DOSE-RELATED EFFECTS OF LAURIC ACID ON ANTROPYLORODUODENAL
MOTILITY, GASTROINTESTINAL HORMONE RELEASE, APPETITE AND
ENERGY INTAKE IN HEALTHY MEN

Short title: Lauric acid, gut motility, gastrointestinal hormones and appetite

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ABSTRACT

We have recently reported that intraduodenal infusion of lauric acid (C12) (at 0.375 kcal/min, 106 mM) stimulates isolated pyloric pressure waves (IPPWs), inhibits antral and duodenal pressure waves (PWs), stimulates the 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 isocaloric 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. 13 healthy males were studied on four days in double-blind, randomised, fashion. APD pressures, plasma CCK and GLP-1 concentrations and appetite perceptions were measured during 90 minute intraduodenal infusion of C12 at either (i) 0.1 (14 mM), (ii) 0.2 (28 mM) or (iii) 0.4 (56 mM) kcal/min, or (iv) saline (control) (rate: 4 ml/min). Energy intake was determined at a buffet meal immediately following the infusion. C12 dose-dependently stimulated IPPWs, decreased antral and duodenal motility, and stimulated secretion of CCK and GLP-1 (r > 0.4, P < 0.05 for all). C12 at 0.4 kcal/min suppressed energy intake compared with control, C12 (0.1) and C12 (0.2) (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, while effects on energy intake were only apparent with the highest dose infused (0.4 kcal/min), possibly because only at this dose modulation of antropyloroduodenal motility and gastrointestinal hormone secretion was sufficient for a suppressant effect on energy intake. These effects occurred in the absence of nausea.
**Key words:** free fatty acid, lauric acid, antropyloroduodenal motility, energy intake, gastrointestinal hormones
INTRODUCTION

Studies utilising pharmacological agents, such as tetrahydrolipstatin (THL), 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) are also dependent on their acyl chain length. Hunt and Knox 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 less carbon atoms (16).

In a recent study from our laboratory 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) (12). Intraduodenal C12 also stimulated the release of cholecystokinin (CCK) (12, 23) and glucagon-like peptide-1 (GLP-1), whilst 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 have recently reported an inhibitory effect of intraduodenal infusion of C12, but not C10, on appetite and energy intake (12), in healthy subjects. In this latter study, infusion of C12 potently attenuated ratings of hunger and desire to eat and suppressed energy intake at a subsequent meal (12). However, in some subjects C12 also induced nausea, and the suppression of energy intake was greater in those subjects (3516 kJ) when compared with those that did not experience nausea (1801 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 CCK1 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 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 upon 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. following ingestion of a meal, fatty acids are present within the small intestine
at much lower concentrations, ranging from approximately 25 – 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 suggest that C12 empties from the stomach at rates ranging from approximately 0.1 to 0.4 kcal/min (16), 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

13 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 $\alpha = 0.05$, with a power of 80%. Subjects had a mean age of $23.4 \pm 1.7$ years (range 19 - 30 years) and were required to have a normal body weight for their height (mean BMI $23.6 \pm 0.5$ kg/m$^2$). 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 prior to their enrolment in the study.

Study design

Each subject was studied on four occasions, separated by 3 – 10 days, in a double-blind, randomised fashion to evaluate the effects of 90 minute intraduodenal infusions of lauric acid (C12) at (i) 0.1 kcal/min, (ii) 0.2 kcal/min, (iii) 0.4 kcal/min, or (iv) 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 in order to ensure both the subject and the investigator (TJL) who analysed 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 pm the previous night and were intubated, via an anaesthetised 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 7 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 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.

