12 (0.1) increased plasma GLP-1 concentrations at $t = 45$ and $90$ min compared with control ($P < 0.05$). There was a positive correlation between the amount of C12 administered and the plasma concentrations of GLP-1 at 90 min such that the greater the dose of C12, the greater the concentration of GLP-1 at 90 min ($r = 0.5, P < 0.001$).

**Appetite Sensations and Energy Intake**

There was no effect of treatment on ratings of appetite, i.e., hunger, desire to eat, fullness, prospective consumption, or gastrointestinal symptoms, i.e., bloating and nausea. There was a significant effect of treatment on energy intake ($P = 0.05$). C12 (0.4) decreased energy intake compared with control, C12 (0.1), and C12 (0.2) ($P < 0.05$ for all). There was, however, no effect on the total amount (g), solid (g) or liquid (g) amount, or macronutrient distribution of food consumed at the buffet meal (Table 2). There was no correlation between energy intake and the amount of C12 administered.

**DISCUSSION**

This study establishes that intraduodenal administration of C12 modulates APD motility and gastrointestinal hormone release in a dose-dependent fashion such that the greater the dose of C12 administered, the greater the stimulation of isolated pyloric PWs, suppression of antral and duodenal PWs and APD PWs, and stimulation of CCK and GLP-1. In contrast, at the doses used, appetite perceptions were not affected, and suppression of energy intake was only apparent with the 0.4 kcal/min dose, perhaps reflecting the greater effects of this dose on APD motility and plasma CCK and GLP-1 secretion. The effects of C12 on motility, CCK, GLP-1, and energy intake occurred in the absence of nausea.

It has been established that the presence of C12 in the small intestinal lumen slows gastric emptying (16, 19), stimulates isolated pyloric PWs (12), increases proximal gastric relaxation (19), suppresses antral (12, 23) and duodenal motility (12), and stimulates the release of CCK (12, 23) and GLP-1 (12). The current study extends these observations by demonstrating that the responses are dependent on the dose of C12 administered to the small intestine and that even very low doses of C12 have potent effects. For example, infusion of C12 at doses as low as 0.1 and 0.2 kcal/min, resulting in a total energy delivery of only 9 and 14 kcal, respectively, over the 90-min infusion period, had substantial effects. In previous studies using intraduodenal lipid infusion at a rate of 2.8 kcal/min, the stimulation of pyloric motility and plasma CCK concentrations were maximal at ~30–45 min, when ~84–126 kcal would have been delivered to the small intestine (10). This contrasts with the current study in which maximal effects of C12 infusion on pyloric motility and plasma hormone secretion were observed after 15 min, when only 1.5, 3, or 6 kcal had been delivered to the small intestine during infusion of C12 at 0.1, 0.2, or 0.4 kcal/min, respectively. This finding provides persuasive evidence that the effects of C12 on pyloric motility and plasma concentrations of CCK and GLP-1 are much more potent than those of long-chain triglycerides. The underlying reasons are currently unknown but may perhaps reflect the fact

![Graph of Plasma CCK](image)

**Fig. 5.** Plasma concentrations of cholecystokinin (CCK; A) and glucagon-like peptide-1 (GLP-1; B) during 90-min intraduodenal infusion of C12 at 0.1, 0.2, and 0.4 kcal/min and control. A: infusion of C12 increased plasma CCK concentrations (treatment × time interaction: $P < 0.001$). Infusion of C12 (0.4) increased plasma CCK concentrations between 15 and 90 min compared with control and C12 (0.1) and between 30 and 90 min compared with C12 (0.2). Infusion of C12 (0.2) increased plasma CCK concentrations between 15 and 90 min compared with control and at 15, 30, 60, and 90 min compared with C12 (0.1). Infusion of C12 (0.1) increased plasma CCK concentrations compared with control at 15, 45, 60, 75, and 90 min. B: infusion of C12 significantly increased plasma GLP-1 concentrations (treatment × time interaction: $P < 0.01$). Infusion of C12 (0.4) increased plasma GLP-1 concentrations from 30 to 90 min compared with control, at 30, 60, 75, and 90 min compared with C12 (0.1), and at 45 and 75 min compared with C12 (0.2). Infusion of C12 (0.2) increased plasma GLP-1 concentrations at 30, 60, and 90 min compared with control and at 30 min compared with C12 (0.1). Infusion of C12 (0.1) increased plasma GLP-1 concentrations at 45 and 90 min compared with control. *$P < 0.05$ vs. control. #$P < 0.05$ vs. C12 (0.1). $\alpha P < 0.05$ vs. C12 (0.2). Data are means ± SE ($n = 13$).
that lauric acid probably accounts for only <2% of daily energy intake (24) so that, under normal conditions, exposure of the small intestine to lauric acid is likely to be limited.

The demonstrated dose-dependent effects of C12 on plasma concentrations of CCK and GLP-1 suggest that the release of CCK and GLP-1 in response to C12 is also dependent on the amount of C12 present in the small intestinal lumen, i.e., infusion of C12 at 0.4 kcal/min resulted in a greater secretion of CCK and GLP-1 than during infusion of C12 at 0.1 or 0.2 kcal/min. The secretion profiles varied between CCK and GLP-1: plasma CCK increased almost immediately after the start of the C12 infusions, with a plateau after 30 min, consistent with release of CCK from enteroendocrine cells in the proximal small intestine (31). In contrast, there was a 30-min delay before plasma GLP-1 concentrations increased from baseline, and this increase was progressive during the entire 90-min infusion period. It is possible that during infusion of C12, the absorption capacity of the proximal small intestine was exceeded, resulting in progressively greater amounts of C12 reaching the distal small intestine, the primary site of CCK and GLP-1 release (9), thereby accounting for the gradual increase in GLP-1 secretion.

