Intraduodenal protein modulates antropyloroduodenal motility, hormone release, glycemia, appetite, and energy intake in lean men

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ABSTRACT
Background: Intraduodenal fat and carbohydrate modulate antropyloroduodenal motility and hormone release and suppress appetite and energy intake in a load-dependent manner. Protein also suppresses energy intake, but its effects on these gastrointestinal factors and their role in the appetite-suppressive effects of protein remain unclear.

Objective: We aimed to characterize the effects of different intraduodenal protein loads on antropyloroduodenal pressures, gastrointestinal hormone release, glucose and insulin concentrations, appetite perceptions, and energy intake.

Design: Sixteen lean, healthy men were studied on 4 occasions in a randomized, double-blind fashion. Antropyloroduodenal pressures, plasma glucagon-like peptide 1 (GLP-1), cholecystokinin, peptide YY (PYY), ghrelin, blood glucose, serum insulin, and appetite were measured during 60-min, 4-mL/min intraduodenal infusions of protein at 0.5, 1.5, or 3 kcal/min or saline (control). Energy intakes at a buffet lunch consumed immediately after the infusion were quantified.

Results: Increases in the load of protein resulted in greater suppression of antral motility, greater stimulation of basal and isolated pyloric pressures and plasma cholecystokinin and GLP-1 concentrations, and greater suppression of energy intake. However, energy intake was reduced only after a protein load of 3 kcal/min compared with after all other treatments (P < 0.05). The suppression of energy intake after adjustment for cholecystokinin, GLP-1, and insulin was related inversely with basal pyloric pressure (r = −0.51, P < 0.001).

Conclusion: The acute effects of intraduodenal protein on antropyloroduodenal motility, gastrointestinal hormone release, glucose, and insulin are load dependent and contribute to the suppression of energy intake. This trial was registered at www.anzctr.org.au as 12610000376044.

INTRODUCTION

Protein is regarded as more satiating than either fat or carbohydrate both acutely (1) and under ad libitum consumption conditions over the longer term (2). These effects have been attributed to increased circulating amino acids (3), postprandial thermogenesis (4), and small intestinal gluconeogenesis (5). Gastrointestinal factors, including antropyloroduodenal motility and hormone release, also play a pivotal role in the regulation of energy intake in response to fat and carbohydrate (6). It has been well established that small amounts of intraduodenal fat and carbohydrate suppress antral and duodenal motility and stimulate pyloric pressures (7), the release of cholecystokinin from the proximal small intestine (8), and the release of peptide YY (PYY) (8, 9) and glucagon-like peptide 1 (GLP-1) (9, 10) from the distal small intestine and suppress ghrelin release from the stomach (11, 12). Inhibitory feedback from these interrelated gastrointestinal factors slows gastric emptying (13) and suppresses energy intake (9–12).

In healthy humans, there is a substantial interindividual, but relatively low intra-individual, variation in gastric-emptying rates, and thus, nutrients enter the intestine at an overall rate of 1–4 kcal/min (14–16). Studies in which intraduodenal loads of fat and glucose have been infused at rates within this range have indicated that a lower threshold of fat is required to modulate gastrointestinal function (7). A pooled analysis of data collected by our group also identified more isolated pyloric pressure waves (IPPWs) and plasma cholecystokinin release as independent predictors of an acute energy intake in healthy men (6). In contrast to what is known about intraduodenal fat and glucose, the effects of protein on gastrointestinal responses, appetite, and energy intake have been less comprehensively characterized (17), which is surprising because of the potent effects of protein on energy intake (1, 2, 18). Oral protein-enriched meals more potently modulate the release of GLP-1 (19), cholecystokinin (20), PYY (1), and ghrelin (20) than do isocaloric fat- or carbohydrate-enriched meals.

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4Abbreviations used: BPP, basal pyloric pressure; GLP-1, glucagon-like peptide 1; IPPW, isolated pyloric pressure wave; MI, motility index; PW, pressure wave; PYY, peptide YY; P0.5, protein load of 0.5 kcal/min; P1.5, protein load of 1.5 kcal/min; P3, protein load of 3 kcal/min; VAS, visual analog scale.

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Protein may also increase insulin concentrations by stimulating GLP-1 and gastric inhibitory polypeptide (21) and by the direct stimulation of β cells by amino acids (21). Therefore, the investigation of the effects of different intraduodenal protein loads has potential relevance for health and type 2 diabetes (22).

The aim of this study was to evaluate the effects of different intraduodenal loads of whey protein hydrolysate on antropyloroduodenal motility, gastrointestinal hormone release, insulin and glucose concentrations, appetite, and energy intake in healthy males. We hypothesized that intraduodenal protein at loads lower than [0.5 kcal/min (P0.5)], similar to [1.5 kcal/min (P1.5)], and toward the upper end [3 kcal/min (P3)] of normal gastric-emptying rates would modulate gastrointestinal parameters in a load-related fashion and suppress appetite and energy intake.

