Effects of intraduodenal lipid and protein on gut motility and hormone release, glycemia, appetite, and energy intake in lean men

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ABSTRACT

Background: Intraduodenal lipid modulates gastrointestinal motility and hormone release and suppresses energy intake (EI) more than does intraduodenal glucose. Oral protein is the most satiating macronutrient and modulates postprandial glycemia; the comparative effects of intraduodenal protein and lipid and their combined effects are unclear.

Objective: We investigated the effects of intraduodenal protein and lipid, alone or in combination, on antropyloroduodenal motility, gastrointestinal hormone release, glycemia, and EI.

Design: Twenty lean men were studied on 5 randomized, double-blind occasions. Intraduodenal lipid or protein were administered over an 80-min period (3 kcal/min) and EI, subjective appetite, and gastrointestinal symptoms were assessed for 3 h post-prandially.

Results: In comparison with the control, all nutrient infusions suppressed antral and duodenal motility and stimulated pyloric pressures (P < 0.05). Cholecystokinin and GLP-1 release and pyloric stimulation were lipid-load dependent (r = 0.39, P < 0.01), insulin and glucagon releases were protein-load dependent (r = 0.83, P < 0.001), and normoglycemia was maintained. L3 but not P3 increased nausea (P < 0.05). Compared with the control, L3 and P3 but not L2P1 or L1P2 suppressed EI (P < 0.05) without major effects on appetite.

Conclusions: In lean men, despite differing effects on gut function, intraduodenal lipid and protein produce comparable reductions in energy intake. The effects of lipid may be a result of nausea. Protein also regulates blood glucose by stimulating insulin and glucagon. In contrast, at the loads selected, lipid:protein combinations did not suppress energy intake, suggesting that a threshold load is required to elicit effects. This trial was registered at Australia and New Zealand Clinical Trial Registry (http://www.anzctr.org.au) as 12609000949280.

INTRODUCTION

An understanding of gastrointestinal sensing of nutrients and its role in the regulation of food intake has major implications for a number of disorders, which are associated with altered gastrointestinal responses to nutrients (1–5). For example, in obese humans, oral and intraduodenal responses to fat are diminished in comparison with lean subjects (1, 2), whereas the satiating effect of oral protein persists (2). Elderly subjects have higher plasma cholecystokinin concentrations, both fasting and in response to intraduodenal lipid, and are less hungry than healthy young subjects (3, 4). In patients with functional dyspepsia, the oral ingestion of a fatty meal or intraduodenal infusion of a triglyceride emulsion induces much greater upper gastrointestinal symptoms than in healthy subjects and compared with carbohydrate (6). The characterization of gastrointestinal effects of nutrients and their relation with the regulation of energy intake, blood glucose, and gastrointestinal symptoms will be an important step to understand the role of dietary nutrients, as well as changes in the diet, for these disorders.

In healthy humans, dietary macronutrients modulate gastrointestinal motor and hormone function in a load-dependent manner (7–9). The stimulation of pyloric motility and release of gut hormones, including cholecystokinin (7, 10) and glucagon-like peptide-1 (GLP-1) (7, 8), slow gastric emptying (11), modulate glycemia (12), and suppress energy intake (7–9, 13). Pyloric pressures and plasma cholecystokinin are independent determinants of acute energy intake in healthy individuals in response to intraduodenal lipid and carbohydrate (14). Although intraduodenal whey protein, infused at loads reflecting average gastric emptying of nutrients (0.5–4 kcal/min) suppressed energy intake...
SUBJECTS AND METHODS

Subjects

Twenty healthy, lean men [mean ± SEM age: 27 ± 3 y (range: 18–58 y); BMI (in kg/m²): 22.4 ± 0.4 (range 18.5–24.8); waist circumference: 79 ± 1 cm] participated in the study (see Supplemental Figure 1 under “Supplemental data” in the online issue). The number of subjects was based on power calculations on the basis of our previous studies and indicated that n = 20 subjects would allow the detection of a 150-kcal difference in energy intake with β = 0.8 and α = 0.05 (9, 22). Participants were recruited from an existing pool of volunteers or through an advertisement in local newspapers. All subjects had been weight stable (≤5% of their screening weight) for ≥3 mo before the study and were untrained eaters [score ≤12 on the eating restraint component of the Three-Factor Eating Questionnaire (23)]. No subject currently smoked, consumed >20 g alcohol/wk, had a history of gastrointestinal symptoms, or was taking medication known to affect energy intake, appetite, or gastrointestinal function. The Royal Adelaide Hospital Research Ethics Committee approved the study protocol (22 October 2009), and all subjects provided written informed consent before their inclusion. Once subjects were enrolled in the study, they were allocated a random sequence of all 5 infusions generated by the #RAN function in the Microsoft Excel program (Microsoft Corp) by a research officer. Subject and investigators who assessed outcomes (ATR and AAS) were blinded to the random allocation sequence. This trial was registered at Australia and New Zealand Clinical Trial Registry (http://www.anzctr.org.au) as 12609000949280.

Study outline

The study compared the effects of 1) 3-kcal lipid/min [pure lipid condition (L3)], 2) 2-kcal lipid/min + 1-kcal protein/min [2:1 lipid:protein condition (L2P1)], 3) 1-kcal lipid/min + 2-kcal protein/min [1:2 lipid:protein condition (L1P2)], 4) 3-kcal protein/min [pure protein condition (P3)], and 5) a saline control [control condition (C)] infused intraduodenally for 90 min on antropyloroduodenal motility, gastrointestinal hormone release, serum insulin, plasma glucagon, blood glucose, appetite, and energy intakes.

Intraduodenal nutrient infusions

We used direct intraduodenal infusion of nutrients in our study. During the oral ingestion of meals, the delivery of nutrients to the small intestine (ie, where receptors that sense nutrients and initiate feedback responses are concentrated) is dependent on the rate of gastric emptying. Because there is substantial interindividual and intraindividual variation in rates of gastric emptying (24) and, thus, varying influences from gastric distension (4), the intraduodenal infusion of nutrients bypasses the stomach, while allowing for the delivery of nutrients to the small intestinal lumen at standardized rates that reflect average rates of gastric emptying. In addition, intraduodenal nutrient delivery allowed us to exclude any orosensory influences (including those from taste, smell, and texture) of foods. Although the study paradigm did not directly reflect all influences that occurred during oral meal ingestion, it enabled us to study the small intestinal contributions in detail, which was the primary focus of this study.

Intralipid (a soy-based emulsion consisting primarily of linoleic, oleic, and palmitic acids resembling fatty acids that are common in the diet; 20%, 300 mOsm/L; energy density: 2 kcal/mL; Baxter Healthcare) was used as the lipid source, and whey protein [hydrolyzed whey protein isolate DH17 Ultra (18.5% hydrolyzed), MyoPure; Muscle Brand Pty Ltd) was used as the protein source. As a component of dairy products, whey is also common in the diet and has generally been shown to be the most satiating protein source when consumed orally (25, 26). We have used both Intralipid and whey protein extensively in our previous studies to evaluate the role of macronutrients on gastrointestinal function and appetite (7, 9).

