Effects of fat, protein, and carbohydrate and protein load on appetite, plasma cholecystokinin, peptide YY, and ghrelin, and energy intake in lean and obese men

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1University of Adelaide Discipline of Medicine, Royal Adelaide Hospital, Adelaide, and 2National Health and Medical Research Council of Australia Centre of Clinical Research Excellence in Nutritional Physiology, Interventions and Outcomes, University of Adelaide, Adelaide, South Australia, Australia; and 3Medizinische Klinik und Poliklinik IV, Klinikum der Universität München, Munich, Germany

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Brennan IM, Luscombe-Marsh ND, Seimon RV, Otto B, Horowitz M, Wishart JM, Feinle-Bisset C. Effects of fat, protein, and carbohydrate and protein load on appetite, plasma cholecystokinin, peptide YY, and ghrelin, and energy intake in lean and obese men. Am J Physiol Gastrointest Liver Physiol 303: G129–G140, 2012. First published April 30, 2012; doi:10.1152/ajpgi.00478.2011.—While protein is regarded as the most satiating macronutrient, many studies have employed test meals that had very high and unsustainable protein contents. Furthermore, the comparative responses between lean and obese subjects and the relationships between energy intake suppression and gut hormone release remain unclear. We evaluated the acute effects of meals with modest variations in 1) fat, protein, and carbohydrate content and 2) protein load on gastrointestinal hormones, appetite, and subsequent energy intake in lean and obese subjects. Sixteen lean and sixteen obese men were studied on four occasions. Following a standardized breakfast, they received for lunch: 1) high-fat (HF), 2) high-protein (HP), 3) high-carbohydrate/low-protein (HC/LP), or 4) adequate-protein (AP) isocaloric test meals. Hunger, fullness, and gut hormones were measured throughout, and at t = 180 min energy intake at a buffet meal was quantified. In lean subjects, hunger was less and fullness greater following HF, HP, and AP compared with HC/LP meals, and energy intake was less following HF and HP compared with HC meals (P < 0.05). In the obese subjects, hunger was less following HP compared with HF, HC/LP, and AP meals, and energy intake was less following HP and AP compared with HF and HC meals (P < 0.05). There were no major differences in hormone responses to the meals among subject groups, but the CCK and ghrelin responses to HP and AP were sustained in both groups. In conclusion, HP meals suppress energy intake in lean and obese subjects, an effect potentially mediated by CCK and ghrelin, while obese individuals appear to be less sensitive to the satiating effects of fat.

diet composition; obesity; protein intake; dietary macronutrients; gut hormone release

THE CURRENT AVERAGE WESTERN diet derives ~50% of energy from carbohydrate, 35% from fat, and 15% from protein (35), which represents a shift toward an increased carbohydrate and reduced protein intake when compared with the diet of our hunter-gatherer ancestors (14). Of the dietary macronutrients, protein is generally regarded as the most satiating nutrient. Accordingly, one dietary strategy for the management of obesity has been to replace some carbohydrate in the diet with protein (2, 19, 49), although the benefit of this approach remains uncertain (24, 29, 48). A recent study indicated that in obese subjects weight regain after a 26-wk period was less (by 930 g) in response to a high-protein diet (25% energy from protein), compared with a low-protein diet (13% energy from protein) (29). There is little definitive information about the comparative effects of the three macronutrients or differences in protein loads on energy intake, which has fundamental implications for the rational development of dietary strategies for the prevention and management of obesity. A substantial caveat in interpreting the outcome of studies that have reported that high-protein meals reduce subsequent appetite and energy intake is that some meals have contained large amounts of protein (~185 g/meal, equating to ~1.8 kg/meal wt) (4) or used protein powder or single foods (e.g., chicken) (43, 46), which makes it difficult, if not impossible, to manipulate test meals covertly and without introducing substantial differences in their texture, taste, and palatability. For example, in one study, lean women were less hungry and had a lower energy intake following consumption of a high-protein (65 g protein), compared with high-carbohydrate or high-fat (7 g protein each) test meals, but the high-protein meal was rated as the least pleasant to consume (43). Furthermore, not all studies that have compared the satiating efficiency of the three macronutrients have found protein to be more satiating than fat or carbohydrate (7, 17, 44, 56). For example, in a study of healthy lean men, isocaloric (~3 MJ) yoghurt preloads rich in either fat (40%, 25 g protein), carbohydrate (60%, 25 g protein), or protein (30%, 51 g protein) and controlled for palatability, volume, and energy density had comparable effects on subsequent energy intake (56). It is also well established that previous dietary intake, particularly a high-fat, high-energy diet can modify the gastrointestinal (GI) and appetite responses to nutrients (16). We have recently demonstrated that both oral and GI sensitivity to fat is diminished in the obese (54); thus, it is conceivable that a high-fat meal is less satiating in obese subjects.

The release of gut hormones, including cholecystokinin (CCK), peptide YY (PYY), and ghrelin, in response to nutrient ingestion is important in mediating satiation (5) and accordingly may mediate any differential effects of macronutrients on energy intake. PYY has received much attention for its apparent role in the regulation of energy intake by protein (3, 4), and in the obese, PYY release in response to meals has been reported to be less compared with lean subjects (31). However, evidence remains inconsistent; for example, while exogenous administration of PYY markedly suppresses energy intake in humans (3), this may reflect supraphysiological concentrations associated with nausea (18). It is, therefore, also possible that
the high PYY responses to protein meals are accounted for by the extreme protein content, leading to reduced palatability of the test meal (4). Macronutrients, particularly protein and fat, also potently stimulate the release of CCK (10, 33), and CCK is known to stimulate the release of PYY and suppress ghrelin (11, 12, 34) and to reduce energy intake (5). It is thus conceivable that these peptides interact to mediate the differential effects on energy intake in response to different macronutrients and protein loads and in obesity. In contrast to CCK and PYY, ghrelin concentrations are high in the fasted state and suppressed in response to meal ingestion (15), and in the obese, there is a diminished postprandial suppression when compared with lean individuals (32). The differential responses of plasma ghrelin in lean and obese subjects to standardized, equally palatable meals varying in their macronutrient composition or protein content are currently unknown.

