The droplet size of intraduodenal fat emulsions influences antropyloroduodenal motility, hormone release, and appetite in healthy males\textsuperscript{1–3}

Radhika V Seimon, Timothy Wooster, Bärbel Otto, Matthew Golding, Li Day, Tanya J Little, Michael Horowitz, Peter M Clifton, and Christine Feinle-Bisset

ABSTRACT

Background: The presence of fat in the small intestine modulates gastrointestinal motility, stimulates plasma cholecystokinin and peptide YY release, and suppresses appetite and energy intake. These effects are dependent on the lipolysis of fat.

Objective: Our aim was to evaluate the hypothesis that increasing the droplet size of a fat emulsion would attenuate these effects.

Design: Ten healthy, lean males were studied on 4 separate occasions in single-blind randomized order. Antropyloroduodenal pressures, plasma triglycerides, cholecystokinin, peptide YY, and appetite were measured during 120-min intraduodenal infusions of fat emulsions comprising 3 different droplet sizes: 1) 0.26 \(\mu\)m (LE-0.26), 2) 30 \(\mu\)m (LE-30), and 3) 170 \(\mu\)m (LE-170) in addition to saline (control). Energy intake at a buffet lunch was quantified immediately after the infusions.

Results: Increasing the droplet size of the lipid emulsion was associated with diminished suppression of antral \((r = 0.75, P < 0.01)\) and duodenal \((r = 0.80, P < 0.01)\) pressure waves and with stimulation of isolated \((r = -0.72, P < 0.01)\) and basal \((r = -0.83, P < 0.01)\) pyloric pressures. Increasing the droplet size was also associated with attenuation of the stimulation of plasma triglycerides \((r = -0.73, P < 0.001)\), cholecystokinin \((r = -0.73, P < 0.001)\), and peptide YY \((r = -0.83, P < 0.001)\) as well as with reductions in the suppression of hunger \((r = 0.75, P < 0.01)\) and energy intake \((r = 0.66, P < 0.001)\).

Conclusions: The acute effects of intraduodenal fat emulsions on gastrointestinal function and appetite are dependent on fat droplet size. These observations have implications for the design of functional foods to maximize effects on those gut functions that are involved in the suppression of appetite. Am J Clin Nutr 2009;89:1729–36.

INTRODUCTION

The dietary macronutrients, carbohydrates, fats, and protein, when ingested in a meal or infused directly into the small intestine, have a number of effects on gut function, including the modulation of gastrointestinal motility [by stimulating isolated pyloric pressure waves (IPPWs) and suppressing contractions in the antrum and duodenum (1), leading to an overall slowing of gastric emptying (2)] and the release of a number of gut hormones [including cholecystokinin (CCK) from the proximal (3), and peptide YY (PYY) from the distal, small intestine (4)]. The presence of nutrients in the small intestine also inhibits appetite and subsequent energy intake (5, 6). The effect of lipid on these functions is dependent on lipolysis; for example, when fat digestion is blocked by the lipase inhibitor orlistat, the effects of lipids on gastric emptying (7, 8), gut hormone release (9–12), appetite, and energy intake (9, 13, 14) are abolished.