On the morning of each study day isosmotic (480 mOsm/L) infusion solutions were prepared in 420-mL batches by a research officer who was not involved in either the analysis or interpretation of data. Protein stock solutions were made by dissolving 28 g whey protein hydrolysate powder (to contribute 1 kcal/min of protein for L2P1 infusion) or 56 g whey protein hydrolysate powder (to contribute 2 kcal/min of protein for L1P2 infusion) in distilled water to a volume of 140 or 280 mL, respectively. Solutions of 1.67%, 1.7%, and 1.8% NaCl were prepared by dissolving 6.9, 7.14, or 7.56 g NaCl in 420 mL distilled water, respectively. The L3 solution consisted of 157.5 mL Intralipid and 262.5 mL 1.67% NaCl solution. To make up the mixed-nutrient solutions (L2P1 and L1P2), 105 mL Intralipid and 87.5 mL 1.8% NaCl solution were added to the 1 kcal/min of protein stock solution, and 52.5 mL Intralipid and 175 mL 1.7% NaCl solution were added to the 2 kcal/min of protein stock solution. The P3 solution consisted of 84 g protein hydrolysate powder in 420 mL distilled water, and the C consisted of 420 mL 1.4% NaCl solution (6 g NaCl in 420 mL distilled water). All solutions were

(7), its effects on gastrointestinal motility and hormone release seemed weaker than with lipid (9), and thus, effects of whey on energy intake may be mediated by alternative mechanisms. Whey protein also stimulates insulin release and reduces postprandial glycemic excursions in both healthy individuals (15) and type 2 patients with diabetes (16, 17). Although the mechanisms that underlie the effects of protein on glycemia are not fully understood, the effect is at least in part because of the release of the incretin hormones, GLP-1, and glucose-dependent insulinotropic peptide (GIP) and the stimulation of pancreatic β cells by circulating amino acids (15, 18). Furthermore, cholecystokinin (19) and GLP-1 (20) slow gastric emptying, which is a major determinant of postprandial glycemia (12, 21).
administered at a rate of 4 mL/min, delivering 360 mL over 90 min, and nutrient-containing infusions provided a total of 270 kcal. The infusion apparatus was covered at all times, and thus, the investigators who performed the studies (ATR and AAS) and subjects were blinded to the treatment.

**Protocol**

Each subject was studied on 5 occasions, each of which was separated by 3–10 d, in a double-blind, randomized, crossover design. Subjects were provided with a standardized meal (beef lasagna; energy content: 519 kcal; McCain Foods) to consume at 1900 the day before each study. Thereafter, subjects were asked to refrain from all solids and liquids and any strenuous physical activity before attending the Department of Medicine at 0830 the next morning.

On arrival, a small-diameter (outer diameter: 3.5 mm) manometric catheter (total length: 100 cm; Dentsleeve International, Mui Scientific) was inserted into the stomach through an anesthetized nostril and allowed to pass into the duodenum by peristalsis. The catheter contained 16 side holes spaced at 1.5-cm intervals, with an additional channel used for intraduodenal infusions located 11.75 cm distal to the end of the sleeve sensor (14.5 cm from the pylorus), and was positioned as described previously (7). Once the manometric catheter was in position (which took between 15–40 min), fasting motility was monitored until the occurrence of phase III of the interdigestive migrating motor complex, which took between 15–40 min (which took between 15–40 min), fasting motility was monitored until the occurrence of phase III of the interdigestive migrating motor complex. Immediately after cessation of phase III activity, an intravenous cannula was placed in a right forearm vein for blood sampling. At $t = -15$ min (during motor quiescence (i.e., phase I of the migrating motor complex)), a baseline blood sample (14 mL) was taken, and a visual analog scale (VAS) questionnaire (27) to assess appetite perceptions and gastrointestinal symptoms completed by the subject. The baseline measurement of antropyloroduodenal motility was commenced. At $t = 0$ min, the 90-min intraduodenal infusion of one of the 5 treatments commenced. During the infusion, antropyloroduodenal motility was monitored continuously, and VAS ratings and blood samples were obtained at 15-min intervals. At $t = 90$ min, the infusion was terminated, and the intraduodenal catheter was removed. Subjects were presented with a standardized cold, buffet-style meal in excess of what they would be expected to consume and instructed to eat freely for $\leq 30$ min ($t = 90–120$ min) until comfortably full (28). The composition of the buffet meal has been described previously (28). After ingestion of the meal, at $t = 120$ min, a final blood sample was taken, and the VAS was completed, before the intravenous cannula was removed, and the subject was allowed to leave the laboratory.

**Measurements**

**Antropyloroduodenal motility**

Antropyloroduodenal pressures were digitized and recorded by using a computer-based system running commercially available software (Flexisoft Version 3; Oakfield Instruments, GS Hebbard) and stored for subsequent analysis. Antropyloroduodenal pressures were analyzed for the number and amplitude of antral, duodenal, and isolated pyloric pressure waves (IPPs) as well as basal pyloric pressure (BPP) with custom-written software modified to our requirements (A Smout, University Medical Centre, Amsterdam, Netherlands). The definition of pressure waves (PWs), and calculation of BPP have been described previously in detail (7).

**Plasma cholecystokinin and GLP-1, serum insulin, plasma glucagon, and blood glucose concentrations**

Venous blood samples (~14 mL) were collected in ice-chilled EDTA-coated tubes. Plasma and serum were separated by centrifugation at 3200 rpm for 15 min at 4°C and stored at −70°C for later analysis of cholecystokinin, GLP-1, insulin, and glucagon. Plasma cholecystokinin-8 (pmol/L) was measured by radioimmunoassay after ethanol extraction by using an adaptation of a previous method (29). Standards were prepared by using synthetic sulfated cholecystokinin-8 (Sigma Chemical) and an anti–cholecystokinin-8 antibody (C2581, Lot 105H4852; Sigma Chemical). This antibody binds all cholecystokinin peptides containing the sulfated tyrosine residue in position 7, shows a 26% cross-reactivity with unsulfated cholecystokinin-8 and <2% cross-reactivity with human gastrin I, and does not bind to structurally unrelated peptides. Sulfated cholecystokinin-8 125I-labeled with Bolton and Hunter reagent (Perkin Elmer) was used as tracer, and samples were incubated for 7 d at 4°C. The antibody-bound fraction was separated by the addition of dextran-coated charcoal containing gelatin, and the radioactivity was determined in the supernatant fluid after centrifugation. Intraspasy and interassay CVs were 5.8% and 21.1%, respectively. The minimum detectable concentration was 1 pmol/L. Values below the detection limit were treated as 0 (in all hormone assays).