The aims of this study were, therefore, to evaluate the acute effects of equally palatable 1) high-fat, high-protein, and high-carbohydrate meals; 2) increasing low, adequate, and high levels of protein on CCK, PYY, and ghrelin release, appetite, and energy intake; and 3) to compare these responses in lean and obese subjects. We hypothesized that 1) the high-protein meal would be more satiating than the high-fat or high-carbohydrate meals in both lean and obese subjects, 2) the high-fat meal would be less satiating in the obese, 3) increasing the protein content of the meal would be associated with greater satiety, and 4) these effects would be related to changes in GI hormone secretion.

MATERIALS AND METHODS

Subjects

Sixteen healthy, lean men (age: 29 ± 2 yr; range, 21 to 47 yr; body mass index: 24 ± 0.4 kg/m²; range, 19.9 to 25 kg/m²) and 16 obese men (age: 38 ± 4 yr; range, 18 to 55 yr; body mass index: 33 ± 0.5 kg/m²; range, 30 to 36.5 kg/m²) were recruited from an existing pool of volunteers through flyers placed around the Royal Adelaide Hospital, University of Adelaide and University of South Australia campuses and advertisements placed in the Adelaide newspaper The Sunday Mail. Based on preliminary data (which showed a within-subject SD of 1,700 kJ, an average correlation between treatments of 0.9, and an effect size among treatments in energy intake of 0.9), we calculated that a sample size of 15 subjects in each group was required to achieve power of 80%. The degree of eating restraint in an individual, i.e., the tendency to restrict food intake to control body weight, was measured in all subjects, using the Three Factor Eating questionnaire (55) but was only used as an exclusion criterion (score >12) in lean subjects, since obese subjects are likely to have some degree of eating restraint. The following exclusion criteria were also applied 1) significant GI, cardiovascular, or respiratory disease, symptoms, or surgery; 2) current use of medication known to affect GI function, appetite, or body weight; 3) other diseases including diabetes mellitus, gallbladder and pancreatic disease, and epilepsy; 4) allergy to anesthetic; 5) cigarette smoking and/or an alcohol intake in excess of 20 g/day; 6) high-performance athletes; and 7) lactose intolerance. Subjects were also required to be weight stable, i.e., < 5% fluctuation in their body weight at study entry as determined by their weight in the preceding 12 wk and were asked to maintain their usual physical activity over the course of the study. The study protocol was approved by the Royal Adelaide Hospital Human Research Ethics Committee, and the study was registered as a clinical trial (http://www.ANZCTR.org.au; ACTR no.: ACTRN12610003150111) and carried out in accordance with the Declaration of Helsinki. All subjects provided informed, written consent prior to their inclusion in the study. To distract from the primary aims of the study, each subject was informed that the study was designed to evaluate the effects of the different test meals on GI hormone release.

Study Protocol

Subjects were provided with a ready-to-eat dinner (beef lasagna, 2,472 kJ; McCain Foods, Victoria, Australia) to be consumed at 1900 h on the evening prior to each study day (to standardize study conditions) after which time they were required to fast. Each subject attended the laboratory in the Division of Medicine, Royal Adelaide Hospital, on four occasions, each separated by 3–7 days, at 0830 h. Subjects were comfortably seated, and an intravenous cannula was placed in a forearm vein for blood sampling. At 0845 h subjects were presented with a standard breakfast (to further standardize study conditions), consisting of a cup of white coffee or tea with 1 teaspoon of sugar, a glass of orange juice, and slices of whole-meal toast with butter and jam, which they consumed within 15 min. The energy content of the breakfast was individualized to comprise ~10% of each subject’s daily energy requirements (lean: 1,255 ± 19 kJ; obese: 1,320 ± 26 kJ), calculated using the Harris-Benedict equation and a physical activity factor between 1.4 and 1.6 based on each subject’s estimated daily activity (26). At 1130 h (t = 30 min), a 10-ml blood sample was taken, and a visual analog scale (VAS) questionnaire to assess appetite-related perceptions, nausea, and bloating was completed. After a 10-min baseline period, i.e., at t = 20 min, subjects ingested, in randomized order, either a 1) high-fat [HF; %energy from fat/protein/carbohydrate 55:15:30; protein content (g/kg body wt): 0.4], 2) high-protein (HP; 25:45:30; 1.35), 3) high-carbohydrate (HC; also low-protein LP; 30:10:60; 0.2), or 4) adequate-protein (AP; 30:30:40; 0.8) test meal within 20 min. The composition of the meals, thus, allowed evaluation of the comparative effects of macronutrients (HF, HC, HP) as well as of increasing protein loads (LP, AP, HP). The energy content of each test meal represented ~30% of each subject’s estimated daily energy requirement (lean: 3,766 ± 58 kJ; obese: 3,959 ± 79 kJ). Immediately following ingestion of the test meal (t = 0 min) another blood sample was taken, and VAS completed. Subsequently, blood samples were taken and VAS completed at 15-min intervals until t = 90 min, and at 30-min intervals until t = 180 min. At t = 180 min, subjects were presented with a cold, buffet-style meal to assess their energy intake. At t = 210 min, a final blood sample was taken, and VAS completed. The intravenous cannula was then removed, and the subject was allowed to leave the laboratory.

Test meal preparation.

Each test meal (ingredients are detailed in Table 1) comprised conventional pasta, with a tomato-based sauce, lean beef mince, onion, olive oil, and mixed dried herbs, and a vanilla yoghurt dessert. To achieve the described macronutrient compositions, whey protein isolate was used to increase the protein, pure cream to increase the fat, and corn flour and raw sugar to increase the carbohydrate content of the meals. All test meals were prepared by the same investigator on the morning of each study day. The four meals were isocaloric and matched for volume, palatability, smell, texture, and appearance. Immediately after ingestion of each meal, subjects were asked to rate pleasantness.

Measurements

Plasma hormone concentrations. Blood samples were collected into ice-chilled EDT (Trayslot; Bayer Australia, Pymble, Australia). Plasma was separated by centrifugation at 3,200 rpm for 15 min at 4°C within 30 min of collection and stored at −70°C until assayed.