Following correct positioning of the catheter, fasting motility was observed until the occurrence of a phase III of the interdigestive migrating motor complex (MMC) (7). Immediately following the end of the 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 analogue 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. t = 0 – 90 min). APD pressures were recorded throughout the infusion; blood samples were collected and VAS completed every 15 min. At t = 90 min, the infusion was terminated and the nasoduodenal catheter removed. The subjects were then offered a standardised, cold, buffet-style meal and allowed 30 min (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 has been described in detail previously (12). Briefly, the meal consisted of 100 g each of white and wholemeal bread, 100 g deli ham, 100 g deli chicken, 4 cheese slices, 100 g sliced tomato, 100 g sliced cucumber and 100 g lettuce, 200 g yoghurt, 150 g custard, 150 g fruit salad, 500 g orange juice, 600 g iced coffee, 600 g water, 1 apple, 1 banana, 20 g margarine and 20 g 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 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 either: (i) 0.1 kcal/min, (C12 (0.1); concentration: 14 mmol; total energy in 90 min: 9 kcal (37.5 kJ)), (ii) 0.2 kcal/min (C12 (0.2); concentration: 28 mmol; total energy: 18 kcal (75 kJ)) or (iii) 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 in which C12 was administered intraduodenally at 0.375 kcal/min (106 mmol) to healthy subjects and shown to potently suppress energy intake (12). 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, USA). 1.13, 2.26 or 4.52 g of C12 were
dissolved with 0.18, 0.36 or 0.75 g of sodium hydroxide (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 digitised and recorded on a computer-based system (PowerMac 7100/75; Apple Computers, Cupertino, CA, USA) running commercially available software (HAD, Associate Prof G.S. Hebbard, Royal Melbourne Hospital, Melbourne, Australia), written in Labview 3.1.1. (National Instruments), and stored for subsequent analysis. APD pressures were analysed for: (i) number and amplitude of antral pressure waves (PWs), (ii) basal pyloric pressure (tone), (iii) number and amplitude of isolated pyloric pressure waves (IPPWs), (iv) number and amplitude of duodenal PWs, and (v) 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, Professor Charles Malbert, Institut National de la Recherche Agronomique (INRA), Rennes, France) (15). Phasic PWs in the antrum and pylorus were defined by pressure increases which lasted 1 to 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 PWSs) 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 ml blood (Trasylol; Bayer Australia Ltd, Pymble, Australia). Plasma was separated by centrifugation at 3200 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 following ethanol extraction using an established radioimmunoassay (21). A commercially available antibody raised in rabbits against synthetic sulphated CCK-8 was employed (C258, Lot 105H4852, Sigma Chemical, St Louis, MO, USA). This antibody binds to all CCK analogues with the sulphated tyrosine residue in position 7, has a cross-reactivity of 26 % with unsulphated CCK-8, less than 2 % cross-reactivity with human gastrin (0.2 % with gastrin I and 1 % with Big gastrin) and does not bind to structurally unrelated peptides. The intra-assay coefficient of variation (CV) was 9 % and the inter-assay CV was 27 %. 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 Professor SR Bloom (Hammersmith Hospital, London) 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(7-36) amide, and it is likely that this antibody also binds the degraded form of GLP-1(9-36) amide. The intra-assay CV was 17 % and the inter-assay 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 were also assessed. Each VAS evaluated a sensation on a 100 mm horizontal line, where 0 mm represented ‘sensation not felt at all’ and 100 mm ‘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, were also assessed to distract from the main purpose of the questionnaire, but 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 (% of energy from carbohydrate, fat and protein) was analysed 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 indices (MI) using the following equation: MI (mmHg*number) = natural logarithm [(sum of amplitudes x number of contractions (PWs)) + 1] (5). For the number, amplitude and motility indices of antral and duodenal PWs, number and amplitude of IPPWs, basal pyloric pressures and number of APD PWSs, baseline values (0) were calculated as the mean of values obtained between t = -15 to 0 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 were
expressed as mean values over 15 min periods during the 90 min infusion period (i.e. 0 – 15, 15 – 30, ..., 75 – 90 min), while the number, amplitude and motility indices of antral and duodenal PWs were expressed as mean values for the entire 90 min infusion period. APD PWSs were expressed as the total number of PWs travelling over 2 (i.e. 1.5 - < 3 cm), 3 (i.e. 3 - < 4.5 cm), ..., 15 (i.e. 21 - < 22.5 cm) channels during the 90 minute infusion period. All data were expressed as changes from baseline values.