**SUBJECTS AND METHODS**

**Subjects**

Sixteen healthy, lean men [mean age: 27 ± 3 y (range: 19–54 y); BMI (in kg/m²): 22.1 ± 0.6 (range 18.5–24.8)] participated in the study. The number of subjects was determined by using power calculations on the basis of our previous work (9, 23). We calculated that n = 16 would allow us to detect a minimum difference in energy intake of 150 kcal with α = 0.05 and β ≥ 0.8. All subjects were unrestrained eaters [score ≤12 on the eating restraint component of the 3-factor eating questionnaire (24)]. Subjects who smoked, consumed >20 g alcohol/wk, had a history of gastrointestinal symptoms, or were currently taking medication known to affect energy intake, appetite, or gastrointestinal function were excluded from the study. The Royal Adelaide Hospital Research Ethics Committee approved the study protocol, and the study was registered as a clinical trial with the Australia and New Zealand Clinical Trial Registry (www.anzctr.org.au; 12610000376044). All subjects provided written informed consent before their inclusion in the study.

**Study outline**

The study compared the effects of different intraduodenal protein loads (P0.5, P1.5, or P3) and a saline control, each of which were infused for 60 min, on antropyloroduodenal motility, gastrointestinal hormone release, blood glucose and insulin concentrations, perceptions of appetite, and energy intake.

**Intraduodenal infusions**

Protein solutions were prepared by dissolving whey protein hydrolysate powder (18.1% Hydrolyzed Whey Protein 821; Fonterra Co-Operative Group Ltd) in varying amounts of saline and water to achieve the desired loads (ie, P0.5, P1.5, or P3), which equated to 8, 24, and 48 g protein, respectively) and ensure that they were iso-osmotic (680 mOsmol/L). Thus, 100 mL of the protein solutions consisted of 1) 3.25 g whey protein, 35.75 mL distilled water, and 61 mL 3% saline (P0.5); 2) 9.75 g whey protein, 46 mL distilled water, and 44.25 mL 3% saline (P1.5); and 3) 19.5 g whey protein, 63.5 mL distilled water, and 16.75 mL 3% saline (P3). The saline control consisted of 28 mL distilled water and 72 mL 3% saline. All infusions were administered at a rate of 4 mL/min (ie, 240 mL over 60 min) and delivered a total of 30 (P0.5), 90 (P1.5), or 180 (P3) kcal. Infusions were prepared on the morning of each study by an investigator who was not involved in the data analysis (AK). The infusion apparatus was covered at all times so that both the primary investigator (ATR) and the subject were blinded to the treatment. Intraduodenal administration was used to bypass orosensory influences and variation in gastric emptying. Whey protein was used because it is a major protein source (in milk and yogurt) in the Australian diet (25). Hydrolyzed whey was selected because it is more likely to resemble partially digested protein (26).

**Protocol**

Subjects were studied on 4 occasions, with each occasion separated by 3–10 d, in a randomized, double-blind, crossover design. Subjects were provided with a standardized evening meal (total energy content: 1160 kcal) that consisted of beef lasagna (McCain Foods), 300 mL orange juice (Daily Juice Co), a chocolate chip muesli bar (Nestle Cereal Partners Australia Ltd), and 150 g fruit salad (SPC Ardmona Operations Ltd) to consume the night before each study at 1900. Subjects were instructed to fast overnight from solids and liquids and to refrain from strenuous physical activity until they attended the laboratory at the University of Adelaide Discipline of Medicine, Royal Adelaide Hospital, at 0830.

On arrival, a small-diameter (3.5-mm) 16-channel 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. Six side holes (channels 1–6) were positioned in the antrum: a 4.5-cm sleeve sensor (channel 7) with 2 side-holes (channels 8 and 9), which was placed on the back of the sleeve, was positioned across the pylorus, and 7 side holes (channels 10–16) were positioned in the duodenum. All side holes were spaced at 1.5-cm intervals. An additional channel, which was used for intraduodenal infusions, was located 11.75 cm distal to the end of the sleeve sensor (14.5 cm from the pylorus); infusion of the nutrient 14.5 cm distal to the pylorus (ie, in the proximal small intestine) yielded both direct and indirect stimulation of pyloric motor activity and the release of proximal small intestinal hormones, which was optimal to maximize a potential suppressive effect on energy intake. The maximum intubation length depended on the height of the individual but did not exceed 75 cm. The correct positioning of the catheter, with the sleeve sensor straddling the pylorus, was maintained by continuous measurement of the transmucosal potential difference between the most distal antral channel (channel 6; approximately −40 mV) and the most proximal duodenal channel (channel 10; ~0 mV) (27). Thereafter, an intravenous cannula filled with sterile saline was positioned subcutaneously in the left forearm and used as a reference electrode. All manometric channels were perfused with degassed, distilled water, except for the 2 transmucosal potential difference channels, which were perfused with degassed 0.9% saline, at a rate of 0.15 mL/min (27). Once the manometric catheter was positioned (ie, which took between 30–100 min), fasting motility was observed until phase III of the interdigestive migrating motor complex occurred. Immediately after cessation of phase III activity, during motor quiescence (phase I of the migrating motor complex), an intravenous cannula was placed in a right-forearm vein for blood sampling, and a baseline blood sample (t = −15 min) was taken for the subsequent determination...
of plasma cholecystokinin, GLP-1, PYY, ghrelin, blood glucose, and serum insulin concentrations, and a validated visual analog scale (VAS) questionnaire (28) was completed to assess perceptions of appetite and gastrointestinal symptoms. At \( t = 0 \) min, \( \sim 15-45 \) min after the correct positioning of the catheter, the 60-min duodenal infusion commenced. During the infusion, antropyloroduodenal motility was measured continuously, and VAS ratings and 14-mL venous blood samples were obtained at 15-min intervals. At \( t = 60 \) min (\( \sim 105-205 \) min after initial intubation), the infusion was terminated, and the intraduodenal catheter was removed. Subjects were presented with a standard, cold, buffet-style meal in excess of what they were expected to consume and instructed to eat freely for up to 30 min (\( t = 60-90 \) min) or until comfortably full as described previously in detail (29). At \( t = 90 \) min, immediately after completion of the meal, a final blood sample was taken, and a VAS was completed, before the intravenous cannula was removed, and the subject was allowed to leave the laboratory.