Plasma CCK concentrations (pmol/l) were determined by a sensitive and specific radioimmunoassay (45). In short, the antibody (CH40IX) raised in rabbits, was specifically directed to the biologically active site of CCK, including the sulfated tyrosyl residue at position 7 from the COOH-terminal end, and showed no cross-reactivity with unsulfated CCK-8, unsulfated gastrin-17, or unsulfated...
Table 1. Ingredients and nutrient composition of the high-fat (HF), high-protein (HP), high-carbohydrate/low-protein (HCLP), or adequate-protein (AP) test meals

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>HF</th>
<th>HP</th>
<th>HCLP</th>
<th>AP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extra virgin olive oil, g</td>
<td>7.7</td>
<td>1.3</td>
<td>3.5</td>
<td>2.2</td>
</tr>
<tr>
<td>Onion, raw, g</td>
<td>3.1</td>
<td>2.4</td>
<td>2.9</td>
<td>2.6</td>
</tr>
<tr>
<td>Premium beef, g</td>
<td>13.7</td>
<td>24.6</td>
<td>2.9</td>
<td>20.5</td>
</tr>
<tr>
<td>Pasta sauce, g</td>
<td>19.9</td>
<td>25.6</td>
<td>29</td>
<td>25.9</td>
</tr>
<tr>
<td>Mixed herbs, g</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Pasta, g</td>
<td>18.3</td>
<td>15.2</td>
<td>32.5</td>
<td>23.7</td>
</tr>
<tr>
<td>Pure cream, g</td>
<td>7.1</td>
<td>1.7</td>
<td>3.8</td>
<td>4.3</td>
</tr>
<tr>
<td>Whey protein powder, g</td>
<td>2.5</td>
<td>14.7</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Corn flour, g</td>
<td>0.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skim milk, g</td>
<td></td>
<td></td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>Full-fat vanilla yoghurt, g</td>
<td>15.3</td>
<td>12.3</td>
<td>17.4</td>
<td>14</td>
</tr>
</tbody>
</table>

Energy content, kJ
- HF: 940
- HP: 888
- HCLP: 892
- AP: 872

Carbohydrate, g
- HF: 18.7
- HP: 13.3
- HCLP: 32.0
- AP: 20.2

Protein, g
- HF: 9.8
- HP: 23.3
- HCLP: 6.2
- AP: 15.2

Fat, g
- HF: 15.8
- HP: 7.3
- HCLP: 6.9
- AP: 7.4

g marginie, 170 g apple, 190 g banana, 200 g strawberry yogurt, 150 g chocolate custard, 140 g fruit salad, 375 ml iced coffee, 300 ml orange juice, and 600 ml water. The amount of food offered was in excess of what the subject was expected to eat, and each subject was allowed up to 30 min to freely consume from the buffet meal until comfortably full. Energy intake (kJ) and macronutrient intake [expressed as %energy and in absolute terms (g)] were analyzed using commercially available software (Foodworks 3.01; Xyris Software, Highgate Hill, QLD, Australia) (22).

**Statistical Analysis**

The statistical analysis was completed in three parts, according to the aims of the study, i.e., evaluation of (1) the effects of the three macronutrients, i.e., HF, HP, and HC meals; and (2) the effects of differences in protein content, i.e., HP, AP, and LP meals; and (3) comparison of lean and obese. All statistical analyses were performed using raw data. Repeated-measures ANOVA was used to evaluate VAS scores and gut hormones with time and treatment as factors. One-way ANOVA was used to analyze energy intake (kJ), weight of food consumed (g), and macronutrient distribution (% and g). Post hoc pair comparisons adjusted for multiple comparisons by Bonferroni’s correction were performed when ANOVAs revealed significant effects. Differences in the magnitude of the response in VAS scores and plasma hormones among test meals (t = 0 min vs. t = −30 min), as well as between lean and obese subjects, were evaluated using paired and unpaired t-tests, as appropriate. Differences among areas under the curves (t = 0–180 min; calculated using the trapezoidal rule) of VAS scores and hormone profiles, with energy intake, between lean and obese subjects, were compared using unpaired t-tests. Within-subjects relationships between energy intake with VAS scores and hormone concentrations at t = 180 min were calculated using the method described by Bland and Altman (6). Statistical significance was accepted at P < 0.05. Data are presented as means ± SE.

**RESULTS**

All subjects completed the four randomized study days and tolerated the experimental conditions well. Pleasantsness ratings (out of 100; 100 = extremely pleasant) for the test meals did not differ among test meals nor between lean and obese subjects (Lean: HF, 76 ± 5; HP, 75 ± 4; HC, 80 ± 4; AP, 76 ± 6. Obese: HF, 80 ± 3; HP, 74 ± 5; HC, 76 ± 6; AP, 77 ± 6).

**Effect of Macronutrient Content**

**Lean subjects. Appetite perceptions.** There was no difference in baseline (t = −30 min) hunger or fullness scores among study days (Fig. 1, A and C). Following all test meals (t = 0 min), hunger decreased (P < 0.001) and fullness increased (P < 0.001) promptly, with no differences among study days. There was a treatment-by-time (t = 0–180 min) interaction for hunger (P < 0.05) (Fig. 1A). Hunger was less following HP at t = 180 min when compared with HC (P < 0.01) and tended to be less compared with HF (P = 0.07) with no significant difference between HF and HC (P = 0.2). Hunger increased progressively on all study days (time effect: P < 0.01) and at t = 180 min was greater than baseline for HC (P < 0.001) but not for HF or HP. There was a treatment-by-time interaction for fullness (P < 0.05) (Fig. 1C). Fullness was greater following HP between t = 150 and 180 min (P < 0.05) and following HF at t = 180 min (P < 0.01) when compared with HC, with no difference between HP and HF. Fullness decreased progressively on all study days (time effect: P < 0.01) and at t = 180 min was still greater than baseline after HF (P = 0.05) and lower than baseline after HC (P < 0.05) with no difference after HP.
ENERGY INTAKE. There was an effect of treatment on energy intake at the buffet meal ($P < 0.01$) (Table 2). Energy intake was $\sim 14\%$ less following HP when compared with HC ($P < 0.01$) with no significant difference between HF and HP. There was no effect of treatment on the weight (g) of food consumed at the buffet meal. There was an effect of treatment on % energy consumed from fat at the buffet meal ($P < 0.05$), which was greater following HF when compared with HP and HC ($P < 0.05$ for both) with no difference between HP and HC (Table 2). There was no difference in % energy from carbohydrate or protein consumed at the buffet meal among treatments.