Plasma GLP-1 (pmol/L) was determined after ethanol extraction by using a radioimmunoassay kit (GLPIT-36HK; Millipore). The antibody used does not cross-react with glucagon, gastric inhibitory peptide, or other gut or pancreatic peptides, and it measures both GLP-1(7–36) amide and GLP-1(9–36) amide. Intrassay and interassay CVs were 6.0% and 8.1%, respectively. The minimum detectable concentration was 3 pmol/L.

Serum insulin (mU/L) was measured by ELISA (10-1113; Mecordia). Intrassay and interassay CVs were 1.6% and 4.6%, respectively. The minimum detectable concentration was 1.0 mU/L.

Plasma glucagon (pmol/L) was measured by using a radioimmunoassay kit (GL-32K; Millipore). The antibody used does not cross-react with insulin, proinsulin, C-peptide, somatostatin, or pancreatic polypeptide and has <0.1% cross-reactivity with oxyntomodulin. Intrassay and interassay CVs were 11.2% and 6.7%, respectively. The minimum detectable concentration was 5.7 pmol/L.

Venous blood glucose (mmol/L) was measured immediately after collection by the glucose oxidase method by using a portable glucometer (Medisense Precision QID; Abbott Laboratories). The accuracy of this method has been confirmed in our laboratory by using the hexokinase technique (30).

**Appetite perceptions and gastrointestinal symptoms**

Perceptions of hunger, prospective consumption, fullness, nausea, and bloating were rated on VAS questionnaires (27). These questionnaires consisted of 100-mm horizontal lines, where 0 mm, which was on the furthest left on the scale, represented the sensation “not felt at all,” and 100 mm, which was on the furthest right on the scale, represented the sensation “felt the greatest possible.” Subjects placed vertical marks on each horizontal line to indicate the strength of each sensation felt at that time point.
Energy intake

The amount eaten (g) was quantified by weighing the buffet meal before and after consumption. Energy (kcal) and macronutrient (g) intakes and the percentage contribution of energy from fat, carbohydrate, and protein were calculated by using commercially available software (Foodworks, version 3.01; Xyris Software) (28). Total energy intake on each study day was quantified as the sum of energy intake at the buffet and the energy content of the infusion (270 kcal for nutrient infusions; 0 kcal for the saline control). The percentage of compensation in response to each nutrient infusion represented the degree to which subjects compensated for the caloric content of the infusion at the subsequent meal and was calculated by using the following formula (31):

\[
\text{Percentage of compensation} = \left( \frac{\text{EI at buffet}_{\text{control}} - \text{EI at buffet}_{\text{after infused}}}{270 \text{ kcal}} \right) \times 100
\]

where EI at buffet\textsubscript{control} represents energy intake from the ad libitum buffet meal on the control day, and EI at buffet\textsubscript{after infused} represents energy intake from the ad libitum buffet meal in response to each infusion. A compensation of 100% represents the full compensation for the caloric load of the infusion.

Data and statistical analyses

Baseline values for the number and amplitude of antral and duodenal PWs, IPPWs, and BPP, VAS scores, and hormone and blood glucose concentrations were calculated as the mean of values obtained between \( t = -15 \) to 0 min. During the 90-min infusion, the number and amplitude of pyloric pressures and BPPs were expressed as mean values over 15-min intervals, and hormone concentrations and VAS scores were expressed as mean values at collection time points. Antral and duodenal motility were expressed as motility indexes (MIs) over 90 min, which were calculated by using the following equation (32):

\[
\text{MI} = \ln(\text{sum of amplitudes} \times \text{no. of phasic PWs} + 1)
\]

Statistical analyses were performed with SPSS software (version 19.0; SPSS Inc, IBM). MIs of antral and duodenal PWs, total number and mean amplitude of IPPWs, mean BPP, AUCs for appetite sensations and hormone concentrations, energy and macronutrient intakes at the buffet meal, and total energy intake (ie, energy intake at the buffet meal plus the energy content of the infusion) were analyzed by 1-factor ANOVA. Post hoc comparisons, adjusted for multiple comparisons by using Bonferroni’s correction, were performed when ANOVAs revealed significant effects, and statistical significance was accepted at \( P < 0.05 \).

Because 9 of 20 subjects experienced nausea (VAS scores increased by \( \geq 30 \) mm for 30–90 min) during L3 (of these participants, 2 subjects also vomited between 75 and 90 min after commencement of the infusion, and missing data were carried forward to 90 min to allow inclusion in the analysis), but only one subject experienced nausea during the L2P1, one subject experienced nausea during the L1P2, and no subjects experienced nausea during the P3, potential effects of nausea on the response to L3 were evaluated by comparing all outcome variables between subjects who experienced nausea (\( N^+; n = 9 \)) subjects who did not experience nausea (\( N^-; n = 11 \)) by using an independent-sample \( t \) test. Energy intake in response to L3 compared with C was also analyzed separately in N+ and N− groups.

The percentage of compensation was examined by using a 1-sample \( t \) test, testing each treatment against a fixed value of 0 (where \( P > 0.05 \) represented no compensation of the energy content of the infusant) and against a fixed value of 100 (where \( P > 0.05 \) represented perfect compensation or overcompensation for the energy content of the infusant) (31).

Relations between loads of protein or lipid with AUCs (calculated by using the trapezoidal rule) for BPP, IPPWs, hormones, blood glucose concentrations, VAS, and energy intake were evaluated by using linear within-subject correlations. Relations of energy intake with mean BPPs, number and amplitude of IPPWs, antral and duodenal MIs, and AUCs for plasma hormones, blood glucose concentrations, and the VAS were evaluated by using linear within-subject correlations (33). Variables with the top-4 ranked \( r \) values were included in a multiple regression analysis to establish determinants of energy intake. Significance was accepted at \( P < 0.05 \); all data are presented as means \( \pm \) SEMs.

RESULTS

Antropyloroduodenal pressures

Baseline values for antral and duodenal MIs, total number and mean amplitude of isolated pyloric PWs, and BPPs did not differ between study days (Table 1).

Antral pressures

There was an effect of treatment on the antral MI (\( P < 0.001 \)) (Figure 1A), which reflected a reduction in both the number and amplitude of antral PWs (Table 2). Compared with the C, all nutrient infusions suppressed the antral MI (all \( P < 0.05 \)), with no difference between any of the nutrient treatments. There was no relation between antral MI with the load of lipid or protein administered. Antral MI during the L3 was slightly greater in N+ than N− (N+: 8.6 \( \pm \) 0.5 mm Hg, N−: 7.0 \( \pm \) 0.5 mm Hg; \( P < 0.05 \)).