Fat digestion is controlled primarily by the ability of lipase to bind to the surface of emulsion droplets, the size of which increases exponentially as droplet size decreases. Accordingly, physicochemical characteristics may influence fat digestion directly (15) and, thus, the effects of fat on gut function and energy intake. There is, however, little information about this, although informal comparisons of data from our previous studies suggest that this may be the case (9, 16). For example, intraduodenal infusion of Intralipid (droplet size of <1 \(\mu\)m; Fresenius Medical Care Australia Pty Ltd, Smithfield, Australia), at a load of 1.5 kcal/min, resulted in a peak plasma CCK concentration of \(\approx\)14 pmol/L and in a peak number of IPPWs of \(\approx\)2.1/min (16), whereas intraduodenal administration of another triglyceride emulsion, with a fatty acid profile identical to that of Intralipid but with a droplet size of \(\approx\)10 \(\mu\)m (17), resulted in a peak plasma CCK concentration of \(\approx\)6.5 pmol/L and in a peak number of IPPWs of \(\approx\)1.3/min, despite being infused intraduodenally at the higher load of 2.8 kcal/min (9). Hence, there is indirect evidence that an emulsion with a smaller droplet size has greater...
modulatory effects on gut function and appetite, probably because of more rapid release of fatty acids. We have reported that the presence of even small amounts of free fatty acids in the small intestinal lumen (<0.5 g) is critical for the modulation of gastric emptying, CCK and PYY release, appetite, and energy intake (18). It is also possible that the rapid digestion of small droplets results in more marked early responses, whereas, as a result of slower and more prolonged digestion of larger droplets, a more delayed and sustained response could occur. Such information is potentially relevant for the food industry, because in many foods fats are contained in a number of forms (for example, in an emulsified form in milk and cream or associated with cellular structures in meat), and modification of these may be fundamental to the design of foods with specific appetite-suppressant properties. Because the prevalence of obesity is increasing markedly, the search continues for strategies that effectively reduce food consumption yet do not have adverse effects on other body functions. We hypothesized that, during a 120-min intraduodenal infusion, increasing the droplet size of a fat emulsion would reduce effects on antropyloroduodenal (APD) motility, hormone release, appetite, and energy intake.

**SUBJECTS AND METHODS**

**Subjects**

Ten healthy males [age: 25 ± 3 y; range: 18–47 y; body mass index (in kg/m²): 22.8 ± 0.4; range: 21–25.3] participated in the study, which conformed to the guidelines set out in the Declaration of Helsinki of 1975 as revised in 1983. The number of subjects was based on power calculations derived from our previous studies (19, 20). We calculated that N = 10 subjects would allow us to detect a 15% difference in energy intake at a = 0.05 with a power of 80%. The study was approved by the Royal Adelaide Hospital Research Ethics Committee and was initiated on 8 April 2008. All subjects were unrestrained eaters, as determined by a score of <12 on the eating-restraint component of the 3-factor eating questionnaire (21). Subjects with significant gastrointestinal symptoms or disease—using medication known to affect gut function or appetite, cigarette smokers, or those consuming >20 g alcohol/d—were excluded. All subjects provided informed, written consent before their inclusion.

**Intraduodenal infusions**

Intralipid (10%, 300 mOsmol/kg, 1.1 kcal/mL, Fresenius Medical Care Australia Pty Ltd, Smithfield, NSW, Australia) was selected for its small droplet size of ≈0.26 μm. The 30- and 170-μm emulsions were made using 1.4 g of food-grade Tween 80 (Kerry Bio-Science, Zwijndrecht, Netherlands), which was dissolved in 461.1 g of water by mixing the solution with a magnetic stirrer at 1000 rpm. After the Tween 80 was dissolved, 43.3 g of canola oil (Crisco, Meadow Foods, Macquarie Park, NSW, Australia) was added gradually under further stirring. The emulsion was mixed for a further 5 min, and the resulting droplet size was ≈170 μm. To obtain a droplet size of 30 μm, the emulsion was then sheared with a mixer-emulsifier (model DR 4895; Silverson Machines Ltd, London, UK) on its lowest setting for 2 min. Droplet sizes of all 3 emulsions (Figure 1) were quantified by laser light scattering using a Mastersizer 2000 (Malvern Instruments, Malvern, UK). The emulsions were kept stable by continually stirring at 1000 rpm throughout the study. To match the fat and energy content of the 30- and 170-μm emulsions, 381.8 ml Intralipid were diluted with 100.6 ml 0.9% saline. All infusions [including the control (0.9% saline)] were administered at a rate of 3.2 ml/min; thus, a total volume of 384 ml was infused on each study day and the lipid emulsions were delivered at 2.8 kcal/min (a total of 336 kcal). On each study day both the infusion pump and the tubing were covered with a sheet so that the subjects were blinded to the treatments.