There was an effect of treatment on macronutrient intake (g) at the buffet meal for carbohydrate ($P = 0.05$) and protein ($P < 0.05$), but not fat (Table 1) so that carbohydrate intake (g) was less following HP when compared with HC ($P < 0.05$), and protein intake (g) was less following HP when compared with HC ($P < 0.05$) and HF ($P = 0.06$).

GUT HORMONE RESPONSES. For plasma CCK, there were no differences in baseline CCK concentrations among study days (Fig. 2A). Following test meal ingestion ($t = 0$ min), plasma
CCK increased following HP \((P = 0.05)\) but not significantly following HF or HC. There was a treatment-by-time interaction for plasma CCK \((P < 0.05)\) (Fig. 2A). There was a sustained elevation in plasma CCK following HP; levels were greater between \(t = 120\) and 180 min when compared with HC \((P < 0.05)\) and tended to be greater at \(t = 120\) min \((P = 0.08)\) when compared with HF with no difference between HF and HC. In contrast, following HF and HC, plasma CCK fell after \(t = 60\) min. At \(t = 180\) min, plasma CCK was lower than baseline following HF and HC (both \(P < 0.05\)) but not following HP.

For plasma PYY, there were no differences in baseline PYY concentrations among study days (Fig. 2C). Plasma PYY rose in response to all test meals \((P < 0.001)\), and the magnitude of the rise was greater following HP \((P < 0.01)\) and tended to be greater following HC \((P = 0.07)\) compared with HF with no difference between HP and HC. There was a treatment-by-time interaction for PYY \((P < 0.01)\) (Fig. 2C). After HF, PYY continued to rise until around \(t = 60\) min after which time concentrations fell on all study days \((P < 0.01)\). PYY was greater following HF at \(t = 90\) min when compared with HC \((P < 0.05)\) with no significant differences between HP and HF or HC although HP tended to be slightly higher at \(t = 180\) min \((P = 0.08)\). At \(t = 180\) min, plasma PYY was greater than baseline following HP \((P < 0.05)\) but not following HF or HC.

For plasma ghrelin, there were no differences in baseline ghrelin concentrations among study days (Fig. 2E). Immediately following test meal ingestion, ghrelin rose slightly following HF and HP \((P = 0.05)\) but not following HC. There was a treatment-by-time interaction for ghrelin \((P < 0.001)\) (Fig. 2E). Plasma ghrelin fell in response to all test meals, and there was a sustained suppression following HP and HF so that ghrelin was lower following HP between \(t = 120\) and 180 min \((P < 0.01)\) and following HF between \(t = 150\) and 180 min \((P = 0.05)\) when compared with HC and lower following HP between \(t = 90\) and 180 min when compared with HF \((P < 0.05)\). At \(t = 180\) min, plasma ghrelin was still less than baseline following HP and HF \((P < 0.01)\), but not following HC.

**Obese subjects.** **APPETITE PERCEPTIONS.** There was no difference in baseline hunger or fullness scores among study days (Fig. 1, B and D). In response to all test meals \((t = 0\) min), hunger decreased \((P < 0.05)\) and fullness increased \((P < 0.01)\) promptly with no differences among study days. There was a treatment-by-time interaction for hunger \((P < 0.01)\) (Fig. 1B). Hunger was less following HP at \(t = 15\) min and between \(t = 150\) and 180 min compared with HF \((P < 0.05)\) and between \(t = 90\) and 180 min compared with HC \((P < 0.01)\) with no difference between HF and HC. Hunger increased progressively on all study days (time effect: \(P < 0.01\)) and at \(t = 180\) min was greater than baseline for HF \((P < 0.01)\) and tended to be greater for HC \((P = 0.08)\) compared with baseline with no difference for HP. There was no effect of treatment but an effect of time \((P < 0.01)\) on fullness scores (Fig. 1D), which decreased progressively on all study days to levels at \(t = 180\) min that did not differ from baseline.

**ENERGY INTAKE.** There were effects of treatment on energy intake \((P < 0.01)\) and the weight of food consumed \((P < 0.05)\) at the buffet meal (Table 2). Energy intake was \(-18\%\) and \(-23\%\) less following HP compared with HF and HC \((P < 0.05\) for both), respectively, with no difference between HF and HC. There was a trend for the weight of food consumed to be less following HP compared with HC \((P = 0.095)\). There was no effect of treatment on %macronutrient distribution. There was an effect of treatment on macronutrient intake \((g)\) at the buffet meal for fat \((P = 0.05)\) and carbohydrate \((P < 0.05)\) but not

### Table 2. Energy and nutrient intake at the buffet meal following ingestion of HF, HP, HC/LP, or AP test meals in lean and obese subjects