IPPWs

There was an effect of treatment on the total number of IPPWs (\( P < 0.001 \)) (Figure 1B, Table 2). Compared with the C, all nutrient infusions increased the number of IPPWs (all \( P < 0.05 \)) with no difference between any of the nutrient treatments. The number of IPPWs was related directly to the load of lipid (\( r = 0.39, P < 0.01 \)) administered, so that IPPWs increased with the amount of lipid in the infusion. There was an effect of treatment on the mean amplitude of IPPWs (\( P < 0.05 \)) (Table 2). All nutrient infusions increased the amplitude of IPPWs compared with the C (all \( P < 0.05 \), and L2P1 increased the amplitude of IPPWs compared with the P3 and L1P2 (all \( P < 0.05 \)). There was no relation between the mean amplitude of IPPWs with the load of lipid or protein administered. There were no differences in the AUC of the number or amplitude of IPPWs during the L3 between N+ and N− (number: N+, 136 \( \pm \) 19 min; N−, 116 \( \pm \) 25 min; amplitude: N+: 39.8 \( \pm \) 5.4 mmHg \( \cdot \) min, N−: 34.2 \( \pm \) 5.4 mmHg \( \cdot \) min).
There was an effect of treatment on mean BPPs ($P < 0.05$); however, the post hoc analysis revealed no significant differences between treatments (Figure 1C, Table 2). There was no relation between BPP and the load of lipid or protein administered. The mean BPP in response to the L3 tended to be greater in N+ than N− (N+: 4.2 ± 1.0 mm Hg; N−: 1.3 ± 1.2 mm Hg; $P = 0.09$).

### TABLE 1

Baseline values for the number and amplitude of antral, pyloric, and duodenal PWs; basal pyloric pressures; and hormone and blood glucose concentrations

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>P3</th>
<th>L1P2</th>
<th>L2P1</th>
<th>L3</th>
<th>$P$</th>
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<tr>
<td><strong>Antral PWs</strong></td>
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<tr>
<td>No.</td>
<td>14 ± 4</td>
<td>12 ± 4</td>
<td>14 ± 5</td>
<td>9 ± 3</td>
<td>13 ± 7</td>
<td>NS</td>
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<tr>
<td>Amplitude (mm Hg)</td>
<td>19 ± 4</td>
<td>18 ± 3</td>
<td>23 ± 6</td>
<td>13 ± 3</td>
<td>16 ± 3</td>
<td>NS</td>
</tr>
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<td><strong>IPPWs</strong></td>
<td></td>
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<tr>
<td>No.</td>
<td>0.9 ± 0.3</td>
<td>0.4 ± 0.2</td>
<td>1.5 ± 0.6</td>
<td>1.7 ± 0.7</td>
<td>0.9 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Amplitude (mm Hg)</td>
<td>5.3 ± 1.9</td>
<td>3.3 ± 1.7</td>
<td>5.8 ± 1.9</td>
<td>10.9 ± 3.8</td>
<td>5.9 ± 2.0</td>
<td>NS</td>
</tr>
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<td>Mean BPP (mm Hg)</td>
<td>−0.1 ± 1.2</td>
<td>0.7 ± 0.9</td>
<td>0.7 ± 1.0</td>
<td>−1.4 ± 0.7</td>
<td>−0.6 ± 0.8</td>
<td>NS</td>
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<tr>
<td><strong>Duodenal PWs</strong></td>
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<tr>
<td>No.</td>
<td>56 ± 12</td>
<td>55 ± 11</td>
<td>88 ± 20</td>
<td>65 ± 13</td>
<td>50 ± 7</td>
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<td>Amplitude (mm Hg)</td>
<td>18 ± 1</td>
<td>23 ± 3</td>
<td>22 ± 2</td>
<td>19 ± 1</td>
<td>18 ± 1</td>
<td>NS</td>
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<td><strong>Hormone and glucose concentrations</strong></td>
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<tr>
<td>Cholecystokinin (pmol/L)</td>
<td>1.9 ± 0.2</td>
<td>1.9 ± 0.2</td>
<td>1.9 ± 0.2</td>
<td>1.9 ± 0.2</td>
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<td>GLP-1 (pmol/L)</td>
<td>24.9 ± 4.3</td>
<td>23.6 ± 3.5</td>
<td>31.2 ± 7.5</td>
<td>22.5 ± 2.4</td>
<td>25.9 ± 4.9</td>
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<td>Serum insulin (mU/L)</td>
<td>2.9 ± 0.4</td>
<td>3.3 ± 0.6</td>
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<td>2.7 ± 0.4</td>
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<tr>
<td>Glucagon (pmol/L)</td>
<td>16.2 ± 0.8</td>
<td>16.6 ± 0.9</td>
<td>16.4 ± 0.8</td>
<td>16.2 ± 1.0</td>
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<td>NS</td>
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<tr>
<td>Blood glucose (mmol/L)</td>
<td>5.3 ± 0.1</td>
<td>5.4 ± 0.1</td>
<td>5.4 ± 0.1</td>
<td>5.5 ± 0.1</td>
<td>5.3 ± 0.1</td>
<td>NS</td>
</tr>
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</table>

$^1$All values are means ± SEs. $n = 20$. Before 90-min intraduodenal infusions of P3, L3, L2P1, or L1P2 (each at 3 kcal/min) or C. Main treatment effects were determined by using 1-factor ANOVA. BPP, basal pyloric pressure; C, saline control; GLP-1, glucagon-like peptide-1; IPPW, isolated pyloric pressure wave; L1P2, intralipid and whey protein hydrolysate in a 1:2 ratio; L2P1, intralipid and whey protein hydrolysate in a 2:1 ratio; L3, pure lipid; PW, pressure wave; P3, whey protein hydrolysate.

### FIGURE 1

Means (±SEMs) of the MI for antral pressure waves (A), IPPWs (B), BPPs (C), and the MI for duodenal pressure waves (D) during 90-min, 3-kcal/min intraduodenal infusions of C (open circles), P3 (open triangles), L1P2 (closed triangles), L2P1 (closed squares), or L3 (open squares). $n = 20$. Treatment effects were determined by using 1-factor ANOVA, and significant differences ($P < 0.05$) were determined by post hoc comparisons by using Bonferroni correction. A: Treatment effect for MI (over 90 min) of antral pressure waves ($P < 0.001$): *significantly different from C, $P < 0.05$. B: Treatment effect for the total number (over 90 min) of IPPWs ($P < 0.001$): *significantly different from C, $P < 0.05$. C: Treatment effect for mean BPP (over 90 min) ($P < 0.05$): no significant differences between treatments. D: Treatment effect for MI (over 90 min) of duodenal waves ($P < 0.001$): *significantly different from C, $P < 0.01$; #significantly different from L2P1, L1P2, and P3 ($P < 0.01$) BPP, basal pyloric pressure; C, saline control; IPPW, isolated pyloric pressure wave; L1P2, intralipid and whey protein hydrolysate in a 1:2 ratio; L2P1, intralipid and whey protein hydrolysate in a 2:1 ratio; L3, pure lipid; MI, motility index; P3, whey protein hydrolysate.
There was an effect of treatment on the duodenal MI (P < 0.001) (Figure 1D), which reflected a reduction in both the number and amplitude of duodenal PWs (Table 2). All nutrients reduced the duodenal MI compared with C (all P < 0.01) and the L3 compared with P3, L1P2, and L2P1 (all P < 0.01). The MI of duodenal PWs was related inversely to the load of lipid (r = -0.54, P < 0.01) administered. The duodenal MI during the L3 was slightly greater in N+ than N− (N+: 10.4 ± 0.2 mm Hg; N−: 9.7 ± 0.2 mm Hg; P < 0.05).