![Graph of droplet size distributions](https://academic.oup.com/ajcn/article-abstract/89/6/1729/4596780)

**Protocol**

Subjects were provided with a standardized meal (beef lasagna: McCain Foods, Wendouree, Victoria, Australia; or spinach and ricotta ravioli: Lean Cuisine, Rhodes, NSW, Australia) to be consumed on the evening before each study day at 2000 and were instructed to fast overnight from solids and liquids thereafter before attending the laboratory at 0830. Each subject was studied on 4 occasions, separated by 3–10 d, on which they received in a single-blind, randomized order, intraduodenal infusions of either isotonic saline (control) or 1 of the 3 lipid emulsions with different droplet sizes: 1) 0.26 μm (LE-0.26), 2) 30 μm (LE-30), or 3) 170 μm (LE-170). Immediately after the subject arrived in the laboratory, a 16-channel manometric APD catheter (Dentsleeve International Ltd, Ontario, Canada) was inserted through an anesthetized nostril into the stomach and positioned across the pylorus as described (1). Fastig motility was then monitored until the occurrence of a phase III of the migrating motor complex and an intravenous cannula was inserted into a forearm vein for blood sample collection. At t = −10 min, a baseline blood sample was taken and a visual analog scale (VAS) questionnaire, which assessed perceptions of appetite (22), was completed. At t = 0 min, duodenal infusions of 1) control, 2) LE-0.26, 3) LE-30, or 4) LE-170 commenced for 120 min. During the infusions, 10-ml blood samples were obtained, and a VAS was completed every 10 min between t = 0 min and t =
30 min, every 15 min between \( t = 30 \) min and \( t = 90 \) min, and at \( t = 120 \) min. At \( t = 120 \) min, the infusion was discontinued, and the subject extubated and offered a cold buffet-style meal to consume freely until comfortably full for \( \leq 30 \) min (\( t = 120–150 \) min) (23). After ingestion of the meal, at \( t = 120 \) min, subjects were allowed to leave the laboratory.

**Data analysis**

Manometric pressures were digitized and recorded on a computer-based system that runs commercially available software (HAD, A/Prof GS Hebbard, Royal Melbourne Hospital, Australia), and stored for subsequent analysis. Using previously described criteria, APD pressures were analyzed for 1) the number and amplitude of antral and duodenal pressure waves (PWs) and 2) the basal pyloric pressure and the number and amplitude of IPPWs (24, 25).

For subsequent analysis of triglycerides, CCK, and PYY, 10-ml venous blood samples were collected in ice-chilled EDTA-treated tubes containing 400 kIU aprotinin (Trasylol; Bayer Australia Ltd, Pymble, Australia) per milliliter of blood. Plasma was obtained by centrifugation of blood samples at 3200 rpm for 15 min at 4°C. The plasma samples were frozen at −70°C.

Plasma CCK concentrations (pmol/L) were determined by a sensitive and specific radioimmunoassay, as described (26). In short, the antibody (CH40IX), raised in rabbits, was specific and sensitive to full-length CCK octapeptide, as well as to the 24-amino acid octapeptide and the 8-amino acid tetrapeptide. The antibody (CH40IX) was used in a two-site radioimmunoassay, utilizing 

\[ ^{125}I \text{Iodine labeled CCK} \] as the tracer and a CCK antiserum to determine the concentration of active CCK by the double antibody/PEG technique. The antiserum was raised in rabbits and recognizes both the C-terminal and N-terminal forms of CCK-8.

Blood samples were collected before and after each infusion, for measurement of plasma CCK concentrations (pmol/L) using a commercially available radioimmunoassay (Linco Research Inc, St Charles, MO) by using \(^{125}\)I-labeled bioactive PYY as the tracer and a PYY antiserum to determine the concentration of active PYY by the double antibody/PEG technique. The PYY antibody was raised in guinea pigs and recognizes both the PYY(1–36) and the PYY(3–36) forms of human PYY; ie, the assay does not distinguish between PYY(1–36) and PYY(3–36). The intraassay CV was 5.3%.

Perceptions of hunger and fullness were rated using a validated VAS questionnaire (22). Nausea and bloating were also assessed. Each VAS consisted of a 100-mm horizontal line, where 0 represented “sensation not felt at all” and 100 represented “sensation felt the greatest.” Subjects placed a vertical mark along the horizontal line to indicate the strength of the sensation felt at that particular time point. The buffet meal was weighed before and after consumption to determine the amount eaten (g), and for evaluation of energy intake (kJ) and macronutrient distribution (percentage of energy from fat, carbohydrate, and protein) using the software program Foodworks (version 3.01; Xyris Software, Highgate Hill, Australia) (23).