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
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<th>Lean</th>
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<tbody>
<tr>
<td>Energy intake, kJ</td>
<td>4,156 ± 358</td>
<td>3,800 ± 404</td>
<td>4,509 ± 341</td>
<td>4,533 ± 384</td>
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<tr>
<td>Amount eaten, g</td>
<td>958 ± 73</td>
<td>957 ± 86</td>
<td>1,040 ± 72</td>
<td>1,061 ± 61</td>
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<tr>
<td>Macronutrient distribution, energy %</td>
<td></td>
<td></td>
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<tr>
<td>Fat</td>
<td>34 ± 1</td>
<td>32 ± 1</td>
<td>30 ± 1</td>
<td>33 ± 1</td>
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<tr>
<td>Carbohydrate</td>
<td>43 ± 2</td>
<td>48 ± 2</td>
<td>45 ± 2</td>
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<tr>
<td>Protein</td>
<td>23 ± 1</td>
<td>20 ± 1</td>
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<td>22 ± 1</td>
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<tr>
<td>Macronutrient intake, g</td>
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<tr>
<td>Fat</td>
<td>38 ± 4</td>
<td>35 ± 5</td>
<td>38 ± 4</td>
<td>40 ± 4</td>
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<tr>
<td>Carbohydrate</td>
<td>105 ± 10</td>
<td>101 ± 6°</td>
<td>119 ± 8</td>
<td>110 ± 8</td>
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<td>Protein</td>
<td>55 ± 5</td>
<td>47 ± 6°</td>
<td>58 ± 5</td>
<td>57 ± 6</td>
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<td>Obese Energy intake, kJ</td>
<td>4,887 ± 357</td>
<td>4,018 ± 452</td>
<td>5,206 ± 362</td>
<td>4,711 ± 334</td>
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<tr>
<td>Amount eaten, g</td>
<td>1,123 ± 77</td>
<td>970 ± 93</td>
<td>1,199 ± 84b</td>
<td>1,146 ± 87</td>
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<td>Macronutrient distribution, energy %</td>
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<td>Fat</td>
<td>34 ± 1</td>
<td>34 ± 2</td>
<td>33 ± 1</td>
<td>32 ± 2</td>
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<tr>
<td>Carbohydrate</td>
<td>43 ± 1</td>
<td>42 ± 3</td>
<td>43 ± 2</td>
<td>46 ± 2</td>
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<td>Protein</td>
<td>23 ± 1</td>
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<td>Macronutrient intake, g</td>
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<tr>
<td>Fat</td>
<td>44 ± 7</td>
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<td>45 ± 4</td>
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<tr>
<td>Carbohydrate</td>
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<td>97 ± 11°</td>
<td>128 ± 9</td>
<td>123 ± 10</td>
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<tr>
<td>Protein</td>
<td>66 ± 6</td>
<td>57 ± 7</td>
<td>67 ± 5</td>
<td>63 ± 5</td>
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</tbody>
</table>

Data are means ± SE, \(n = 16\) lean and \(n = 16\) obese subjects. \(^a\)Significantly different from HC/LP, treatment effect: \(P < 0.05\); \(^b\)significantly different from AP, treatment effect: \(P < 0.05\); \(^c\)trend for significant difference from HF, \(P = 0.06\); \(^d\)significantly different from HC/LP and AP, treatment effect: \(P < 0.05\); \(^*\)significantly different from HP and HC/LP, treatment effect: \(P < 0.05\); \(^\dagger\)trends for significant difference from lean subjects, \(P = 0.08\) (energy intake), \(P = 0.06\) (amount eaten); \(^\ddagger\)significantly different from HF and HC/LP, treatment effect: \(P < 0.01\); \(^\star\)trends for significant difference from lean subjects, \(P = 0.09\) (energy intake), \(P = 0.07\) (amount eaten); \(^\dagger\)significantly different from HP and HC/LP, treatment effect: \(P < 0.01\); \(^\ddagger\)significantly different from HF, HC/LP and AP, treatment effect: \(P < 0.05\).
protein (Table 2) so that fat intake (g) tended to be less following HP when compared with HC ($P < 0.07$), and carbohydrate intake (g) was less following HP when compared with both HC and HF ($P < 0.05$ for both).

**Gut hormone responses.** For plasma CCK, there were no differences in baseline CCK concentrations among study days (Fig. 2B). Following test meal ingestion ($t = 0$ min), plasma CCK increased following HP ($P < 0.01$) but not following HF or HC. There was a treatment-by-time interaction for plasma CCK concentrations ($P < 0.05$) (Fig. 2B). Following HP, plasma CCK was relatively stable from $t = 30$ to $t = 180$ min and greater compared with HF and HC between $t = 150$ and $180$ min ($P < 0.05$ for all) with no difference between HF and HC. In contrast, plasma CCK fell following HF and HC after $t = 60$ min. At $t = 180$ min plasma CCK was lower than baseline following HF and HC (both $P < 0.05$) but not following HP.

For plasma PYY, there were differences in baseline plasma PYY concentrations among study days ($P < 0.05$) (Fig. 2D). PYY concentrations tended to be greater on the HP compared with HC day ($P = 0.07$). Plasma PYY increased immediately following ingestion of all meals ($P < 0.01$) with no differences among meals. There was a treatment-by-time interaction for PYY ($P < 0.05$) (Fig. 2D). PYY concentrations rose until $t = 60–90$ min, after which time they fell on all study days. PYY was greater following HP compared with HC between $t = 90$ and $120$ min and at $t = 180$ min ($P < 0.05$) and greater following HF at $t = 150$ min ($P < 0.01$) when compared with HC. At $t = 180$ min, plasma PYY was still greater than baseline on all study days ($P < 0.05$ for all).

For plasma ghrelin, there were no differences in baseline ghrelin concentrations among study days (Fig. 2F). Plasma ghrelin increased immediately following all test meals ($t = 0$ min) ($P < 0.05$). There was a treatment-by-time interaction for ghrelin ($P < 0.001$) (Fig. 2F). Plasma ghrelin fell in response to all test meals: ghrelin was lower following HP between $t = 120$ and $180$ min ($P < 0.05$) and following HF at $t = 180$ min ($P < 0.05$) when compared with HC. At $t = 180$ min, plasma ghrelin was still less than baseline following HP and HF ($P < 0.01$) but not following HC.

**Effect of Protein Load**

**Lean subjects.** Appetite perceptions. There were no differences in baseline hunger or fullness scores among study days (Fig. 1, E and G). Immediately following the test meal, hunger...
decreased ($P < 0.001$) and fullness increased ($P < 0.01$) in response to all meals with no differences among study days. There was a treatment-by-time interaction for hunger ($P < 0.01$) (Fig. 1E). Hunger was less following HP and AP between $t = 75$ and 180 min compared with LP ($P < 0.05$), with no difference between HP and AP. Hunger increased progressively on all study days (time effect: $P < 0.01$) (Fig. 1). Hunger was less following HP and AP between $t = 75$ and 180 min compared with LP ($P < 0.05$), with no difference between HP and AP. There was a treatment-by-time interaction for fullness ($P < 0.05$) (Fig. 1G). Fullness was greater following HP between $t = 150$ and 180 min compared with LP ($P < 0.05$) and following AP at $t = 150$ min compared with LP ($P < 0.05$). Fullness decreased progressively on all study days (time effect: $P < 0.01$) and at $t = 180$ min was lower than baseline for LP ($P < 0.05$) but not for HP and AP.