**Measurements**

**Energy intake**

The amount eaten (g) was quantified by weighing the buffet meal before and after consumption. Energy (kcal) and macronutrient (g) content and the percentage contribution to energy from fat, carbohydrate, and protein were calculated with commercially available software (Foodworks, version 3.01; Xyris Software) (29). Total energy intake was defined as the sum of energy intake from the buffet and energy content of the infusion. The percentage of compensation for a given protein infusion was calculated using the equation (30):

\[
\text{Percentage of compensation} = \left[ \frac{\text{energy intake}_{\text{buffer; control infusion}} - \text{energy intake}_{\text{test infusion}}}{\text{energy content}_{\text{buffer; control infusion}} - \text{energy content}_{\text{test infusion}}} \right] \times 100 \tag{1}
\]

where energy intake represents the energy consumed from the ad libitum buffet-style meal in response to the control and respective comparative test infusions (ie, P0.5, P1.5, or P3), and energy content represents the energy consumed from the respective comparative test infusion or the control infusion. The factor of 100 was used to express the result as an index of 100%, where 100% represents the full compensation of the caloric load of the infusion.

**Appetite perceptions and gastrointestinal symptoms**

Perceptions of hunger, fullness, desire to eat, and prospective consumption, as well as nausea and bloating, were rated by using validated VAS questionnaires (28). These questionnaires consisted of 100-mm horizontal lines, where 0 represented that the sensation was “not felt at all,” and 100 represented that the sensation was “felt the greatest.” Subjects placed a vertical mark on each horizontal line to indicate the strength of each sensation felt at the specified time points.

**Antropyloroduodenal motility**

Antropyloroduodenal pressures were recorded continuously over the 60-min infusion and digitized by using a computer-based system that ran commercially available software (Flexisoft v3; Oakfield Instruments, GS Hebbard) and stored for subsequent analysis. Data were analyzed for J the number and amplitude of antral pressure waves (PWs), 2 basal pyloric pressure (BPP), 3 the number and amplitude of IPPWs, and 4 the number and amplitude of duodenal PWs. PWs were defined by an amplitude >10 mm Hg with a minimum time interval of 15 s between peaks for antral and pyloric waves and 3 s for duodenal waves (31). BPP was calculated by subtracting the mean basal pressure (with phasic pressures excluded) recorded at the most distal antral channel from the mean basal pressure recorded at the sleeve (27) with custom-written software modified to our requirements (A Smout, University Medical Centre).

**Plasma gut hormone, serum insulin, and blood glucose concentrations**

Ten-milliliter venous blood samples were collected in ice-chilled tubes treated with EDTA for plasma hormone analysis, and 4-mL samples in serum Z tubes that contained clotting beads (Sarstedt Australia Pty Ltd) were used for serum insulin. 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, PYY, ghrelin, and insulin.

Plasma cholecystokinin-8 concentrations (pmol/L) were measured after ethanol extraction by using an adapted radioimmunoassay (32). Standards were prepared by using synthetic sulfated cholecystokinin-8 (Sigma-Aldrich) and an antibody (C2581, Lot 105H4852; Sigma-Aldrich). Sulfated cholecystokinin-8 labeled with Bolton and Hunter reagent (Perkin Elmer) was used as a tracer. The antibody-bound fraction was separated by the addition of dextran-coated charcoal that contained gelatin and the radioactivity determined in the supernatant fluid after centrifugation. Intrassay and interassay CVs were 6.8% and 9.2%, respectively. The minimum detectable concentration was 1 pmol/L.

Plasma GLP-1 concentrations (pmol/L) were determined after ethanol extraction by using a radioimmunoassay kit (GLPIT-36HK; Millipore) (9). The antibody used did not crossreact with glucagon, gastric inhibitory peptide, or other gut or pancreatic peptides, and it measured intact GLP-1(7–36) amide as well as the degraded form GLP-1(9–36) amide. Intraassay and interassay CVs were 4.2% and 10.5%, respectively. The minimum detectable concentration was 3 pmol/L.

