Energy Expenditure, Satiety, and Plasma Ghrelin, Glucagon-Like Peptide 1, and Peptide Tyrosine-Tyrosine Concentrations following a Single High-Protein Lunch$^{1,2}$

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Abstract

High-protein (HP) foods are more satiating and have a higher thermogenic effect than normal protein foods over the short-term as well as the long-term. We hypothesized that acute effects of higher protein intake on satiety may be related to acute metabolic and hormonal responses. The study was a single-blind, randomized, crossover design. Subjects underwent 2 indirect calorimetry tests for measurement of energy expenditure (EE) and substrate oxidation. After a standard subject-specific breakfast, subjects received 1 of 2 randomly assigned treatments: an appropriate protein (AP) lunch (10% energy (E) protein, 60%E carbohydrate, 30%E fat), or a HP lunch (25%E protein, 45%E carbohydrate, 30%E fat). The increase in postlunch EE tended to be greater after the HP lunch (0.85 ± 0.32 kJ/min) than after the AP lunch (0.73 ± 0.22 kJ/min) ($P = 0.07$). The respiratory quotient did not differ between the HP (0.84 ± 0.04) and the AP (0.86 ± 0.04) treatments. Satiety visual analogue scales (VAS) scores were significantly higher 30 and 120 min after the HP lunch than after the AP lunch. The area under the curve of the VAS score for satiety was higher after the HP lunch (263 ± 61 mm/h) than after the AP lunch (AP 236 ± 76 mm/h) ($P < 0.02$). Effects of the meals on satiety and diet-induced thermogenesis did not occur simultaneously with changes in plasma ghrelin, glucagon-like peptide 1, and peptide tyrosine-tyrosine concentrations. A single HP lunch, therefore, does not exert its acute effect on satiety through increased concentrations of satiety-related hormones. Other factors, which may explain the HP effect on satiety, may be metabolites or amino acids. J. Nutr. 138: 698–702, 2008.

Introduction

High-protein (HP)$^{6}$ foods are more satiating and have a higher thermogenic effect than normal protein foods over the short-term as well as the long-term (1–13). However, the mechanisms or factors that may play a role in the observed effects of HP foods are yet to be discovered. Gut hormones and their effects on hunger and metabolic speed are the first candidate factors to be studied. Ghrelin is a peptide secreted from the stomach and appears to be a hunger signal (14,15); i.v. infusion of ghrelin increases food intake and enhances appetite (15,16). In addition, plasma ghrelin concentrations rise gradually before a meal and decrease immediately after eating (17,18). Glucagon-like peptide 1 (GLP-1) is a 30-amino acid peptide hormone that is released from intestinal L-cells into the circulation after a mixed meal (19–21). Peripheral GLP-1 administration compared with saline infusions reduced food intake and suppressed appetite in normal-weight subjects (22) and led to lower hunger ratings and decreased energy intake after obese subjects consumed a meal (19–21). Peripheral GLP-1 administration compared with saline infusions reduced food intake and suppressed appetite in normal-weight subjects (22) and led to lower hunger ratings and decreased energy intake after obese subjects consumed a meal ad libitum (21). Peptide tyrosine-tyrosine (PYY) is a gut-derived hormone. Like proglucagon-derived peptides, PYY is synthesized and released from endocrine L-cells from the distal gut in response to food consumption (23). Fat is a strong stimulus for PYY release (24,25).

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6 Abbreviations used: AP, appropriate protein; DIT, diet-induced thermogenesis; E, energy; EE, energy expenditure; GLP-1, glucagon-like peptide 1; HP, high protein; PYY, peptide tyrosine-tyrosine; RO, respiratory quotient; TBW, total body water; VAS, visual analogue scale.
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the HP condition, satiety was related to protein intake and incidentally to plasma ghrelin and GLP-1 concentrations. It has not been determined whether these effects of a HP diet occur with exposure to 1 meal. Therefore, the aim of this study was to test the acute effects of a HP lunch on energy expenditure (EE), diet-induced thermogenesis (DIT), substrate oxidation, satiety related hormones (GLP-1, ghrelin, and PYY), and satiety. We hypothesized that a single HP meal compared with a single appropriate-protein (AP) meal may increase satiety, DIT, plasma GLP-1 and PYY concentrations, and fat oxidation, and decrease plasma ghrelin concentrations and respiratory quotient (RQ).