The effects of C12 on appetite and energy intake were not dose dependent at the doses evaluated in this study. Infusion of C12 at 0.4 kcal/min, but not 0.1 and 0.2 kcal/min, decreased energy intake by 831 kJ compared with control, without inducing nausea. The reduction in energy intake was not associated with a change in the amount (weight) of food consumed, that is, subjects consumed a less energy-dense meal. Although the highest dose suppressed energy intake, this occurred in the absence of changes in appetite perceptions. This suggests that perceptions of appetite may be regulated by mechanisms different from those involved in the control of acute energy intake and may perhaps require higher energy loads. The effects of C12 on energy intake, however, were much more marked in our previous study, in which C12 reduced ratings of hunger and suppressed subsequent energy intake by 2,781 kJ when compared with control (12). However, as discussed, the inhibition of hunger and energy intake after infusion of C12 (0.375 kcal/min, 106 mM) was associated with a marked increase in nausea (12). Although the decrease in hunger and energy intake in our earlier study could not altogether be attributed to nausea, because the subjects who did not experience nausea still also decreased their energy intake, these new observations provide further support for a physiological effect of C12 on the suppression of energy intake.

Although it has been suggested that the suppressive effects of nutrients on subsequent energy intake are mediated by changes in gastrointestinal motility and gastrointestinal hormone release (19, 37), the current study suggests that the stimulation of IPPWs and secretion of CCK and GLP-1 have to reach a “threshold” to result in suppression of energy intake. The motor patterns associated with infusion of C12 at 0.2 and 0.4 kcal/min are known to be associated with the slowing of gastric emptying (14), which is thought to play a role in suppressing energy intake (34). Recent evidence suggests that electrical stimulation of the pylorus suppresses food intake in dogs (37), thus implying an important role for the pylorus in the regulation of energy intake. It is, however, interesting to note that despite C12 at 0.2 kcal/min having a more prolonged stimulatory effect on IPPWs than C12 at 0.4 kcal/min, there was no effect on energy intake. Furthermore, at the time of the meal, the effects of C12 at 0.4 kcal/min on IPPWs had returned to baseline, yet energy intake was suppressed only after this infusion. Hence, factors other than the stimulation of isolated pyloric PWs are probably required to inhibit energy intake. The discrepant effects of C12 seen in our study may be attributable to the different patterns of secretion of the gastrointestinal hormones CCK and GLP-1, because intravenous infusion of both CCK and GLP-1 suppress energy intake in humans (13, 17). C12 at 0.4 kcal/min stimulated the secretion of CCK and GLP-1 to a greater extent than C12 at 0.2 kcal/min. It is interesting to note that whereas C12 at 0.2 kcal/min stimulated the release of CCK to an extent similar to that previously observed in studies using intraduodenal infusion of a long-chain triglyceride emulsion in which there was a significant suppression of energy intake (10), it did not suppress energy intake. This may potentially reflect the number of subjects studied, i.e., a type II statistical error; however, it seems unlikely that increasing the number of subjects would show an effect of the lower doses on energy intake, because there was no trend at all toward decreased energy intake with these doses. Rather, it is likely that there are different threshold requirements for the effects observed on motility, gastrointestinal hormone release, appetite, and energy intake, i.e., whereas the lower doses were sufficient to stimulate motility and hormone release, only the 0.4 kcal/min infusion suppressed energy intake, and this may have been due to appropriate modulation of motility and gastrointestinal hormones. Likewise, although none of the doses had an effect on perceptions of appetite, i.e., hunger, fullness, desire to eat, and prospective consumption, C12 at 0.4 kcal/min suppressed subsequent energy intake. Although we have demonstrated a clear dose-responsive effect of C12 on APD motility and gastrointestinal hormone release, it remains unclear whether the concentration, or the energy load, of C12 administered mediated these effects of C12, because the concentration of the solutions was varied to keep the volume of the infusion identical on all study days.
Canine studies have suggested that inhibition of gastric emptying or stimulation of pancreatic enzyme secretion by intestinal oleate is length and load dependent at luminal concentrations near or above 20 mM but that concentration becomes increasingly important for these responses as it drops below 10 mM (26). The effects of oleate and lauric acid on food intake, at concentrations ranging from 20 to 80 mM, have been shown in rats to be load, but not concentration, dependent (20). In our previous study, during infusion of C12 at 0.375 kcal/min, we observed a more marked increase in the number of IPPWs, basal pyloric pressure, and secretion of CCK and GLP-1 as well as decreased perceptions of hunger, desire to eat, and energy intake with a concentration of 106 mM (12), compared with our current observations for C12 at 0.4 kcal/min and 56 mM, suggesting that concentration may be important. Increasing the load and/or concentration of C12 could suppress energy intake by increasing the effects of C12 on gastrointestinal motility and hormone secretion. This issue warrants further investigation.

In conclusion, our study has demonstrated a dose-dependent effect of acute intraduodenal C12 on APD motility, gastrointestinal hormone secretion, and, at the highest dose used, suppression of energy intake. This latter observation adds to the concept that C12 has the potential to provide an effective nutrient-based treatment for weight loss in obesity.

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