Plasma PYY (pmol/L) was measured by using a radioimmunoassay with an antiserum raised in rabbits against human PYY(1–36) (Sigma-Aldrich); thus, the assay measured both PYY(1–36) and PYY(3–36). The antiserum showed <0.001% crossreactivity with human pancreatic polypeptide or sulfated cholecystokinin-8 and 0.0025% crossreactivity with human neuropeptide Y. Standards (1.6–50 fmol/tube) or samples (200 µL plasma) were incubated in 200 µL assay buffer (50 mmol/L NaPO4, 10 mmol/L EDTA, 2 g/L gelatin, and 0.1 g/L Na-Azide; pH 7.4) and a 1/12,000 dilution of antisera for 24 h followed by an incubation with 100 µL 10,000-cpm tracer (NEX3410; Perkin Elmer) for 24 h. Antibody-bound tracer was separated from free tracer by a second antibody precipitation that was followed by incubation for 2 h at room temperature and centrifugation at 4000 rpm for 20 min. The supernatant fluid was discarded, and pellets were counted in a γ counter (33). Intrassay and interassay CVs were 6.5% and 4.2%, respectively. The minimum detectable concentration was 1.5 pmol/L.

Plasma ghrelin concentrations (pg/mL) were measured by using a radioimmunoassay according to a method that was modified from...
a previously published method (12). The radiolabel (NEX388) was purchased from Perkin Elmer. Briefly, the sensitivity of the curve was improved by modification to a disequilibrium assay. The standard and samples were incubated with the antibody for 3–4 d before incubation with the radiolabel for an additional 24 h at 4°C. Intraassay and interassay CVs were 5.2% and 7.5%, respectively. The minimum detectable concentration was 40 pg/mL.

Venous blood glucose concentrations (mmol/L) were measured immediately by using the glucose oxidase method with a portable glucometer (Medisense Precision QID; Abbott Laboratories) (34). The accuracy of this method has been confirmed in our laboratory by using the hexokinase technique (34).

Serum insulin concentrations (mU/L) were measured by using an ELISA (10-1113; Mercodia) (9). Intraassay and interassay CVs were 2.6% and 4.8%, respectively. The minimum detectable concentration was 1.0 mU/L.

Data and statistical analyses

Statistical analyses were performed with SPSS software (version 19.0; IBM). Baseline values for VAS scores and plasma hormone, blood glucose, and serum insulin concentrations were calculated as the means of values obtained at t = -15 min and t = 0 min. Baseline values for numbers and amplitudes of antral and duodenal PWs and IPPWs and BPP were calculated as means of values obtained between t = -10 to 0 min. To characterize antral and duodenal PWs, motility indexes (MIs) were calculated by using the following equation (9, 23):

$$MI = \ln(\frac{\text{sum of amplitudes \times number of phasic PWs}}{+1})$$

(2)

BPPs and the number and amplitude of IPPWs were expressed as mean values over 15-min intervals.

Appetite perceptions and gut hormone, insulin, and glucose concentrations were analyzed by using repeated-measures ANOVA.

Energy and macronutrient intakes at the buffet meal; the total energy intake (ie, energy intake at the buffet meal plus the energy content of infusion); the total number, mean amplitude, and MI of antral and duodenal PWs; and tonic and phasic pyloric pressures were analyzed by using 1-factor ANOVA. Post hoc comparisons, which were adjusted for multiple comparisons by using Bonferroni’s correction, were performed when ANOVAs revealed significant effects. The percentage of compensation was examined by using a 1-sample t test and testing each treatment against a fixed value of zero (which represented no compensation of the energy content of the infusant) and against a fixed value of 100 (which represented perfect compensation for the energy content of the infusant) (30).

Relations of energy intake with AUCs (which were calculated by using the trapezoidal rule) for BPPs, IPPWs, plasma hormones, serum insulin, and blood glucose concentrations and VAS were evaluated by using linear within-subject correlations (r) (35). Statistical significance was accepted at P < 0.05; all data are presented as means ± SEMs.

RESULTS

All 16 subjects completed all 4 study visits, and experimental procedures were tolerated well.

Antypyloroduodenal pressures

Antral pressures

Baseline values for antral PWs did not differ between study days. There was an effect of treatment on the total number, mean amplitude, and MI of antral PWs (P < 0.05) (Table 1). Post hoc comparisons revealed no difference between treatments for the number of antral PWs. The mean amplitude of antral PWs was reduced during P3 compared with during control (P < 0.05). The mean antral MI was reduced during both P3 and P1.5 compared

### TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>P0.5 (30 kcal)</th>
<th>P1.5 (90 kcal)</th>
<th>P3 (180 kcal)</th>
<th>P</th>
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<tr>
<td><strong>Antral PWs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>96 ± 16</td>
<td>78 ± 22</td>
<td>56 ± 14</td>
<td>34 ± 11</td>
<td>&lt;0.05</td>
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<tr>
<td>Amplitude (mm Hg)</td>
<td>33 ± 4</td>
<td>29 ± 4</td>
<td>20 ± 3</td>
<td>16 ± 3^2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MI</td>
<td>7.3 ± 0.5</td>
<td>6.9 ± 0.5</td>
<td>5.1 ± 0.4^2</td>
<td>4.0 ± 0.8^2,3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>BPP</td>
<td>0.4 ± 0.4</td>
<td>0.2 ± 0.6</td>
<td>0.7 ± 0.5</td>
<td>2.2 ± 0.7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><strong>IPPWs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>32 ± 6</td>
<td>36 ± 8</td>
<td>50 ± 9</td>
<td>62 ± 11^3,4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Amplitude (mm Hg)</td>
<td>19 ± 3</td>
<td>21 ± 4</td>
<td>26 ± 4</td>
<td>28 ± 4</td>
<td>0.17</td>
</tr>
<tr>
<td>Duodenal PWs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>59 ± 27</td>
<td>64 ± 21</td>
<td>48 ± 16</td>
<td>38 ± 18^5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Amplitude (mm Hg)</td>
<td>26 ± 2</td>
<td>26 ± 1</td>
<td>25 ± 1</td>
<td>25 ± 2</td>
<td>0.84</td>
</tr>
<tr>
<td>MI</td>
<td>8.4 ± 0.3</td>
<td>7.9 ± 0.7</td>
<td>7.7 ± 0.3</td>
<td>7.1 ± 0.4</td>
<td>0.09</td>
</tr>
</tbody>
</table>

^1 All data are means ± SEMs. Intraduodenal infusions consisted of P0.5, P1.5, P3, or C for 60 min (all at 4 mL/min). P values are for main treatment effects determined by 1-factor ANOVA. Significant differences were determined by using post hoc comparisons with Bonferroni’s correction. BPP, basal pyloric pressure; C, saline control; IPPWs, isolated pyloric pressure waves; MI, motility index; PWs, pressure waves; P0.5, whey protein hydrolysate load of 0.5 kcal/min; P1.5, whey protein hydrolysate load of 1.5 kcal/min; P3, whey protein hydrolysate load of 3 kcal/min.

^2 Significantly different from C, P < 0.05.

^3 Significantly different from P0.5, P < 0.05.

^4 Trend for significant difference from P0.5, P = 0.09.

^5 Trend for significant difference from C, P = 0.09.
with during control, and during P3 compared with during P0.5 ($P < 0.05$ for all). The antral MI was related inversely to the load of protein administered ($r = -0.44$; $P < 0.01$).

### BPPs

Baseline values for BPPs did not differ between study days. There was no effect of treatment on the mean amplitude of IPPWs ($P > 0.05$) (Table 1); post hoc comparisons, however, revealed no difference between treatments. The mean BPP was related directly to the load of protein administered ($r = 0.4$, $P < 0.05$).

### IPPWs

Baseline values for IPPWs did not differ between study days. The total number of IPPWs was greater during P3 than during control ($P < 0.05$) (Table 1) and also tended to be greater with P3 than with P0.5 ($P = 0.09$). There was no effect of treatment on the mean amplitude of IPPWs (Table 1). The total number of IPPWs was related directly to the load of protein administered ($r = 0.48$, $P < 0.01$).

### Duodenal pressures

Baseline values for duodenal PWs did not differ between study days. There was an effect of treatment on the total number of duodenal PWs ($P < 0.05$) (Table 1); fewer duodenal PWs tended to be present after P3 than after control ($P = 0.1$) (Table 1). There was no effect of treatment on the mean amplitude of duodenal PWs. There was a trend for an effect of treatment on duodenal MI ($P = 0.09$) (Table 1). There was no relation between duodenal pressures and the load of protein administered.

### Gut hormone, glucose, and insulin concentrations

There were no differences in baseline values between study days for plasma cholecystokinin, GLP-1, PYY, ghrelin, blood glucose, or serum insulin (Figure 1, A–F).

#### Cholecystokinin

There was a treatment $\times$ time interaction for plasma cholecystokinin concentrations ($P < 0.001$), which were greater during P3 than during control, P0.5, and P1.5 at $t = 60$ min ($P < 0.05$ for all) (Figure 1A). The plasma cholecystokinin AUC was related directly to the load of protein administered ($r = 0.42$, $P < 0.01$).

#### GLP-1

There was a treatment $\times$ time interaction for plasma GLP-1 concentrations ($P < 0.001$) (Figure 1B), which were greater during P3 than during control between $t = 15–60$ min ($P < 0.05$ for all) and compared with during P1.5 and P0.5 at $t = 60$ (both $P < 0.05$), during P1.5 compared with during control between $t = 15–45$ min (both $P < 0.05$), and during P0.5 compared with during control at $t = 15$ min ($P < 0.05$). The plasma GLP-1 AUC was related directly to the load of protein administered ($r = 0.64$, $P < 0.01$).

#### PYY

There was a treatment $\times$ time interaction for plasma PYY concentrations ($P < 0.05$) (Figure 1C), which were greater during P3 and P1.5 than during control at $t = 15$ min ($P < 0.05$). There was no relation between the plasma PYY AUC and load of protein administered.