Subjects and Methods

Subjects. Thirty healthy subjects (19 women and 11 men) aged 18–60 y with a BMI of 20–30 kg/m² were recruited by advertisements in local newspapers and on notice boards at Maastricht University. All subjects underwent a medical screening and all were in good health, nonsmokers, not using medication, and at most moderate alcohol users. Eating behavior was assessed using a validated Dutch translation of the Three Factor Eating Questionnaire. Cognitive restrained and unrestrained eating behavior (factor 1), emotional eating and disinhibition (factor 2), and the subjective feeling of hunger (factor 3) were scored (26). The baseline characteristics of the subjects are presented in Table 1. Written informed consent was obtained from all participants. The Medical Ethics Committee of the Academic Hospital in Maastricht approved the study.

Experimental sessions. The study had a single-blind, randomized, crossover design. The subjects underwent 2 indirect calorimetry tests for the measurement of EE and substrate oxidation. On the morning before the test (0800), the subjects received a subject-specific breakfast (Drinkontbijt, Campina), which provided 15% of each subject’s individual daily energy requirements and consisted of a yogurt, bread, jam, and coffee or tea without milk or sugar. One hour before the test lunch was not allowed to eat or drink during the morning except water and 1 cup of coffee or tea without milk or sugar. One hour before the test lunch was served (1100), baseline EE and substrate oxidation were measured by means of an open-circuit, ventilated-hood system with subjects lying supine for at least 30 min. At 1200, the subjects received a lunch of 1 of 2 meals. The increase in postlunch EE tended to be greater after the HP lunch (0.85 ± 0.32 kJ/min) than after the AP lunch (0.73 ± 0.22 kJ/min) (P = 0.07). Total postlunch EE above baseline EE over a period of 210 min tended to be greater after the HP lunch (177.60 ± 67.30 kJ) than after the AP lunch (153.50 ± 46.40 kJ) (P = 0.07).

Blood sampling. One hour before the lunch was served (1100), a polytetrafluoroethylene catheter was placed in the antecubital vein for blood sampling. During each test day, we took 1 blood sample just before the lunch (at 0 min) and 4 blood samples after lunch (at 45, 60, 120, and 180 min) for measurement of plasma ghrelin, GLP-1, and PYY concentrations. Blood samples were collected in tubes containing EDTA to prevent clotting. Plasma was obtained by centrifugation (4°C, 1000 × g; 10 min) and stored at −80°C until analyzed. We measured plasma concentrations of active ghrelin using radioimmunoassay (EGLP-35K; Linco Research) and analyzed plasma active GLP-1 samples by enzyme-linked immunoradiometric assay (EGLP-35K; Linco Research). PYY was measured with a specific and sensitive radioimmunoassay, which measures both the full length (PYY1–36) and the fragment (PYY3–36) (Linco Research).

Appetite profile. We measured appetite profile using anchored 100-mm visual analogue scales (VAS). During each test day, questionnaires were completed at several time points before and after the lunch. The questions were, “How hungry are you?” and “How satiated are you?” and were anchored by “not at all” and “very.”

Body composition. Body composition was measured using the deuterium dilution technique. ²H₂O dilution was used to measure total body water (TBW). Deuterium was measured in the urine samples with an isotope ratio mass spectrometer (VG-Isoga Aquas Sira; VG Isogas). We obtained TBW by dividing the measured deuterium dilution space by 1.04. Fat-free mass was calculated by dividing TBW by the hydration factor 0.73. Fat mass was determined as body weight – fat-free mass (30–32).