### Duodenal PWs

| MI (mm Hg) | 211 ± 31 | 57 ± 17 | 80 ± 28 | 73 ± 25 | 71 ± 16 | 46 ± 6 | 14 ± 2 | 14 ± 2 | 83 ± 15 | 100 ± 11 | 130 ± 15 | 125 ± 16 | 0.8 ± 1.0 | 1.3 ± 0.8 | 2.1 ± 1.0 | 1.4 ± 0.7 | 2.7 ± 0.9 | 0.8 ± 1.0 | 1.3 ± 0.8 | 2.1 ± 1.0 | 1.4 ± 0.7 | 2.7 ± 0.9 |
| C | P3 | L1P2 | L2P1 | L3 | | | | | | | | | | | | | | | | | | | | | |

### Gut hormone, insulin, glucagon, and blood glucose concentrations

Baseline values for plasma cholecystokinin and GLP-1, serum insulin, plasma glucagon, and blood glucose concentrations did not differ between study days (Table 1).

### Plasma cholecystokinin

There was an effect of treatment on the plasma cholecystokinin AUC (P < 0.001) (Figure 2A). Compared with the C, all nutrient infusions increased the plasma cholecystokinin AUC. The L3 also increased the plasma cholecystokinin AUC compared with all other nutrients, the L2P1 compared with P3 and L1P2, and the L1P2 compared with P3 (all P < 0.05). The cholecystokinin AUC (Table 3) was related directly to the load of lipid (r = 0.79, P < 0.001) administered. There was no difference in the cholecystokinin AUC during the L3 between N+ and N− (N+: 613 ± 67 pmol · L⁻¹ · min; N−: 518 ± 69 pmol · L⁻¹ · min).

### GLP-1

There was an effect of treatment on the plasma GLP-1 AUC (P < 0.001) (Figure 2B). Compared with the C, all nutrient infusions increased the plasma GLP-1 AUC. The L3 also increased the plasma GLP-1 AUC compared with all other nutrients, the L2P1 compared with L1P2 and P3, and the L1P2 compared with P3 (all P < 0.05). The GLP-1 AUC (Table 3) was related directly to the load of lipid (r = 0.82, P < 0.001) administered. There was no difference in the GLP-1 AUC during the L3 between N+ and N− (N+: 482 ± 10 pmol · L⁻¹ · min; N−: 464 ± 11 pmol · L⁻¹ · min).

### Serum insulin

There was an effect of treatment on the serum insulin AUC (P < 0.001) (Figure 2C). Compared with the C, all nutrient infusions increased the serum insulin AUC. P3 also increased serum insulin compared with all other nutrients, the L1P2 compared with L2P1 and L3, and the L2P1 compared with L3 (all P < 0.05). The serum insulin AUC (Table 3) was directly related to the load of protein (r = 0.83, P < 0.001) administered. There was no difference in the insulin AUC during the L3 between N+ and N− (N+: 486 ± 40 mU · L⁻¹ · min; N−: 458 ± 50 mU · L⁻¹ · min).

### Glucagon

There was an effect of treatment on the plasma glucagon AUC (P < 0.001) (Figure 2D). Compared with the C, all nutrient infusions increased the plasma glucagon AUC. P3 also increased the plasma glucagon AUC compared with the L2P1 and L3, the L1P2 compared with L2P1 and L3, and the L2P1 compared with L3 (all P < 0.001). The plasma glucagon AUC (Table 3) was related directly to the load of protein (r = 0.83, P < 0.001) administered. There was no difference in glucagon AUC during the L3 between N+ and N− (N+: 2035 ± 96 pmol · L⁻¹ · min; N−: 1980 ± 104 pmol · L⁻¹ · min).

### Blood glucose

There was an effect of treatment on blood glucose AUC (P < 0.05) (Figure 2E); however, there were no significant differences

---

**Table 2**

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>P3</th>
<th>L1P2</th>
<th>L2P1</th>
<th>L3</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antral PWs No.</td>
<td>211 ± 31</td>
<td>57 ± 17</td>
<td>80 ± 28</td>
<td>73 ± 25</td>
<td>71 ± 16</td>
<td>—</td>
</tr>
<tr>
<td>Amplitude (mm Hg)</td>
<td>46 ± 6</td>
<td>15 ± 2</td>
<td>14 ± 2</td>
<td>14 ± 2</td>
<td>13 ± 2</td>
<td>—</td>
</tr>
<tr>
<td>MI (mm Hg)</td>
<td>10.7 ± 0.3</td>
<td>8.2 ± 0.4²</td>
<td>7.8 ± 0.5²</td>
<td>7.6 ± 0.7²</td>
<td>7.7 ± 0.5²</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

---

²Significantly different from C, P < 0.05.
³Significantly different from P3, P < 0.05.
⁴Significantly different from L1P2, P < 0.05.
⁵Significantly different from L2P1, P < 0.05.

---

All values are means ± SEs. n = 20. Intraduodenal infusions were P3, L3, L2P1, or L1P2 (each at 3 kcal/min) or C. There was an effect of treatment on the plasma cholecystokinin AUC (P < 0.001) (Figure 2A), which reflected a reduction in both the number and amplitude of duodenal PWs (Table 2). All nutrients reduced the duodenal MI compared with C (all P < 0.01) and the L3 compared with P3, L1P2, and L2P1 (all P < 0.01). The MI of duodenal PWs was related inversely to the load of lipid (r = -0.54, P < 0.01) administered. The duodenal MI during the L3 was slightly greater in N+ than N− (N+: 10.4 ± 0.2 mm Hg; N−: 9.7 ± 0.2 mm Hg; P < 0.05).

Baseline values for plasma cholecystokinin and GLP-1, serum insulin, plasma glucagon, and blood glucose concentrations did not differ between study days (Table 1).

There was an effect of treatment on the plasma cholecystokinin AUC (P < 0.001) (Figure 2A). Compared with the C, all nutrient infusions increased the plasma cholecystokinin AUC. The L3 also increased the plasma cholecystokinin AUC compared with all other nutrients, the L2P1 compared with L1P2 and P3, and the L1P2 compared with P3 (all P < 0.05). The cholecystokinin AUC (Table 3) was related directly to the load of lipid (r = 0.79, P < 0.001) administered. There was no difference in the cholecystokinin AUC during the L3 between N+ and N− (N+: 613 ± 67 pmol · L⁻¹ · min; N−: 518 ± 69 pmol · L⁻¹ · min).