ENERGY INTAKE. There was an effect of treatment on energy intake at the buffet meal ($P < 0.01$) (Table 2). Energy intake was less following HP compared with AP and LP ($P < 0.01$ for both) with no difference between AP and LP. There was no effect of treatment on the weight of food consumed at the buffet meal or %macronutrient distribution. There was an effect of treatment on macronutrient intake (g) at the buffet meal for carbohydrate ($P < 0.05$) and protein ($P < 0.05$) but not fat (Table 2), so that carbohydrate intake (g) was less following HP when compared with LP ($P < 0.05$) and protein intake (g) was less following HP when compared with both LP and AP ($P < 0.05$ for both).

GUT HORMONE RESPONSES. For plasma CCK, there were no differences in baseline CCK concentrations among study days (Fig. 3A). Immediately following the test meal ($t = 0$ min), plasma CCK increased after HP and AP ($P < 0.001$) but not significantly after LP ($P = 0.1$). There was a treatment-by-time interaction for plasma CCK ($P < 0.05$) (Fig. 3A). Plasma CCK was greater following HP between $t = 120$ and 180 min compared with LP ($P < 0.05$) and greater following AP at $t = 180$ min compared with LP ($P < 0.05$) with no difference between HP and AP. Following LP, but not HP or AP, plasma CCK fell markedly after $t = 60$ min so that at $t = 180$ min levels for LP were below baseline ($P < 0.05$).

For plasma PYY, there were no differences in baseline PYY concentrations among study days (Fig. 3C). Plasma PYY rose in response to all test meals ($P < 0.001$) with no differences in the magnitude of the responses. From approximately $t = 60$ min, levels declined (time effect: $P < 0.01$). At $t = 180$ min, plasma PYY was greater than baseline following HP ($P < 0.01$) (Fig. 3).
0.05) and tended to be greater following AP (P = 0.08) but not following LP.

For plasma ghrelin, there were no differences in baseline ghrelin concentrations among study days (Fig. 3E). Immediately following test meal ingestion, ghrelin rose following HP and AP (P < 0.05) but not following LP. There was a treatment-by-time interaction for ghrelin (P < 0.001) (Fig. 3E). Plasma ghrelin fell in response to all test meals, and there was a sustained suppression following HP and AP, so that ghrelin was lower following HP and AP between t = 120 and 180 min (P < 0.01) when compared with LP and tended to be lower following HP at t = 180 min when compared with AP (P = 0.09). At t = 180 min plasma ghrelin was still less than baseline following HP and AP (P < 0.01) but not following LP.

Obese Subjects

Appetite perceptions. There were no differences in baseline hunger or fullness scores among study days (Fig. 1, F and H). Immediately following the test meal, hunger decreased and fullness increased (P < 0.05 for all) with no differences in the magnitude of the responses. There was a treatment-by-time interaction (P < 0.05) for hunger scores and an effect of time (P < 0.001) but not treatment on fullness scores. Hunger was less following HP between t = 90 and 180 min compared with LP (P < 0.01). Overall, there was a progressive rise in hunger and reduction in fullness. At t = 180 min, hunger scores for HP did not differ from baseline, whereas they tended to be lower for AP (P = 0.06) and LP (P = 0.08), while fullness scores did not differ from baseline for any meal.

Energy intake. There were effects of treatment on energy intake (P < 0.01) and the weight of food consumed at the buffet meal (P < 0.05) (Table 2). Energy intake was less following HP compared with AP (P < 0.01) and LP (P < 0.01) with no difference between AP and LP. The weight of food consumed at the buffet meal tended to be less following HP compared with LP (P = 0.095) with no differences between HP and AP or AP and LP. There was no effect of treatment on %macronutrient distribution. There was an effect of treatment on macronutrient intake (g) at the buffet meal for fat (P = 0.05) and carbohydrate (P < 0.05) but not protein (Table 2), so that fat intake (g) tended to be less following HP when compared with LP (P = 0.07) and carbohydrate intake (g) was less following HP when compared with both LP and AP (P < 0.05 for both).

Gut hormone responses. For plasma CCK, there were no differences in baseline CCK concentrations among study days (Fig. 3B). Immediately following test meal ingestion (t = 0 min), plasma CCK increased following HP and AP (P < 0.05) but not following LP. There was a treatment-by-time interaction for plasma CCK concentrations (P < 0.05) (Fig. 3B). Plasma CCK was greater following HP at t = 90 min and at t = 180 min compared with LP (P < 0.05) and greater following AP between t = 90 and 120 min compared with LP (P < 0.05) with no difference between HP and AP. Plasma CCK rose slightly until t = 30 min after which time levels fell following LP but not AP or HP, so that at t = 180 min levels were lower than baseline for LP (P < 0.01) with no significant differences for AP or HP.

For plasma PYY, there were modest differences in baseline plasma PYY concentrations among study days (Fig. 3D). PYY concentration tended to be greater on the HP compared with the LP day (P = 0.07). Plasma PYY increased immediately following ingestion of all meals (P < 0.001) with no differences in the magnitude of responses among meals. There was a trend for a treatment-by-time interaction for PYY concentrations (P = 0.055) (Fig. 3D). On all study days, plasma PYY rose until t = 60–90 min after which time levels fell. Plasma PYY was greater following HP at t = 90 min, t = 120 min, and t = 180 min (P < 0.05) and tended to be greater at t = 150 min (P = 0.079) compared with LP and greater following AP between t = 120 and 180 min compared with LP (P < 0.05) with no difference between HP and AP. At t = 180 min plasma PYY was still greater than baseline on all study days (P < 0.05 for all).