The number and amplitude of IPPWs, basal pyloric pressures, VAS scores and plasma hormone concentrations were analysed by repeated measures analysis of variance (ANOVA) with time (t = 0 – 15, 15 – 30, ..., 75 – 90 min for IPPWs and basal pyloric pressures, and t = 0, 15, 30, ..., 90 min for VAS scores and plasma hormone concentrations) and treatment as factors. The number of APD PWSs was analysed by repeated measures ANOVA with length of propagation (1.5 - < 3, 3 - < 4.5, ..., 21 - < 22.5 cm) and treatment as factors. One-way ANOVA was used to analyse the effects of treatment on the number, amplitude and motility indices of antral and duodenal PWs, energy intake (kJ), macronutrient distribution, the total amount (g), and the amount of solid (g) and liquid (g), of food consumed at the buffet meal. Post-hoc paired comparisons, corrected for multiple comparisons by 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 minutes of the number, amplitude and motility indices of antral and duodenal PWs, the number of IPPWs, basal pyloric pressure, APD PWSs, energy intake, as well as the 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 ± SEM.
RESULTS

All subjects completed the four randomised study days, and the study protocol was tolerated well by these subjects. The tube was correctly positioned and the infusion commenced within an average of $146 \pm 45$ (range $45 – 210$) minutes.

Antro- pyloroduodenal 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 was also a significant effect of treatment on the MI of antral PWs ($P < 0.001$) (Figure 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 motility index 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 motility index 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) (Figure 2 A). 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 ($P < 0.01$) (Figure 2 B). C12 (0.4) increased the number between 0 – 60 minutes compared with control and between 0 – 45 min compared with C12 (0.1), but decreased the number between 60 – 75 min compared with C12 (0.2) ($P < 0.05$ for all). C12 (0.2) increased the number between 0 – 90 minutes compared with control, and between 0 – 45 min and 75 – 90 min compared with C12 (0.1) ($P < 0.05$ for all). C12 (0.1) increased the number between 0 – 15 min compared with control ($P < 0.01$). There was a positive correlation between the dose of C12 administered and the total number of IPPWs over 90 min, such that the greater the dose of C12, the greater the number of IPPWs ($r = 0.4$, $P < 0.05$). There was no significant effect of treatment on, or a relationship between the dose of C12 administered and, the amplitude of IPPWs.

**Duodenal pressures**

There was a significant effect of treatment on the number of duodenal pressure waves ($P < 0.01$) (Table 1). Infusion of C12 (0.4) decreased the number 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 ($P < 0.05$) (Figure 3). Infusion of C12 (0.4) decreased the MI compared with both control and C12
(0.1) \( (P < 0.05 \text{ for both}) \). 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 \( (r = -0.4, P < 0.01) \).

**Antropyloroduodenal sequences**

There was a significant effect of treatment on the number of pressure wave sequences travelling over 2 (i.e. \( 1.5 < 3 \text{ cm} \)), 3 (i.e. \( 3 < 4.5 \text{ cm} \)), 4 (i.e. \( 4.5 < 6 \text{ cm} \)), 5 (i.e. \( 6 < 7.5 \text{ cm} \)), 6 (i.e. \( 7.5 < 9 \text{ cm} \)) and 7 (i.e. \( 9 < 10.5 \text{ cm} \)) channels \( (P < 0.001) \) (Figure 4). Infusion of C12 (0.4) decreased the number of PWSs travelling over 2, 3, 4 and 5 channels compared with control, C12 (0.1) and C12 (0.2), the number of PWSs travelling over 6 channels compared with control, and the number of PWSs travelling over 7 channels compared with C12 (0.1) \( (P < 0.05 \text{ for all}) \). Infusion of C12 (0.2) decreased the number of PWSs travelling over 2 channels compared with C12 (0.1) \( (P < 0.001) \). Infusion of C12 (0.1) increased the number of PWSs travelling over 2, 3 and 4 channels compared with control \( (P < 0.01) \). PWSs travelling over 8 and more (i.e. \( \geq 10.5 \text{ cm} \)) channels were not analysed statistically, as they were very infrequent (a total of 26 waves travelled over 8 – 15 channels, 9 during the control infusion, 14 during C12 (0.1), 2 during C12 (0.2) and < 1 during C12 (0.4)).