There was an effect of treatment on the plasma GLP-1 AUC (P < 0.001) (Figure 2B). Compared with the C, all nutrient infusions increased the plasma GLP-1 AUC. The L3 also increased the plasma GLP-1 AUC compared with all other nutrients, the L2P1 compared with L1P2 and P3, and the L1P2 compared with P3 (all P < 0.05). The GLP-1 AUC (Table 3) was related directly to the load of lipid (r = 0.82, P < 0.001) administered. There was no difference in the GLP-1 AUC during the L3 between N+ and N− (N+: 482 ± 10 pmol · L⁻¹ · min; N−: 464 ± 11 pmol · L⁻¹ · min).

There was an effect of treatment on the serum insulin AUC (P < 0.001) (Figure 2C). Compared with the C, all nutrient infusions increased the serum insulin AUC. P3 also increased serum insulin compared with all other nutrients, the L1P2 compared with L2P1 and L3, and the L2P1 compared with L3 (all P < 0.05). The serum insulin AUC (Table 3) was directly related to the load of protein (r = 0.83, P < 0.001) administered. There was no difference in the insulin AUC during the L3 between N+ and N− (N+: 486 ± 40 mU · L⁻¹ · min; N−: 458 ± 50 mU · L⁻¹ · min).

There was an effect of treatment on the plasma glucagon AUC (P < 0.001) (Figure 2D). Compared with the C, all nutrient infusions increased the plasma glucagon AUC. P3 also increased the plasma glucagon AUC compared with the L2P1 and L3, the L1P2 compared with L2P1 and L3, and the L2P1 compared with L3 (all P < 0.001). The plasma glucagon AUC (Table 3) was related directly to the load of protein (r = 0.83, P < 0.001) administered. There was no difference in glucagon AUC during the L3 between N+ and N− (N+: 2035 ± 96 pmol · L⁻¹ · min; N−: 1980 ± 104 pmol · L⁻¹ · min).

There was an effect of treatment on blood glucose AUC (P < 0.05) (Figure 2E); however, there were no significant differences.
between treatments. There was no relation between the blood glucose AUC (Table 3) and load of lipid or protein administered. There was no difference in the blood glucose AUC during the L3 between N+ and N− (N+: 486 ± 10 mmol · L⁻¹ · min; N−: 466 ± 14 mmol · L⁻¹ · min).

Appetite perceptions and gastrointestinal symptoms

Hunger

There was no difference in baseline hunger ratings between study days. There was a trend for an effect of treatment on the hunger AUC (P = 0.09) (see Supplemental Figure 2A under “Supplemental data” in the online issue). There was no difference in the hunger AUC during the L3 between N+ and N− (N+: 4702 ± 392 mm · min; N−: 5059 ± 640 mm · min).

Prospective consumption

There was no difference in baseline prospective consumption ratings between study days. There was an effect of treatment on the prospective consumption AUC (P < 0.05) (see Supplemental Figure 2B under “Supplemental data” in the online issue); however, there were no significant differences between treatments. The prospective consumption AUC was related weakly to the load of lipid (r = 0.28, P < 0.05) administered. There was no difference in the prospective consumption AUC during the L3 between N+ and N− (N+: 4876 ± 643 mm · min; N−: 5375 ± 519 mm · min).

Fullness

There was no difference in baseline fullness ratings between study days. There was a trend for an effect of treatment on the
fullness AUC ($P = 0.076$) (see Supplemental Figure 2C under “Supplemental data” in the online issue). The fullness AUC was related weakly to the load of lipid ($r = 0.28, P < 0.05$) administered. There was no difference in the fullness AUC during the L3 between N+ and N− (N+: 2176 ± 604 mm · min; N−: 2075 ± 444 mm · min).

Nausea
There was no difference in baseline nausea ratings between study days. There was an effect of treatment on the nausea AUC ($P < 0.01$); nausea was greater during the L3 than C and L1P2 (both $P < 0.05$) and tended to be greater than during the P3 ($P = 0.07$) (see Supplemental Figure 2D under “Supplemental data” in the online issue). The nausea AUC was related weakly to the load of lipid ($r = 0.32, P < 0.05$) administered.

Bloating
There was no difference in baseline bloating ratings between study days. There was a trend for an effect of treatment on bloating AUC ($P = 0.07$) (see Supplemental Figure 2E under “Supplemental data” in the online issue). The bloating AUC was related weakly to the load of lipid ($r = 0.30, P < 0.05$) administered. There was no difference in the bloating AUC during the L3 between N+ and N− (N+: 2570 ± 602 mm · min; N−: 1658 ± 517 mm · min).

Energy and macronutrient intakes
There was an effect of treatment on energy intake (kcal) from the buffet meal ($P < 0.001$) (Table 4). Compared with the C, energy intake was less after the L3 ($P < 0.01$) and P3 ($P < 0.05$) and tended to be less after the L1P2 ($P = 0.08$), with no significant differences between any of the nutrient intusions.

There was an effect of treatment on the amount (g) of food consumed at the buffet ($P < 0.001$), which was less after the P3 and L3 than after the C (both $P < 0.01$). There was an effect of treatment on intakes (g) of protein and carbohydrate (both $P < 0.001$) but not fat. Compared with the C, the intake of protein (g) was less after the P3, L3, and L1P2 (all $P < 0.05$), whereas the intake of carbohydrate (g) was less after the P3 and L3 (both $P < 0.05$). There was an effect of treatment on the percentage of energy consumed from protein ($P < 0.05$); however, post hoc comparisons revealed no significant differences between treatments. There was no effect of treatment on the percentage of energy consumed from carbohydrate or fat. Subjects compensated for the caloric content of the P3 ($P < 0.01$) and L3 ($P < 0.01$) (P3: 77 ± 20%; L3: 95 ± 21%; one-sample $t$ test against 0) (P3: $P = 0.287$; L3: $P = 0.975$; one-sample test against 100), with no differences in the percentage of compensation between the L3 and P3. The energy load of L2P1 and L1P2 infusions were not significantly compensated for (L2P1: 20 ± 19%; L1P2: 38 ± 19%), and the L3 was compensated for better than the L2P1 ($P < 0.05$).