For plasma ghrelin, there were no differences in baseline ghrelin concentrations among study days (Fig. 3F). Plasma ghrelin increased immediately following all test meals (t = 0 min) (P < 0.05). There was a treatment-by-time interaction for ghrelin (P < 0.01) (Fig. 3F). Plasma ghrelin fell in response to all test meals, and there was a sustained suppression following HP and AP so that ghrelin was lower following HP between t = 120 and 180 min (P < 0.05) and following AP between t = 150 and 180 min (P < 0.01) when compared with LP. At t = 180 min, plasma ghrelin was still lower than baseline following HP and AP (P < 0.01) but not following LP.

Comparison Between Lean and Obese Subjects

Appetite perceptions. There were differences in baseline fullness but not hunger scores between lean and obese, so that fullness was greater in the obese on HF (P < 0.05) and HP (P < 0.05) days and tended to be greater on AP (P = 0.09) and HC/LP (P = 0.07) days compared with the lean (Fig. 1). There were trends for the magnitude of the reduction (t = −30 min to 0 min) in hunger in response to HF (P = 0.08) and HP (P = 0.1) and for the increase in fullness in response to HF (P = 0.09) and HP (P = 0.01) to be greater in the lean compared with the obese with no differences in response to HC/LP or AP. There were no differences in areas under the curves (t = 0–180 min) of hunger or fullness profiles in response to the test meals between lean and obese.

Energy intake. There were trends for energy intake and the weight of food consumed to be greater in the obese following HF (energy intake: P = 0.08; amount eaten: P = 0.06) and HC/LP (energy intake: P = 0.09; amount eaten: P = 0.07) compared with lean subjects (Table 2).

Plasma CCK. There were no differences in baseline CCK concentrations or in response to the test meals between lean and obese subjects (Figs. 2 and 3).

Plasma PYY. Baseline PYY concentrations were slightly lower in the obese compared with the lean (P < 0.05); however, there were no differences in plasma PYY in response to the test meals between lean and obese (Figs. 2 and 3).

Plasma ghrelin. There were no differences in baseline ghrelin concentrations or in response to the test meals between lean and obese subjects (Figs. 2 and 3).

Relationships Between Energy Intake with Appetite and Gut Hormones

In lean subjects, there was a trend for a direct relationship between energy intake at the buffet meal and hunger (r = 0.26,
protein intake is 0.8 g·kg$^{-1}$·day$^{-1}$ (4). In obese subjects, the AP meal ($r = -0.35, P < 0.05$) but not PYY or ghrelin and between energy intake with plasma CCK ($r = -0.46, P < 0.01$) and plasma PYY ($r = -0.38, P < 0.01$) but not ghrelin. There was a direct relationship between plasma CCK and plasma PYY ($r = 0.6, P < 0.001$) and an inverse relationship between plasma ghrelin and plasma PYY ($r = -0.30, P < 0.05$) but not CCK at $t = 180$ min.

In the obese, there were no relationships between energy intake with hunger or fullness. There were inverse relationships between hunger and plasma CCK ($r = -0.35, P < 0.05$) but not PYY or ghrelin and between energy intake with plasma CCK ($r = -0.46, P < 0.01$) and plasma PYY ($r = -0.38, P < 0.01$) but not ghrelin. There was a direct relationship between plasma CCK and plasma PYY ($r = 0.6, P < 0.001$) and an inverse relationship between plasma ghrelin and plasma PYY ($r = -0.30, P < 0.05$) but not CCK at $t = 180$ min.

DISCUSSION

We aimed to address a number of the important inconsistencies that exist in the literature regarding the relative effects of macronutrients and the required load of protein to suppress energy intake in humans, as well as the underlying GI mechanisms. Thus, in our study we employed meals that can be regarded as every-day foods and that were varied covery for their macronutrient content within a realistic range and without compromising palatability. We were able to demonstrate that 1) the HP meal was most potent in reducing hunger and energy intake in both lean and obese subjects; 2) the HF meal reduced energy intake in lean but not obese subjects when compared with the HC/LP meal; 3) the HP and AP meals were associated with more sustained stimulation of CCK and suppression of ghrelin in both lean and obese subjects; 4) the HP, AP, and HF meals all stimulated PYY concentrations with no differences between lean and obese subjects; and 5) the obese subjects tended to have greater energy intakes after HF and HC/LP but not HP or AP meals when compared with lean subjects.

That the HP meal resulted in the lowest energy intake in both lean and obese subjects establishes clearly that protein is the most satiating macronutrient, at least acutely, and that the protein content of a meal is a key factor for satiety and appetite regulation (50, 57). The current recommended dietary advice for adequate protein intake is 0.8 g·kg$^{-1}$·day$^{-1}$ (defined as the minimum daily needs for protein to maintain short-term nitrogen balance in healthy people with moderate physical activity) (30). The HP meal ($\sim 1.35$ g/kg) resulted in a reduction in energy intake by $\sim 14$ and $22\%$ in lean and obese subjects, respectively, compared with the HC/LP test meal, consistent with previous studies (4, 43, 46). In obese subjects, the AP meal ($\sim 0.8$ g/kg) also markedly reduced energy intake, indicating that even a moderate load of protein may be sufficient to suppress energy intake in this group but not in the lean who appear to be less sensitive to the satiating effect of this amount of protein. While some studies have employed test meals with much higher protein contents, i.e., $\sim 1.85$ g/kg (4) in the context of the dietary management of obesity and associated comorbidities, including type 2 diabetes, levels of protein of this magnitude are probably unsafe for chronic consumption. In contrast, there is no evidence for detrimental effects of protein intake up to 1.4 g/kg on renal or cardiovascular function, as well as bone metabolism, when consumed for up to 15 mo (13, 28). Studies to investigate the chronic effects of protein intake in the range of 0.8 to 1.6 g/kg on appetite and energy intake and their regulation are now indicated.