**Plasma CCK and GLP-1 concentrations**

Baseline plasma CCK concentrations did not differ between study days (Control: \( 4.0 \pm 0.4 \text{ pmol/l} \), C12 (0.1): \( 3.9 \pm 0.3 \text{ pmol/l} \), C12 (0.2): \( 3.8 \pm 0.3 \text{ pmol/l} \) and C12 (0.4): \( 3.9 \pm 0.3 \text{ pmol/l} \)). There was a significant treatment * time interaction for plasma CCK concentrations.
Plasma concentrations of CCK peaked at approximately 15 min. Infusion of C12 (0.4) increased plasma CCK concentrations between 15 – 90 minutes compared with control and C12 (0.1), and between 30 – 90 minutes compared with C12 (0.2) (P < 0.01 for all). Infusion of C12 (0.2) increased plasma CCK concentrations between 15 – 90 minutes compared with control, and at t = 15, 30, 60 and 90 min compared with C12 (0.1) (P < 0.05 for all). Infusion of C12 (0.1) increased plasma CCK concentrations at t = 15 and 45 - 90 minutes 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): 16.7 ± 3 pmol/l, C12 (0.4): 14.9 ± 1.8 pmol/l). There was a significant treatment * time interaction for plasma GLP-1 concentrations (P < 0.01) (Figure 5 B). Infusion of C12 (0.4) increased plasma GLP-1 concentrations from 30 – 90 min compared with control, at t = 30 and 60 - 90 min compared with C12 (0.1), and at t = 45 and 75 min compared with C12 (0.2) (P < 0.05 for all). Infusion of C12 (0.2) 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). Infusion of 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 either the total amount (g), solid (g), liquid (g), or the 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 antropyloroduodenal 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 pressure waves, suppression of antral and duodenal pressure waves and APD PWSs, 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 antropyloroduodenal motility and plasma CCK and GLP-1 secretion. The effect 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 pressure waves (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 upon 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 about ~ 30 – 45 min when approximately 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 minutes, when only 1.5, 3 or 6 kcal had been delivered to the small intestine during infusion of C12 at 0.1, 0.2 and 0.4 kcal/min,
respectively. This 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 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 minutes, consistent with release of CCK from enteroendocrine cells in the proximal small intestine (31). In contrast, there was a 30-minute delay before plasma GLP-1 concentrations increased from baseline, and this increase was progressive during the entire 90 minute 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 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. While 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 differing to those involved in the control of acute energy intake, and may perhaps require higher energy loads. The effects of C12 on energy intake were, however, much more marked in our previous study, in which C12 reduced ratings of hunger and suppressed subsequent energy intake by 2781 kJ when compared with control (12). However, as discussed, the inhibition of hunger and energy intake following infusion of C12 (0.375 kcal/min, 106 mM) was associated with a marked increase in nausea (12). Whilst the decrease in hunger and energy intake in our earlier study could not altogether be attributed to nausea, as the subjects who did not experience nausea still also decreased their food intake, these new observations provide further support for a physiological effect of C12 on the suppression of energy intake.

 Whilst 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 following this infusion. Hence, factors other than the stimulation of isolated pyloric pressure
waves 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, as 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 while C12 at 0.2 kcal/min stimulated the release of CCK to a similar extent 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, as there was no trend at all towards decreased energy intake using 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. while 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, while 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.

While we have demonstrated a clear dose-responsive effect of C12 on antropyloroduodenal motility and gastrointestinal hormone release, it remains unclear whether the concentration, or the energy load, of C12 administered mediated these effects of C12, as in order to keep the volume of the infusion identical on all study days, the concentration of the solutions varied. 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 20-80 mM, were 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, secretion of CCK and GLP-1, as well as decreased perceptions of hunger and 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 antropyloroduodenal motility and 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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REFERENCES


FIGURE LEGENDS

**Figure 1**: Motility index (MI) of antral pressure waves (PWs) during 90 min intraduodenal infusion of lauric acid (C12) at 0.1, 0.2 and 0.4 kcal/min, and control. There was an effect of C12 infusion on the MI of antral PWs (treatment effect: \( P = 0.001 \)). C12 (0.4) decreased the MI compared with both control and C12 (0.1). C12 (0.2) decreased the MI compared with control. C12 (0.1) appeared to reduce the MI of antral PWs compared with control; however, this was not significant \( (P = 0.16) \). * C12 (0.4) and C12 (0.2) vs. control: \( P < 0.01 \), # C12 (0.4) vs. C12 (0.1): \( P < 0.01 \). Data are means ± SEM (n = 13).