**Statistical analysis**

Motility and VAS data were expressed as changes from the baseline and plasma triglyceride and hormone concentrations as absolute values. Areas under the curve (AUCs) were calculated by using the trapezoidal rule for basal pyloric pressures, IPPWs, plasma triglyceride and hormone concentrations, and VAS scores and were analyzed using one-factor analysis of variance (ANOVA). Treatment by time interactions for these variables were also assessed using repeated-measures ANOVA with time and treatment as factors. The number and amplitude of antral and duodenal PWs and energy intake were analyzed by one-factor ANOVA. Post hoc comparisons, adjusted for multiple comparisons by Bonferroni’s correction, were performed if ANOVAs revealed significant effects. Statistical significance was accepted at \( P < 0.05 \).

**RESULTS**

All subjects tolerated the experimental conditions well, except one who vomited at 110 min after commencement of the LE-0.26 infusion, at which time the infusion was discontinued. For the purpose of the statistical analysis, data were extrapolated for the final 10 min.

**Antral pressures**

There was a significant effect of treatment on the number (\( P < 0.001 \)) and amplitude (\( P < 0.001 \)) of antral PWs (Table 1). Both were less during administration of LE-0.26 (\( P < 0.001 \)), LE-30 (\( P < 0.001 \)), and LE-170 (\( P < 0.001 \)) compared with the control, and the number of antral PWs was also greater during administration of LE-170 compared with LE-0.26 (\( P < 0.05 \)), with no difference between LE-30 and LE-0.26 or between LE-170 and LE-30. There was a direct relation between the number (\( r = 0.83, P = 0.01 \)) and amplitude (\( r = 0.75, P = 0.01 \)) of antral PWs with the emulsion droplet size.

**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>Total no.</th>
<th>Mean amplitude (mm Hg)</th>
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</thead>
<tbody>
<tr>
<td>Antral PWs</td>
<td>Duodenal PWs</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>205 ± 38</td>
<td>1134 ± 184</td>
</tr>
<tr>
<td>LE-0.26</td>
<td>21 ± 6(^i)</td>
<td>328 ± 39(^j)</td>
</tr>
<tr>
<td>LE-30</td>
<td>33 ± 15(^j)</td>
<td>540 ± 64(^j)</td>
</tr>
<tr>
<td>LE-170</td>
<td>116 ± 36(^j)</td>
<td>744 ± 97(^j)</td>
</tr>
<tr>
<td>Antral PWs</td>
<td>Duodenal PWs</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>61 ± 9</td>
<td>26 ± 1</td>
</tr>
<tr>
<td>LE-0.26</td>
<td>22 ± 3(^j)</td>
<td>23 ± 3</td>
</tr>
<tr>
<td>LE-30</td>
<td>20 ± 3(^j)</td>
<td>23 ± 2</td>
</tr>
<tr>
<td>LE-170</td>
<td>3 ± 9(^j)</td>
<td>25 ± 1</td>
</tr>
</tbody>
</table>

\(^i\) Values are means ± SEMs; \( n = 10 \).

\(^j\) Significantly different from control, \( P < 0.05 \) (one-factor ANOVA).

\(^k\) Significantly different from LE-0.26, \( P < 0.05 \) (one-factor ANOVA).
Pyloric pressures

Basal pyloric pressure

There was a significant effect of treatment on AUCs for basal pyloric pressures ($P < 0.01$) (Table 2, Figure 2). Basal pyloric pressure was greater during administration of LE-0.26 compared with the control ($P < 0.01$) and lower during administration of LE-30 ($P < 0.05$) and LE-170 ($P < 0.01$) compared with LE-0.26, with no difference between LE-30, LE-170, and the control or between LE-170 and LE-30. There was an inverse relation between basal pyloric pressure with the emulsion droplet size ($r = -0.83, P = 0.01$).