Interestingly, there were differences in the responses to the different macronutrient- and protein-enriched meals between lean and obese subjects. In the lean, both HP and HF but not AP meals, reduced energy intake when compared with the HC/LP meal, while in the obese HP reduced energy intake compared with HF, HC/LP, and AP meals, and AP also reduced energy intake compared with the HC/LP meal. In addition, the obese tended to have greater energy intakes in response to HF (by $\sim 17.5\%$) and HC/LP (by $\sim 13\%$), but not HP or AP meals when compared with lean subjects. These observations suggest that the obese remain sensitive to the effects of protein (even moderate levels), while unlike the lean, their capacity to adjust their intake in response to a HF meal is impaired. This diminished response to dietary fat is not surprising given the reported effects of chronic exposure to a high-fat diet in both humans and experimental animals (36) and is also consistent with our recent observations of increased oral taste thresholds for and reduced small intestinal responses to oleic acid in obese compared with lean subjects associated with increased habitual energy and fat intakes (54). Whether similar adaptive responses occur in response to a moderately high-protein diet warrants investigation.

The mechanisms underlying the observed effects of macronutrients on energy intake remain unclear, despite much research in recent years. Differences between lean and obese individuals in the release of GI hormones have been implicated, and PYY, in particular, has received considerable attention (4, 9, 18). Our data do not suggest a major role for PYY, while there were differences among HF, HP, and AP meals with the HC/LP meal, there were no significant differences between lean and obese subjects except for modestly reduced baseline concentrations and a possible trend for an overall slightly reduced response to meals. Our observations are consistent with those of Smeets et al. (53) who reported that the effects of a single high-protein meal are not due to changes in plasma gut hormone concentrations, including ghrelin, GLP-1, and PYY. While we did not measure GLP-1 in the present study, its pattern of secretion is often similar to that of PYY (20, 21), which is not surprising given the sites of release. We did not observe any differences between lean and obese in the CCK or ghrelin responses to the meals. A striking feature in our data, however, was the sustained response of both CCK and ghrelin to the HP and AP meals in both subject groups. This confirms previous data from studies using liquid test meals (10) that showed that drinks containing protein, but not those containing carbohydrates, were associated with more prolonged suppression of ghrelin and stimulation of CCK, thus offering a potential explanation for the superior satiating capacity of protein. This previous study did not evaluate the comparative effects of fat, and it is somewhat surprising that in our study the HF meal did not result in the same CCK release, given that fat is regarded generally as the most potent CCK stimulus (33). Nevertheless, our data suggest that CCK may account for the more potent suppressive effects of protein on energy intake (51). Our findings relating to ghrelin contrast
those of previous studies (23, 32, 40) in that there was no difference in fasting or postprandial ghrelin concentrations between lean and obese, while obese subjects have been reported to have reduced fasting and postprandial levels (32). Furthermore, in our present study, ghrelin was suppressed most potently by the HP meal, while the HF meal had an intermediate effect and the HC meal the least effect, whereas previous studies reported carbohydrate and protein to be more effective than protein (23, 40). The reason(s) for the discrepancies among studies are unclear, but could relate to meal choice and composition, previous dietary patterns of subjects, and subject inclusion criteria. Interestingly, our findings relating to the hierarchy of ghrelin suppression by our test meals correspond to their effects on energy intake, i.e., the meals with the greatest ghrelin suppression were associated with the lowest energy intakes.

At the time the buffet meal was served (i.e., at t = 180 min) for the assessment of energy intake, hunger scores were much lower following the HP meal in both lean and obese subjects, suggesting that if the subjects had been able to self-determine the timing of the subsequent meal, this may have occurred at a later time point as has been reported (37). Despite these differences in hunger scores, significant relationships between energy intake with hunger and fullness at t = 180 min, i.e., immediately prior to the buffet meal were evident only in lean subjects but not in the obese. This supports previous findings that the obese are less sensitive to internal hunger and satiety signals and more reactive to external cues, i.e., time; the presence, quality, and palatability of food, and situational events (8, 38). Activation of reward pathways may also play a greater role in the obese in modulating food intake (27). There was a direct relationship in the obese and a trend in the lean, between plasma CCK and PYY levels as well as inverse relationships between CCK or PYY and ghrelin concentrations, indicating that the communication between upper GI regions, including stomach and proximal and distal small intestine was intact (11, 12, 34).

Rather than providing all subjects with a standardized amount of each test meal, we took into account individual daily energy requirements, thus ensuring that subjects were fed according to their metabolic needs. Obese individuals only required ~5% more energy than their lean counterparts, and our observations indicate that when in (or close to) energy balance, there are no major differences in the GI hormone responses to meals. This provides an additional explanation as to why other investigators may have found more striking differences in the obese.

Some limitations of the study should be recognized. Only male volunteers were studied because they have been reported to have a greater capacity to adjust their energy intake in response to dietary manipulation when compared with elderly men, healthy women and obese individuals (1, 47, 52). Hence, the observations may not be fully applicable to women. Moreover, since all subjects were either lean or obese, it remains unknown whether the observations apply to individuals who are overweight or morbidly obese. Our buffet-style meal is appropriate for the evaluation of energy intake but less effective in evaluating macronutrient distribution or food choice (39), i.e., when this meal was given to volunteers repeatedly on three separate occasions, energy intake was shown to be highly reproducible, but the foods eaten and thus the macronutrients that contributed to the energy consumed, varied considerably among days. Thus our data relating to macronutrient consumption should be viewed circumspectly. Finally, only the acute effects of macronutrients were evaluated. Whether the observed effects on appetite and energy intake are sustained following chronic dietary manipulation is unknown, although this is likely to be the case given the observed longer-term effects of a high-protein diet on body weight (57) and recent findings in lean subjects (25) that reducing the protein content of a diet for 4 days from 25% to 10% increases hunger and energy content, supporting the protein leverage hypothesis that energy intake will be increased until a sufficient protein intake is achieved.

In summary, this study has demonstrated that palatable, moderately high-protein test meals suppress energy intake and result in sustained CCK stimulation and ghrelin suppression but are without major effects on PYY release in both lean and obese subjects. Furthermore, obese subjects appear to be less sensitive to the satiating effects of fat. Since successful weight-loss diets should result in sustained suppression of appetite and food intake, chronic studies are now required to determine whether the acute effects of a moderate protein intake are sustained over a prolonged period of time.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES

MEAL MACRONUTRIENT CONTENT AND APPETITE IN LEAN AND OBESE MEN