#### Ghrelin

There was a treatment $\times$ time interaction for plasma ghrelin concentrations ($P < 0.001$) (Figure 1D), which were less during P3 than during control at $t = 60$ min ($P < 0.01$). There was no relation between the plasma ghrelin AUC and load of protein administered.

#### Blood glucose

There was a treatment $\times$ time interaction for blood glucose concentrations ($P < 0.01$) (Figure 1E), which were lower during P3 than during control and P0.5 at $t = 60$ min ($P = 0.05$ and $P < 0.05$, respectively). There was no relation between the blood glucose AUC and load of protein administered.

#### Serum insulin

There was a treatment $\times$ time interaction for serum insulin concentrations ($P < 0.001$) (Figure 1F), which were greater during P3 than during control and P0.5 between $t = 15–60$ min ($P < 0.05$ for all) and compared with during P1.5 between $t = 45–60$ min ($P < 0.05$), during P1.5 compared with during control.
control between $t = 45$–60 min ($P < 0.05$), and compared with during P0.5 at $t = 30$ and $t = 60$ min ($P < 0.05$). The serum insulin AUC was related directly to the load of protein administered ($r = 0.41$, $P < 0.01$).

**Appetite perceptions and gastrointestinal symptoms**

There was no difference between protein loads in the scores of any of the appetite perceptions or gastrointestinal symptoms at baseline or during duodenal infusions. Ratings of fullness and bloating were weakly increased over the 60-min infusion period (both $P < 0.05$), but hunger, desire to eat, prospective consumption, and nausea did not change over time (see Online Supplemental Material under “Supplemental data” in the online issue).

**Energy and macronutrient intakes**

There was an effect of treatment on energy intake (kcal) from the buffet meal ($P < 0.01$). P3 reduced energy intakes compared with all other treatments (all $P < 0.05$), and P1.5 tended to reduce energy intake compared with P0.5 ($P = 0.069$) (Table 2). After all loads of protein, subjects compensated the energy consumed at the subsequent ad libitum meal (1-sample t-test against 0; $P < 0.001$), and in response to P3, there was a trend for overcompensation (ie, $\geq 100\%$) (1-sample t-test against 100; $P = 0.09$) (Table 2). There was no significant effect of treatment on the total energy intake (the energy consumed at buffet plus the energy content of infusion) ($P = 0.2$), despite an $\sim 140$-kcal reduction with P3 compared with control. There was a trend for an effect of treatment on the amount (g) of food consumed at the buffet ($P = 0.078$) (Table 2). There was an effect of treatment on grams of food consumed as protein ($P < 0.001$), fat, and carbohydrate (both $P < 0.05$). P3 reduced the amount (g) of food consumed as protein compared with control and P0.5 (both $P < 0.05$), the amount of food consumed as fat compared with control and P0.5 ($P = 0.69$ and $P < 0.05$, respectively), and the amount of food consumed as carbohydrate compared with all other treatments (all $P < 0.05$). There was no effect of treatment on the percentage of total energy from protein, fat, or carbohydrate. The energy intake at the buffet meal was related inversely to the load of protein administered ($r = -0.59$, $P < 0.001$).

**Relations between antropyloroduodenal motility, gut hormones, insulin, and glucose with energy intake**

The energy intake from the buffet was related inversely to the BPP AUC ($r = -0.57$, $P < 0.001$), number of IPPWs ($r = -0.4$, $P < 0.05$), number of antral PWs ($r = -0.37$, $P < 0.05$), cholecystokinin ($r = -0.42$, $P < 0.05$), GLP-1 ($r = -0.44$, $P < 0.05$), and insulin AUC ($r = -0.51$, $P < 0.001$). The energy intake, after adjustment for cholecystokinin, GLP-1, and insulin, was related inversely to BPP ($r = -0.51$, $P < 0.001$).

**DISCUSSION**

This study showed that the intraduodenal whey protein hydrolysate, when infused at loads that encompassed the normal range of gastric emptying (15), suppressed antral motility; stimulated pyloric pressures and plasma cholecystokinin, GLP-1, and insulin

### TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>P0.5 (30 kcal)</th>
<th>P1.5 (90 kcal)</th>
<th>P3 (180 kcal)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy intake (buffet; kcal)</td>
<td>1237 ± 111</td>
<td>1191 ± 113</td>
<td>1077 ± 125</td>
<td>912 ± 120$^{2,4}$</td>
<td>$&lt;0.05$</td>
</tr>
<tr>
<td>Percentage of compensation$^2$</td>
<td>NA</td>
<td>153 ± 316</td>
<td>178 ± 101</td>
<td>181 ± 46$^{0.7}$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Total energy intake (kcal)$^3$</td>
<td>1237 ± 111</td>
<td>1221 ± 113</td>
<td>1166 ± 125</td>
<td>1091 ± 120</td>
<td>0.21</td>
</tr>
<tr>
<td>Amount eaten (g)</td>
<td>1261 ± 102</td>
<td>1196 ± 104</td>
<td>1109 ± 118</td>
<td>1009 ± 107</td>
<td>0.08</td>
</tr>
<tr>
<td>Fat g</td>
<td>50 ± 7</td>
<td>45 ± 4</td>
<td>40 ± 5</td>
<td>33 ± 5$^{3,4}$</td>
<td>$&lt;0.05$</td>
</tr>
<tr>
<td>Percentage of energy</td>
<td>35 ± 3</td>
<td>33 ± 2</td>
<td>31 ± 2</td>
<td>31 ± 2</td>
<td>0.19</td>
</tr>
<tr>
<td>Carbohydrate g</td>
<td>134 ± 13</td>
<td>134 ± 14</td>
<td>123 ± 15</td>
<td>108 ± 14$^{2,3,9}$</td>
<td>$&lt;0.05$</td>
</tr>
<tr>
<td>Percentage of energy</td>
<td>45 ± 2</td>
<td>46 ± 2</td>
<td>48 ± 3</td>
<td>50 ± 3</td>
<td>0.13</td>
</tr>
<tr>
<td>Protein g</td>
<td>63 ± 6</td>
<td>63 ± 7</td>
<td>55 ± 7</td>
<td>45 ± 7$^{2,3}$</td>
<td>$&lt;0.05$</td>
</tr>
<tr>
<td>Percentage of energy</td>
<td>21 ± 1</td>
<td>21 ± 1</td>
<td>20 ± 1</td>
<td>19 ± 1</td>
<td>0.37</td>
</tr>
</tbody>
</table>

$^1$All data are means ± SEMs ($n = 16$). The buffet meal was consumed immediately after 60-min intraduodenal infusions of P0.5, P1.5, P3, or C (all infused at 4 mL/min). $P$ values are for main treatment effects determined by 1-factor ANOVA. Significant differences were determined by using post hoc comparisons with Bonferroni’s correction. C, saline control; NA, not applicable; P0.5, whey protein hydrolysate load of 0.5 kcal/min; P1.5, whey protein hydrolysate load of 1.5 kcal/min; P3, whey protein hydrolysate load of 3 kcal/min.

$^2$Significantly different from C, $P < 0.05$.

$^3$Significantly different from P0.5, $P < 0.05$.

$^4$Trend for significant difference from C, $P = 0.09$.

$^5$Percentage of compensation = $[\text{energy content}_{\text{test infusion}} - \text{energy content}_{\text{control infusion}}]^{2} [\text{energy content}_{\text{test infusion}} - \text{energy content}_{\text{control infusion}}] + \text{energy content}_{\text{test infusion}} - \text{energy content}_{\text{control infusion}}] \times 100$ and was determined by using a 1-sample t-test against a fixed value of 100, which represents perfect compensation for the energy content of the test infusion.

$^6$Significantly different from a fixed value of 0% of compensation ($P < 0.05$).

$^7$Trend for compensation was different from a fixed value of 100% of compensation ($P = 0.09$).

$^8$Sum of energy intake at the buffet meal and energy content of the respective test infusion.

$^9$Significantly different from P1.5, $P < 0.05$. 
concentrations; reduced glucose concentrations; and suppressed energy intakes. Compared with the control, only P3 maximally modulated all gastrointestinal parameters and resulted in a substantial reduction in energy intakes while maintaining normoglycemia. After adjustment for the effects of GLP-1, cholecystokinin, and insulin, BPPs were the major determinant of the reduction in energy intakes. Appetite perceptions were not altered by increased loads of protein and did not appear to influence energy intakes.

A major finding of this study was the magnitude of compensation in energy intakes of −153%, 178%, and 181% in response to increasing loads of protein (ie, 8 g for P0.5, 24 g for P1.5, and 48 g for P3) but particularly in response to P3. Although a protein load–dependent reduction in energy intakes at a subsequent meal has been reported by Astbury et al (36), the actual percentage reductions observed were substantially less. In addition, other authors (37) reported no load-dependent effect of protein. In the 2-part study of Astbury et al (36), the effects of protein loads of 13, 25, and 50 g in a 1.6-MJ mixed meal (part 1), and 7, 13, and 25 g in a 1-MJ mixed meal (part 2) were compared. Compared with a control (0 g protein), the authors showed a compensation of 37%, 45%, and 77% (part 1) and 57%, 78%, and 96% (part 2), respectively. Because the magnitude of compensation that we observed was greater at all loads and, particularly, in response to P3, for which it was >100% (which reflected overcompensation for the energy content of the infusion), it is plausible that protein administered directly and quickly into the small intestine, rather than when consumed orally, more potently suppresses energy intake, at least in lean, healthy individuals. If this is correct, it would imply that small intestinal factors may be as important as signals that originated from the oral cavity and stomach in the regulation of energy intake. We also acknowledge that the mixed-nutrient nature of the test drinks used in the study of Astbury et al (36) may have contributed to the reduced response they showed compared with that in our study.