Isolated pyloric pressure waves

There was a significant effect of treatment on AUCs for the number ($P < 0.001$), but not for the amplitude, of IPPWs (Table 2, Figure 2B). The number of IPPWs was greater during administration of LE-0.26 ($P < 0.001$) and LE-30 ($P < 0.01$) compared with the control and lower during administration of LE-170 ($P < 0.001$) and LE-30 ($P < 0.05$) compared with LE-0.26, with no difference between LE-170 and the control or between LE-170 and LE-30. There was an inverse relation between the number ($r = -0.72, P = 0.01$), but not the amplitude, of IPPWs with the emulsion droplet size.

Duodenal pressure waves

There was a significant effect of treatment on the number ($P < 0.001$), but not on the amplitude, of duodenal PWs (Table 1). The number of duodenal PWs was lower during administration of LE-0.26 ($P < 0.001$), LE-30 ($P < 0.01$), and LE-170 ($P < 0.05$) compared with the control and greater during administration of LE-170 compared with LE-0.26 ($P < 0.01$), with no difference between LE-30 and LE-0.26 or between LE-170 and LE-30. There was a direct relation between the number ($r = 0.80, P = 0.01$), but not the amplitude, of duodenal PWs with emulsion droplet size.

Plasma triglyceride, CCK, and PYY concentrations

Plasma triglycerides

There was a significant effect of treatment on AUCs for plasma triglycerides between $t = 60$ min and $t = 120$ min ($P < 0.001$) (Table 2, Figure 3). Plasma triglyceride was greater during administration of LE-0.26 ($P < 0.001$) compared with the control, with no other differences. There was an inverse relation between plasma triglyceride concentrations ($r = -0.73, P = 0.001$) and emulsion droplet size.

Plasma CCK

There was a significant effect of treatment on AUCs for plasma CCK ($P < 0.001$) (Table 2, Figure 3B). Plasma CCK was greater during administration of LE-0.26 ($P < 0.001$) compared with the control and lower during administration of LE-170 ($P < 0.001$) and LE-30 ($P < 0.01$) compared with LE-0.26, with no difference between LE-30, LE-170, and the control or between LE-170 and LE-30. There was an inverse relation between plasma CCK concentrations ($r = -0.73, P = 0.001$) and emulsion droplet size.

Plasma PYY

There was a significant effect of treatment on AUCs for plasma PYY ($P < 0.001$) (Table 2, Figure 3C). Plasma PYY was greater during administration of LE-0.26 ($P < 0.001$), LE-30 ($P < 0.001$), and LE-170 ($P < 0.05$) compared with the control and lower during administration of LE-170 ($P < 0.001$) and LE-30 ($P < 0.001$) compared with LE-0.26, with no difference between LE-170 and LE-30. There was an inverse relation between plasma PYY concentrations ($r = -0.83, P = 0.001$) and emulsion droplet size.

Appetite perceptions and nausea

Hunger and fullness

There was a significant effect of treatment on AUCs for hunger ($P < 0.01$) (Table 2, Figure 4A). Hunger was lower during

<p>| TABLE 2 |</p>
<table>
<thead>
<tr>
<th>Areas under the curve for basal pyloric pressure; number and amplitude of isolated pyloric pressure waves (IPPWs); plasma triglyceride, cholecystokinin (CCK), and peptide YY (PYY) concentrations; and scores for hunger, fullness, bloating, and nausea during 120-min duodenal infusions of saline (control) or lipid emulsions (at 2.8 kcal/min) with different droplet sizes: 0.26 μm (LE-0.26), 30 μm (LE-30), or 170 μm (LE-170)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>Basal pyloric pressure (mm Hg × min)</td>
</tr>
<tr>
<td>No. of IPPWs (min)</td>
</tr>
<tr>
<td>Amplitude of IPPWs (mm Hg × min)</td>
</tr>
<tr>
<td>Plasma triglyceride (mmol/L × min)</td>
</tr>
<tr>
<td>Plasma CCK (pmol/L × min)</td>
</tr>
<tr>
<td>Plasma PYY (pmol/L × min)</td>
</tr>
<tr>
<td>Hunger (mm × min)</td>
</tr>
<tr>
<td>Fullness (mm × min)</td>
</tr>
<tr>
<td>Bloating (mm × min)</td>
</tr>
<tr>
<td>Nausea (mm × min)</td>
</tr>
</tbody>
</table>

1 Values are means ± SEMs; $n = 10$. Areas under the curve were calculated using the trapezoidal rule. Negative values are due to the expression of data as changes from the baseline.