Energy intake in response to the L3 was reduced by ~370 kcal in N+ than in N− (energy intake: N+, 769 ± 190 kcal; N−, 1139 ± 81 kcal); however, while of physiologic relevance, the result was NS ($P = 0.07$). In both N+ and N−, energy intake in response to the L3 compared with control was significantly reduced (N+: L3, 769 ± 190 kcal; C, 1153 ± 158 kcal; $P < 0.01$; N−: L3, 1139 ± 81 kcal; C, 1312 ± 69 kcal; $P < 0.05$). The amount of food (g) eaten after the L3 did not differ between N+ and N− (food intake: N+, 962 ± 143 g; N−, 1120 ± 113 g). N+ consumed less protein (N+: 35 ± 10 g; N−: 59 ± 6 g; $P < 0.05$) and carbohydrate (N+: 88 ± 21 g; N−: 135 ± 11 g; $P = 0.05$) than did N−. The percentage of total energy consumed as protein (N+: 15 ± 2%; N−: 21 ± 1%; $P < 0.05$) and carbohydrate (N+: 59 ± 4%; N−: 48 ± 2%; $P < 0.05$) was also less in N+ than N−. The percentage of compensation for N+ during the L3 tended to be greater (142 ± 35%) than for N− (64 ± 21%) ($P = 0.07$).

Relations between antropyloroduodenal motility, cholecystokinin, GLP-1, insulin, glucagon, and glucose concentrations and perceptions of appetite and gastrointestinal symptoms with energy intake
Energy intake from the buffet was related inversely to fullness ($r = −0.37, P < 0.05$), nausea ($r = −0.59, P < 0.001$), and bloating ($r = −0.54, P < 0.001$) at t = 90 min and related directly to prospective consumption ($r = 0.46, P < 0.001$) at t = 90 min. It was also related inversely to cholecystokinin AUC ($r = −0.30, P < 0.05$). When nausea, prospective consumption, and cholecystokinin AUCs were included in a multiple regression analysis, nausea was the most significant predictor of energy intake.
TABLE 4
Energy content, weight, and macronutrient distribution (percentage of energy derived from fat, carbohydrate, or protein) of food consumed at a buffet meal and the percentage of compensation in response to 90-min intraduodenal infusions

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>P3</th>
<th>L1P2</th>
<th>L2P1</th>
<th>L3</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buffet (kcal)</td>
<td>1241 ± 80</td>
<td>1032 ± 83¹</td>
<td>1138 ± 87</td>
<td>1185 ± 86²</td>
<td>973 ± 103²</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total (kcal)</td>
<td>1241 ± 80</td>
<td>1302 ± 83¹</td>
<td>1408 ± 87</td>
<td>1455 ± 86</td>
<td>1243 ± 103²</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Amount eaten (g)</td>
<td>1277 ± 73</td>
<td>1043 ± 71¹</td>
<td>1166 ± 85</td>
<td>1168 ± 78</td>
<td>1049 ± 89³</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Protein intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g</td>
<td>62 ± 5</td>
<td>52 ± 5²</td>
<td>55 ± 5</td>
<td>62 ± 5²</td>
<td>49 ± 6²</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Percentage of energy</td>
<td>20 ± 1</td>
<td>20 ± 1</td>
<td>19 ± 1</td>
<td>21 ± 1</td>
<td>18 ± 1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Carbohydrate intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g</td>
<td>153 ± 8</td>
<td>126 ± 10²</td>
<td>137 ± 9</td>
<td>141 ± 10</td>
<td>114 ± 13²</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Percentage of energy</td>
<td>51 ± 2</td>
<td>51 ± 2</td>
<td>50 ± 2</td>
<td>49 ± 1</td>
<td>53 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>Fat intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g</td>
<td>43 ± 4</td>
<td>36 ± 4</td>
<td>42 ± 4</td>
<td>42 ± 4</td>
<td>39 ± 6</td>
<td>NS</td>
</tr>
<tr>
<td>Percentage of energy</td>
<td>29 ± 1</td>
<td>26 ± 1</td>
<td>31 ± 1</td>
<td>30 ± 1</td>
<td>29 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>Percentage of compensation</td>
<td>—</td>
<td>78 ± 21⁶</td>
<td>38 ± 20</td>
<td>21 ± 20</td>
<td>95 ± 22⁶</td>
<td>—</td>
</tr>
</tbody>
</table>

¹All values are means ± SEs. n = 20. The buffet meal was consumed immediately after 90-min intraduodenal infusions of P3, L3, L2P1, or L1P2 (each at 3 kcal/min) or C. Main treatment effects were determined by using 1-factor ANOVA; significant differences were determined by post hoc comparisons by using Bonferroni correction. C, saline control; L1P2, intralipid and whey protein hydrolysate in a 2:1 ratio; L3, pure lipid; P3, whey protein hydrolysate.
²Significantly different from C, P < 0.05.
³Trend for difference compared with C, P < 0.09.
⁴Calculated as the sum of energy intake at the buffet meal and the caloric content of the infusion.
⁵Percentage of compensation [(EI at buffet — EI at buffet after infusion) × 270 kcal] × 100 was determined by using a 1-sample t test against a fixed value of 0, which represented the compensation for the energy content of the test infusion, and against a fixed value of 100, which represented perfect compensation for the energy content of the test infusion.
⁶Significantly different from fixed value of 0% of compensation (P < 0.05).

intake at the buffet (r = 0.48, P < 0.001). No other significant relations were identified.

Relations between antropyloroduodenal motility, cholecystokinin, GLP-1, insulin, glucagon, and glucose concentrations

The blood glucose AUC was related inversely to the cholecystokinin AUC (r = −0.27, P < 0.05) and glucagon AUC (r = −0.26, P < 0.05). The glucagon AUC was related to the insulin AUC (r = 0.82, P < 0.001).

DISCUSSION

This study described the effects of isocaloric, intraduodenal infusions of pure lipid and their combinations on antropyloroduodenal motility, cholecystokinin, GLP-1, insulin, glucagon, blood glucose, appetite, and energy intake in healthy lean men. We report that 1) intraduodenal lipid and protein comparably suppressed subsequent energy intake, 2) protein had lesser effects on pyloric pressures and plasma cholecystokinin and GLP-1 than did lipid, 3) protein potently stimulated both insulin and glucagon, thus maintaining normoglycemia, 4) lipid and protein in combination did not have additive effects, and 5) lipid, but not protein, induced nausea.

We showed that infusions of lipid and protein, infused at 3 kcal/min, comparably suppressed subsequent energy intake. For lipid, the suppression of energy intake was associated with a potent stimulation of pyloric pressures and gastrointestinal hormone release as well as nausea, in line with our previous findings (9, 14). Compared with lipid, protein also suppressed energy intake, despite weaker effects on cholecystokinin, GLP-1, and gastrointestinal motility, and thus, the suppression of energy intake by protein is likely a result of other mechanisms. Mechanisms that have been suggested include stimulation of insulin release (34), postprandial thermogenesis (35), intestinal gluconeogenesis (36), and direct effects of amino acids, particularly branched-chain amino acids, in regions of the brain, including the hypothalamus (37); however, definitive evidence for a role of these mechanisms remains to be established. Peptide YY has also been suggested as a mediator of the effects of protein on food intake (38); however, this study used unphysiologically high loads of protein, and by using more-moderate amounts, our recent study was unable confirm those findings (2). The suppression of energy intake in the L3 or P3 was not associated with any substantial changes in appetite suppression. This result confirmed our frequent previous observations (7, 39, 40) that, in the absence of gastric distension, intraduodenal nutrients can potently suppress energy intake, with little to no effect on appetite perceptions.