**Figure 2**: (A) Basal pyloric pressure (tone) and (B) number of isolated pyloric pressure waves (IPPWs) during 90 min intraduodenal infusion of lauric acid (C12) at 0.1, 0.2 and 0.4 kcal/min, and control. (A) There was a trend for C12 (0.4) to increase basal pyloric pressure compared with control, C12 (0.1) and C12 (0.2). (B) Infusion of C12 increased the number of IPPWs (treatment * time interaction: \( P < 0.01 \)). C12 (0.4) increased the number of IPPWs between 0 – 60 minutes when compared with control, and between 0 – 45 min compared with C12 (0.1), and decreased the number between 60 – 75 min compared with C12 (0.2). C12 (0.2) increased the number between 0 – 90 minutes when compared with control, and between 0 – 45 min and 75 – 90 min compared with C12 (0.1). C12 (0.1) increased the number between 0 – 15 min compared with control. * C12 (0.4), C12 (0.1) and C12 (0.2) vs. control: \( P < 0.05 \), # C12 (0.4) and C12 (0.2) vs. control: \( P < 0.05 \), α C12 (0.4) vs. C12 (0.2): \( P < 0.01 \). Data are means ± SEM (n = 13).

**Figure 3**: Motility index of duodenal pressure waves (PWs) during 90 min intraduodenal infusion of lauric acid (C12) at 0.1, 0.2 and 0.4 kcal/min, and control. There was an effect of
C12 infusion on the MI of duodenal PWs (treatment effect: P < 0.05). Infusion of C12 (0.4) decreased the MI of duodenal PWs compared with both control and C12 (0.1). There was no difference between C12 (0.1) or C12 (0.2) and control. * C12 (0.4) vs. control: P = 0.01, # C12 (0.2) and C12 (0.4) vs. C12 (0.1): P < 0.05, α C12 (0.4) vs. C12 (0.2): P < 0.05. Data are means ± SEM (n = 13).

**Figure 4:** Antropyloroduodenal pressure wave sequences during 90 min intraduodenal infusion of lauric acid (C12) at 0.1, 0.2 and 0.4 kcal/min, and control. Infusion of C12 decreased the number of APD PWSs (treatment effect: P < 0.001). Infusion of C12 (0.4) decreased the number of PWSs travelling over 2, 3, 4 and 5 channels compared with control, C12 (0.1) and C12 (0.2), the number of PWSs travelling over 6 channels compared with control, and the number of PWSs travelling over 7 channels compared with C12 (0.1). Infusion of C12 (0.2) decreased the number of PWSs travelling over 2 channels compared with C12 (0.1). Infusion of C12 (0.1) increased the number of PWSs travelling over 2, 3 and 4 channels compared with control (P < 0.01). * C12 (0.4), C12 (0.2) and C12 (0.1) vs. control: P < 0.05, # C12 (0.4) and C12 (0.2) vs. C12 (0.1): P < 0.05, α C12 (0.4) vs. C12 (0.2): P < 0.05. Data are means ± SEM (n = 13).