2 Significantly different from control, $P < 0.05$ (one-factor ANOVA).

3 Significantly different from LE-0.26, $P < 0.05$ (one-factor ANOVA).
administration of LE-0.26 ($P < 0.01$) compared with the control and greater during administration of LE-170 ($P < 0.01$) and LE-30 ($P < 0.01$) compared with administration of LE-0.26, with no difference between LE-30, LE-170, and the control or between LE-170 and LE-30. There was a direct relation between hunger ($r = 0.75$, $P = 0.05$) and emulsion droplet size. There was no significant effect of treatment on AUCs for fullness (Table 2).

**Bloating**

There was a significant effect of treatment on AUCs for bloating ($P < 0.01$) (Table 2, Figure 4B). Bloating was greater during administration of LE-0.26 ($P < 0.01$) compared with the control and lower during administration of LE-170 ($P < 0.01$) and LE-30 ($P < 0.01$) compared with administration of LE-0.26, with no difference between LE-30, LE-170, and the control or between LE-170 and LE-30. There was an inverse relation between bloating ($r = -0.72$, $P = 0.01$) and emulsion droplet size.

**Nausea**

There was a significant effect of treatment of nausea ($P < 0.05$) (Table 2, Figure 4C). Although scores were very low, nausea was greater during administration of LE-0.26 ($P < 0.01$) compared with the control and lower during administration of LE-170 ($P < 0.01$) and LE-30 ($P < 0.01$) compared with LE-0.26, with no difference between LE-30, LE-170, and the control or between LE-170 and LE-30. There was an inverse

**FIGURE 2.** Mean ($\pm$ SEM) basal (A) and isolated (B) pyloric pressures (IPPWs) during 120-min duodenal infusions ($t = 0–120$ min) of saline (control) or lipid emulsions (at 2.8 kcal/min) with different droplet sizes: 0.26 $\mu$m (LE-0.26), 30 $\mu$m (LE-30), or 170 $\mu$m (LE-170). Pressures were recorded using high-resolution manometry. Repeated-measures ANOVA was used to determine statistical difference. Treatment $\times$ time interaction, $P < 0.001$: *$P < 0.05$ compared with control; #$P < 0.05$ compared with LE-30; and §$P < 0.01$ compared with LE-170. Data are expressed as changes from the baseline; $n = 10$.

**FIGURE 3.** Mean ($\pm$ SEM) plasma concentrations of triglycerides (A), cholecystokinin (CCK) (B), and peptide YY (PYY) (C) during 120-min duodenal infusions ($t = 0–120$ min) of saline (control) or lipid emulsions (at 2.8 kcal/min) with different droplet sizes: 0.26 $\mu$m (LE-0.26), 30 $\mu$m (LE-30), or 170 $\mu$m (LE-170). Blood samples were collected at the time points indicated throughout the study, and the plasma was frozen for later analysis by an enzymatic colorimetric test (for triglycerides) or by radioimmunoassay (for CCK and PYY). Repeated-measures ANOVA was used to determine statistical difference. A: Treatment $\times$ time interaction, $P < 0.001$: *$P < 0.05$ compared with control; 6$P < 0.05$ compared with control; 7$P < 0.05$ compared with control; 6$P < 0.01$ compared with control; 7$P < 0.01$ compared with LE-170; C: Treatment $\times$ time interaction, $P < 0.001$: *$P < 0.05$ compared with control; 6$P < 0.01$ compared with control; 7$P < 0.01$ compared with LE-30; 8$P < 0.01$ compared with LE-170. Data are absolute values; $n = 10$. 
relation between nausea \((r = -0.72, P = 0.01)\) and emulsion droplet size.

**Energy intake and amount eaten**

There was a trend for an effect of treatment on energy intake and the amount eaten \((P = 0.1)\) (Table 3), and energy intake tended to be less after administration of LE-0.26 \((P = 0.08)\), LE-30 \((P = 0.05)\), and LE-170 \((P = 0.16)\) when compared with the control. There were weak direct relations between both energy intake \((r = 0.66, P = 0.001)\) and the amount eaten \((r = 0.53, P = 0.001)\) and emulsion droplet size. There was no effect of treatment on the percentage of energy from fat, carbohydrate, or protein consumed at the buffet meal (Table 2).