The primary aim of this study was to determine interrelated gastrointestinal factors that underlie the protein-induced suppression of energy intake. We report that protein that entered the duodenum at 1.5 kcal/min was sufficient to suppress antral motility, whereas a higher load (3 kcal/min) appeared necessary to maximally stimulate tonic and phasic pyloric motility. Duodenal motor activity was also suppressed in a load-dependent manner, but the suppression did not reach significance at any load because of substantial within-subject variation. Furthermore, intraduodenal protein stimulated cholecystokinin and GLP-1, but not PYY or ghrelin, release in a load-dependent fashion. It appears that a protein load ≥3 kcal/min is required to stimulate cholecystokinin, whereas GLP-1 was stimulated, albeit transiently, by as little as 0.5 kcal protein/min. These thresholds agreed with previous observations with intraduodenal glucose (23) but appeared to be higher than those observed with lipid (9, 38). For example, 1.5 kcal lipid/min had potent effects on antral, pyloric, and duodenal pressures and cholecystokinin and PYY release (9). Collectively, these data indicate that there are regional and macronutrient-specific sensitivities within the gastrointestinal tract. In a broader context, it appears that in a given individual, the effects of protein are critically dependent on the rate of gastric emptying within that individual. These findings have implications for the use of food-based approaches to target factors involved in the regulation of energy intake.

The temporal profiles of the motility and hormonal responses, particularly in response to P1.5 and P3, suggest that the effects of protein are potentiated as the protein moves beyond the first 15 cm of the duodenum and, thereby, exposes more of the distal small intestine to the nutrient. As distal signals are released, they feedback and enhance the modulation of signals in the proximal gastrointestinal tract (39). Had we monitored responses beyond 60 min, the observed changes in antropyloroduodenal motility, cholecystokinin, PYY, ghrelin, and glucose may have increased, and responses to loads ≥1.5 kcal/min may have achieved significance.

The current data add to the evidence that pyloric motility is a major determinant of acute energy intake (6). Increased pyloric activity regulates nutrient delivery to the small intestine, which prolongs the exposure of endoenterocrinic cells and, thereby, increases neural and hormonal feedback to slow gastric emptying and reduces food intake. An indirect comparison of the current data with our previous findings suggested that intraduodenal protein reduces energy intake more than does fat (23, 38) or glucose (9), despite greater effects of fat on pyloric motility and gut hormone release. Accordingly, it is likely that the protein-induced reduction in energy intake was also mediated by factors that were not quantified in our study, including postprandial thermogenesis (40) and intestinal gluconeogenesis (41).

Also, as an extension of previous reports (3, 42), we have shown that whey protein, when delivered at a load that corresponds to the lower end of the normal range of gastric emptying, caused a prompt and substantial insulin release. The response was most likely caused by the direct stimulation of B cells by absorbed amino acids, especially branched-chain amino acids (21). It may have also been affected by the protein-induced inhibition of dipeptidyl peptidase-4 activity in the proximal small intestine, which would explain the protein load–dependent increase in intact GLP-1(7–36) amide (43). Although insulin was released within the first 15 min, glucose concentrations declined, only modestly, from t = 30 min. Because the insulin response was 4- to 8-fold greater with P1.5 and P3 than with the control, the reduction in glucose concentrations with these loads may have been greater; however, it is likely that increased glucagon release (3) and/or small intestinal gluconeogenesis (41) may have prevented a decline in peripheral glucose concentrations. Taken together, these findings imply that increased insulin concentrations induced by whey protein delivered at a mid-to-high rate of gastric emptying are unlikely to be problematic in individuals with normal glucose sensing. In type 2 patients with diabetes, particularly patients who have delayed gastric emptying, a protein-induced increase in insulin may aid the management of postprandial glycemia (21).

We anticipated that increased fullness and reduced hunger may contribute to a reduction in energy intake; however, the observed changes in appetite perceptions and gastrointestinal symptoms did not differ between treatments. This was not unexpected; one study that used a barostat to mimic the intragastric presence of food (44) and another recent study that compared intragastric and intraduodenal administrations of nutrients (45) suggested that the stomach is integral in the short-term regulation of appetite. Several aspects of our study design should be recognized when interpreting our results. Only healthy, lean males were included because they are more sensitive to dietary manipulation than are women (46). A 60-min infusion ensured that the
total protein loads delivered (ie, 8, 24, and 48 g, respectively) were representative of protein loads typically consumed at a snack or within a main meal (47). In conclusion, these findings indicate that the effects of protein on gastrointestinal function, glycemia, and appetite are critically dependent on the rate of delivery to the small intestine so that intraduodenal administration at the upper end of the normal rate of gastric emptying exerts potent effects on gastrointestinal mechanisms and satiety, while maintaining normoglycemia, in healthy men. Additional gastrointestinal factors, including thermogenesis and intestinal gluconeogenesis, may have also been involved, and an additional characterization of the effects of protein along the entire gastrointestinal tract may elucidate optimal strategies for sustaining a reduced appetite in obesity.

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The authors’ responsibilities were as follows—CF-B, PMC, MH, and NDL-M: designed the research; ATR, AK, and NDL-M: conducted the re-search; ATR and NDL-M: analyzed data and performed the statistical analy-ses; JMW: performed the hormone analyses; ATR, CB-B, PMC, MH, and NDL-M: contributed to data interpretation and wrote the manuscript; and NDL-M: had primary responsibility for the final content of the manuscript. None of the authors had a conflict of interest.

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