**Figure 5:** Plasma concentrations of (A) CCK and (B) GLP-1 during 90 min intraduodenal infusion of lauric acid (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 – 90 minutes compared with control and C12 (0.1), and between 30 – 90 minutes compared with C12 (0.2). Infusion of C12 (0.2) increased plasma CCK concentrations between 15 – 90 minutes compared with control, and at t = 15, 30, 60 and 90 min compared with C12 (0.1). Infusion of C12 (0.1)
increased plasma CCK concentrations compared with control at t = 15, 45, 60, 75 and 90 minutes. (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 – 90 minutes compared with control, at t = 30, 60, 75 and 90 minutes compared with C12 (0.1), and at t = 45 and 75 minutes compared with C12 (0.2). Infusion of C12 (0.2) increased plasma GLP-1 concentrations at t = 30, 60 and 90 compared with control, and at t = 30 minutes compared with C12 (0.1). Infusion of C12 (0.1) increased plasma GLP-1 concentrations at t = 45 and 90 minutes compared with control. * C12 (0.4), C12 (0.2) and C12 (0.1) vs. control: P < 0.05, # C12 (0.4) and C12 (0.2) vs. C12 (0.1): P < 0.05, α C12 (0.4) vs. C12 (0.2): P < 0.05. Data are means ± SEM (n = 13).
**Table 1:** Total number and amplitude of antral and duodenal pressure waves during 90 min intraduodenal infusions of lauric acid (C12) at 0.1, 0.2 and 0.4 kcal/min, or control.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Antral pressure waves</th>
<th></th>
<th>Duodenal pressure waves</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Amplitude</td>
<td>Number</td>
<td>Amplitude</td>
</tr>
<tr>
<td></td>
<td>(mmHg)</td>
<td>(mmHg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>91.1 ± 19.5</td>
<td>38.7 ± 6.4</td>
<td>688.8 ± 104.0</td>
<td>31.9 ± 3.1</td>
</tr>
<tr>
<td>C12 (0.1)</td>
<td>127.4 ± 69.6</td>
<td>39.6 ± 6.6</td>
<td>850.8 ± 92.9</td>
<td>30.0 ± 2.0</td>
</tr>
<tr>
<td>C12 (0.2)</td>
<td>26.5 ± 13.0</td>
<td>21.2 ± 3.5 *#</td>
<td>575.7 ± 89.0 #</td>
<td>30 ± 1.8</td>
</tr>
<tr>
<td>C12 (0.4)</td>
<td>5.5 ± 6.3</td>
<td>19.2 ± 3.3 *#</td>
<td>293.1 ± 87.4 *#α</td>
<td>30.8 ± 4.2</td>
</tr>
</tbody>
</table>

Data are means ± SEM (n = 13). * vs. control, # vs. C12 (0.1), α vs. C12 (0.2): P < 0.05.
Table 2: Energy intake from the buffet meal, and macronutrient distribution, in response to 90 min intraduodenal infusions of lauric acid (C12) at 0.1, 0.2 and 0.4 kcal/min, or control.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Energy (kJ)</th>
<th>Amount consumed (g)</th>
<th>% Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Solid</td>
<td>Liquid</td>
</tr>
<tr>
<td>Control</td>
<td>5932 ± 495</td>
<td>1369 ± 118</td>
<td>829 ± 79</td>
</tr>
<tr>
<td>C12 (0.1)</td>
<td>5902 ± 475</td>
<td>1367 ± 99</td>
<td>874 ± 84</td>
</tr>
<tr>
<td>C12 (0.2)</td>
<td>5815 ± 520</td>
<td>1399 ± 118</td>
<td>885 ± 92</td>
</tr>
<tr>
<td>C12 (0.4)</td>
<td>5101 ± 521*</td>
<td>1305 ± 108</td>
<td>800 ± 96</td>
</tr>
</tbody>
</table>

Data are means ± SEM (n = 13). * C12 (0.4) vs. control, C12 (0.1) and C12 (0.2): P < 0.05. CHO, carbohydrate.
Figure 1

Motility index of antral PWs

MI (mmHg)

Control  0.1  0.2  0.4

Treatment

*  **  #
Figure 2

A

**Basal pyloric pressure**

- Control
- 0.1 kcal/min
- 0.2 kcal/min
- 0.4 kcal/min

*Change from baseline (mmHg)*

**Time (min)**

B

**Number of IPPWs**

**No./ 15 min**

**Time (min)**
Figure 3

Motility index of duodenal PWs

MI (mmHg)

Control 0.1 0.2 0.4

Treatment

*#
Figure 4

Pressure wave sequences

Total no./90 min

Distance of propagation (cm)

Control
0.1 kcal/min
0.2 kcal/min
0.4 kcal/min
Figure 5

A
Plasma CCK

Conc. (pmol/l)

Time (min)

B
Plasma GLP-1

Conc. (pmol/l)

Time (min)