**Relation among APD pressures, hormone release, appetite, and energy intake**

There were no significant relations among these variables (data not shown).

**DISCUSSION**

In Western society, the prevalence of obesity has more than doubled over the last 20 y, meaning that 53\% of women and 68\% of men are overweight, and of these >20\% are obese. Hence, there is an urgent need for effective prevention and treatment strategies for obesity that are not associated with significant adverse effects. Accordingly, there is increasing interest in manipulating the structure of food components to maximize suppressant effects on appetite and energy intake. Our study establishes that, when administered intraduodenally, the droplet size of a lipid emulsion has major effects on APD motility, gastrointestinal hormone release, and appetite in healthy, lean males. Increased droplet size was associated with significant reductions in the suppression of antral and duodenal pressures, the stimulation of isolated and basal pyloric pressures, plasma CCK and PYY concentrations, and the suppression of appetite and energy intake effects known to be dependent on fat digestion (7, 9, 12). The observed effects of droplet size on gastrointestinal motor and hormonal function were marked, such that the emulsion with the smallest droplet size had much more potent effects, presumably because this emulsion, due to its large surface area, showed the most efficient digestion. This concept is also supported by the plasma triglyceride responses; concentrations rose most markedly during LE-0.26 infusion.

It is well established that small intestinal fat stimulates IPPWs and suppresses antral and duodenal pressures (1, 27). The effects on pyloric pressures were evident shortly after commencement of infusion with all 3 emulsions, although the effect of the larger droplet emulsions (LE-30 and LE-170) was markedly less, and LE-170 failed to stimulate basal pyloric pressures. Despite the continued nutrient influx, there was a gradual reduction in the number of IPPWs, particularly during LE-0.26 after the initial peak was reached, which suggests an

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>LE-0.26</th>
<th>LE-30</th>
<th>LE-170</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy intake (kJ)</td>
<td>5072 ± 611</td>
<td>4514 ± 619</td>
<td>4445 ± 468</td>
<td>4623 ± 599</td>
</tr>
<tr>
<td>Amount eaten (g)</td>
<td>1286 ± 112</td>
<td>1124 ± 130</td>
<td>1078 ± 63</td>
<td>1163 ± 111</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>32 ± 2</td>
<td>30 ± 2</td>
<td>34 ± 2</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>47 ± 3</td>
<td>48 ± 3</td>
<td>43 ± 3</td>
<td>48 ± 3</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>21 ± 2</td>
<td>22 ± 1</td>
<td>23 ± 2</td>
<td>21 ± 1</td>
</tr>
</tbody>
</table>

\(^{7}\) Values are means ± SEMs; \(n = 10\). There was no significant effect of treatment of any of the variables (one-factor ANOVA).

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**FIGURE 4.** Mean (±SEM) scores for hunger (A), bloating (B), and nausea (C) during 120-min duodenal infusions \((t = 0–120\,\text{min})\) of saline (control) or lipid emulsions (at 2.8 kcal/min) with different droplet sizes: 0.26 \(\mu\text{m}\) (LE-0.26), 30 \(\mu\text{m}\) (LE-30), or 170 \(\mu\text{m}\) (LE-170). Appetite scores were measured using visual analog scale questionnaires and are expressed as changes from the baseline. Repeated-measures ANOVA was used to determine statistical difference. A: Treatment × time interaction, \(P < 0.01\); \(\ast P < 0.01\) compared with control; \(\ast \# P < 0.01\) compared with LE-30; \(\ast \# \# P < 0.05\) compared with LE-170; \(\ast \# \# \#\) applies to \(t = 10–120\,\text{min} \) for LE-0.26. B: Treatment × time interaction, \(P < 0.001\); \(\ast P < 0.01\) compared with control; \(\ast \# P < 0.001\) compared with LE-30; and \(\ast \# \# P < 0.001\) compared with LE-170. C: Treatment effect, \(P < 0.05\); \(\ast P < 0.01\) compared with control; \(\ast \# P < 0.01\) compared with LE-30; \(\ast \# \# P < 0.01\) compared with LE-170. Data are expressed as changes from the baseline; \(n = 10\).
EMULSION DROPLET SIZE, GUT FUNCTION, AND APPETITE