Lipid induced nausea in ~45% of subjects, in whom energy intake was markedly less than in N−. Thus, nausea contributed, at least in some subjects, to the energy intake–suppressant effect of lipid, confirming that nausea affects energy intake in humans (14), possibly by causing a subtle aversion through activation of 5-hydroxytryptamine receptor mechanisms (41). However, nausea was not associated with any major effects on gastrointestinal motility or hormone (particularly cholecystokinin) release and, thus, may have been because of a hypersensitivity to lipid or its digestion products, fatty acids, in the small intestinal...
lumen or to cholecystokinin. Changes in the sensitivity to lipid (both hypersensitivity and hyposensitivity) have been shown in various disorders, including functional dyspepsia (42) and obesity (1).

We have established that intraduodenal infusions of long-chain triglycerides, glucose, and partially hydrolyzed whey protein dose-dependently modulate antropyloroduodenal motility (7–9), and lipid appears to have the most potent effects (7–9, 22). The current study confirmed that the effects of protein, particularly on pyloric and duodenal pressures, are weaker. Our data also showed major differences in the stimulation of cholecystokinin and GLP-1 release. Although both the L3 and P3 stimulated cholecystokinin and GLP-1 compared with the control, concentrations were much higher during the L3, indicating that lipid is a much more potent stimulus than protein. The potent effects of lipid on gastrointestinal motility and hormone release are a result of the release of fatty acids during fat digestion (43, 44). For example, the entry of 0.1–0.4 kcal fatty acids/min into the small intestine is sufficient to increase plasma cholecystokinin by ∼7 pmol within 10 min (43), whereas cholecystokinin in response to the P3 only increased by ∼1 pmol in the first 15 min. Similar differences applied to GLP-1, although the gradual pattern of the rise reflected its release from enteroendocrine L cells located in the distal small intestine. Thus, a marked elevation in plasma GLP-1 in response to the L3 was evident within 30 min, whereas comparable elevations in response to P3 were achieved at 90 min. There is evidence that, like fat, protein digestion to dipeptides or tripeptides and free amino acids enhances gastrointestinal hormone release (45), and because whey protein is considered a fast protein (46), it would be expected that these digestion products would be liberated quickly within the gastrointestinal tract. Amino acids in whey with effects on gastrointestinal motility include arginine, glutamate, glycine, and L-tryptophan (L-Trp). For example, a 20-min intraduodenal infusion of 50 mmol L-Trp/L (≈20.4 mg/min) stimulates tonic and phasic pyloric pressures in healthy humans (47). By comparison, our P3 infusion delivered ∼17 mg L-Trp/min over 90 min, which, thus, may not have reached the required luminal threshold concentration. L-Trp and L-phenylalanine stimulate cholecystokinin (48), and L-Trp, as well as leucine, glutamine, alanine and asparagine (all present in whey) stimulate GLP-1 in human cell lines and in lean, obese, and type 2 diabetic humans (18, 49–52). Thus, the reasons for the relatively weak effects of whey on gastrointestinal function are unclear.

We observed a significant stimulation of both insulin and glucagon release by protein than lipid. The slight, but significant, reduction in blood glucose during the P3 at t = 90 min suggested that the glucose-lowering effect of insulin was relatively stronger than the glucose-stimulatory effect of glucagon. Thus, the potential hypoglycemic effect of the insulin response to protein was counteracted by the glucagon response, which was probably mediated by amino acids (50, 52), ensuring that, even after a relatively high protein load, blood glucose concentrations remain in the normoglycemic range. Whey protein has potent insulinotropic effects (18), which are glucose-independent and likely driven by the direct stimulation of pancreatic β cells by insulinogenic (18), particularly branched-chain (50), amino acids and may, thus, reduce postprandial blood glucose in both health and type 2 diabetes (15, 18). Moreover, a recent study showed that, after oral protein consumption, hepatic insulin excretion was reduced, which led to a persistent elevation of serum insulin, in the absence of increased insulin release, even after the termination of feeding (51), which may also be the case for intraduodenal protein. GLP-1 and GIP are the 2 known incretin hormones; however, the release of insulin by GLP-1 is glucose-dependent. Thus, because subjects were euglycemic, GLP-1 should not have had insulinotropic or glucagonostatic effects. We did not evaluate GIP release, but a study showed that lipid and protein both stimulate GIP, and the early GIP release after protein was correlated with the insulin rise (53).

At the loads used, the effects of lipid-protein combinations on gut hormones were related directly to the amount of lipid, and the effects on insulin and glucagon were related to the amount of protein, in the infusion, whereas no clear pattern was apparent for effects on motility, and the lipid-protein combinations did not suppress energy intake compared with the control. The reasons for these outcomes are not clear, but if our assumption that critical loads of amino acids and fatty acids, rather than a specific caloric load, within the intestinal lumen are required is correct, it is likely that these thresholds were not reached. It is also possible that, in the combinations, protein and lipid formed complexes, which diminished their effects; however, because combinations displayed intermediate effects between those of the isolated nutrients, particularly on hormones, this outcome seemed unlikely. Thus, additional studies are warranted to define nutrient thresholds for optimal effects on both energy intake and blood glucose control.

Several aspects of our study design warrant recognition. Only healthy, lean men were included because they are more sensitive to dietary manipulation than women (54); hence, our findings may not extend to women. In addition, the number of study conditions (n = 5) may have compromised the power of the study to detect significant differences between treatments for some variables. The appetite-suppressive effects of protein have been shown to vary between sources of protein (25, 26), and thus, our results may not apply to alternative sources of protein.

In conclusion, despite differing effects on gastrointestinal function, intraduodenal lipid and protein comparably suppress energy intake. Intraduodenal protein also regulates glycemia by stimulating insulin and glucagon. In contrast, at the loads chosen, lipid:protein combinations did not suppress energy intake. Additional research needs to identify effective loads of lipid and protein or their digestion products that offer optimal energy intake suppression and blood glucose control while avoiding adverse effects, thereby offering nutritional benefits for a number of diet-related disorders.

We thank Asimina Kallas and Brionhy Bartlett for preparing infusions and assisting on study days, and Kylie Lange for statistical support.

The authors’ responsibilities were as follows—CF-B: accepted full responsibility for the overall conduct of the study and had primary responsibility for the final content of the manuscript; NDL-M, TJL, MH, and CF-B: designed the research; ATR and AAS: conducted the research, analyzed data, and conducted the statistical analysis; SS: performed the hormone analysis; MH, NDL-M, and TJL: assisted with the interpretation and preparation of the manuscript; ATR and CF-B: wrote the manuscript; and all authors: read and approved the final manuscript. None of the authors had any conflicts of interest to declare.

REFERENCES