CCK and PYY are released potently by fat from the proximal and distal small intestine, respectively (3, 4). The pattern of CCK release was similar to that of pyloric stimulation, although only LE-0.26 and LE-30 stimulated plasma CCK over time, and LE-0.26 was much more potent. Moreover, during LE-30 infusion, CCK concentrations were much lower than those during LE-0.26 infusion where CCK increased almost immediately, presumably because the smaller droplet size emulsion facilitated digestion in the proximal small intestine where CCK is released (29). With increasing droplet size, it would be anticipated that feedback slowing of small intestinal transit would be diminished, so that the contact time with CCK-releasing cells would be increasingly less with increasing droplet size because a greater proportion of nutrient is transported further downstream. These concepts are supported by a previous study in our laboratory in which CCK release in response to glucose in the small intestine was shown not to differ whether glucose was allowed access into the proximal (first 60 cm) or the entire small intestine (30). Patterns of PYY release differed significantly from that of CCK, in that PYY was released more slowly and progressively throughout the infusion period, which was consistent with the release of PYY predominantly from the distal small intestine and colon (4). During LE-0.26 infusion, PYY release was greatest and commenced within 20 min of the start of the infusion. This initial rise may relate to the stimulatory effect of endogenous CCK on PYY release (31, 32).

Previous studies suggest that pyloric stimulation (33), endogenous CCK (34), and exogenous PYY (35) all contribute to the suppression of appetite and energy intake. The results from our study establish that the emulsion with the smallest droplet size, which most potently modulated gastrointestinal motor and hormonal responses, caused the greatest suppression of hunger.

Although there were no significant differences in either energy intake or the amount eaten among the 4 treatments, there were inverse relationships between both energy intake and the amount eaten with droplet size, which suggests that modulation of fat structure may be a meaningful approach to the design of foods with specific appetite-suppressant properties.

The limitations of our study should be recognized. Only healthy males were included, because they have been reported to be more sensitive to dietary manipulation than females (36), and all had a normal body weight. Hence, we cannot extend our findings to female and/or overweight subjects. Although it is intuitively probable that similar observations would be evident in the obese, specific studies are warranted, given that this is likely to be the most relevant target population. We used intraduodenal infusion in this study to bypass orosensory and gastric influences on gut function and appetite. We are unable to comment on the potential effects of the infusions beyond 120 min; for example, the release of PYY during LE-30 and LE-170 infusion most likely continued to increase with associated effects on energy intake. This issue warrants evaluation in future studies. We did not measure other gut hormones, including ghrelin and glucagon-like peptide 1, but it is very likely that these would have exhibited similar patterns of release to those of CCK and PYY on the basis of outcomes from our previous studies (9, 11, 23, 37). Further studies are also needed to characterize the fatty acid thresholds for effects on motility, hormone release, and appetite. Our observations suggest that these are likely to differ (eg, the threshold for the stimulation of CCK may be greater than that required to stimulate IPPWs). We have shown that gastric emptying of only a small amount of fatty acid slows further gastric emptying (18). The interactions among gastrointestinal motility, hormone release, appetite, and energy intake are of considerable interest, and the absence of any significant correlations may reflect the relatively small number of subjects. Our study was powered to detect differences among the emulsions, which was shown to be the case. Although our emulsions were designed to ensure the stability of the droplets in the small intestinal environment, it is feasible to create structured emulsions that form particles of specific sizes in the intragastric environment, which are, in turn, associated with differential effects on gastric emptying and gut hormone release (J Keogh, T Wooster, M Golding, H French, M Xu, and Clifton PM; unpublished observations, 2008).

In conclusion, our results establish that, when administered directly into the small intestine, the effects of fat emulsions on gastrointestinal motility, hormone release, appetite, and energy intake are related to their droplet size. These findings have potential implications for the design of functional foods to maximize effects on the gut functions that are involved in the suppression of appetite and could lead to novel approaches to the prevention and management of obesity.

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