Statistical analysis. Data are presented as means ± SD unless otherwise indicated. We used repeated measures ANOVA to compare the HP and AP data. Factorial ANOVA was used to analyze possible differences between gender and BMI groups. Post hoc comparisons were made with the Fisher’s protected least significant difference test. Linear regression analysis was performed to determine the relations between selected variables and Pearson correlations are reported. All statistical tests were performed using Statview SE Graphics software (version 4.5; Abacus Concepts).

Results

EE and substrate oxidation. Baseline EE did not differ before the HP (4.80 ± 0.71 kJ/min) and AP (4.79 ± 0.81 kJ/min) lunches. The increase in postlunch EE tended to be greater after the HP lunch (0.85 ± 0.32 kJ/min) than after the AP lunch (0.73 ± 0.22 kJ/min) (P = 0.07). Total postlunch EE above baseline EE over a period of 210 min tended to be greater after the HP lunch (177.60 ± 67.30 kJ) than after the AP lunch (153.50 ± 46.40 kJ) (P = 0.07). Total postlunch EE above baseline EE > 210 min as percentage of the energy content of the lunch tended to be

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Subject characteristics1</th>
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<tbody>
<tr>
<td>Age, y</td>
<td>31 ± 14</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>72 ± 12</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.73 ± 0.09</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.8 ± 2.8</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>25.6 ± 8.8</td>
</tr>
<tr>
<td>Dietary restrained²</td>
<td>4.3 ± 2.6</td>
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1 Values are means ± SD, n = 30.

2 Factor 1 of the Three-Factor Eating Questionnaire was used (26).
greater after the HP lunch (4.93 ± 1.56%) than after the AP lunch (4.32 ± 1.20%) (P = 0.1).

The RQ did not differ between the HP (0.84 ± 0.04) and the AP (0.86 ± 0.04) treatments and the nonprotein RQ also did not differ between the HP (0.85 ± 0.05) and the AP (0.87 ± 0.05) treatments. Substrate oxidation did not differ between the HP condition (protein oxidation: 17.4 ± 5.9 g; carbohydrate oxidation: 34.2 ± 11.5 g; fat oxidation: 15.8 ± 6.9 g) and the AP condition (protein oxidation: 16.9 ± 6.2 g; carbohydrate oxidation: 37.3 ± 12.4 g; fat oxidation: 13.2 ± 6.4 g).

Plasma hormones. The plasma hormone concentrations did not differ between the 2 treatments at baseline before the lunches were consumed. Changes in hormone concentrations in response to the lunches are expressed as the change from baseline. Plasma GLP-1 responses were lower 15 min after the HP lunch than after the AP lunch (P < 0.01; Fig. 1A). Plasma active ghrelin responses to the HP and AP lunches did not differ at any of the time points measured (Fig. 1B). The plasma active ghrelin response 15 min after the AP lunch differed between men [−35.4 ± 36.8 pg/mL (−10.6 ± 11.1 pmol/L)] and women [6.9 ± 55.5 pg/mL (2.1 ± 16.7 pmol/L); P < 0.05]. Plasma PYY responses to the HP and AP lunches did not differ at any of the time points measured (Fig. 1C). The plasma PYY response 15 min after the HP lunch differed between subjects with a BMI > 25 kg/m² [−7.9 ± 16.5 pg/mL (−1.8 ± 3.8 pmol/L)] and subjects with a BMI < 25 kg/m² [12.8 ± 22.8 pg/mL (3.0 ± 5.3 pmol/L); P < 0.05].

Appetite profile. Immediately before and after lunch, satiety VAS scores did not differ when subjects consumed the HP and AP lunches, but they were higher 30 and 120 min after the HP lunch than after the AP lunch. The area under the curve of the VAS score for satiety was higher after the HP lunch (263 ± 61 mm/h) than after the AP lunch (236 ± 76 mm/h) (P < 0.02; Fig. 2). Satiety VAS scores were not correlated with plasma GLP-1, ghrelin, and PYY concentrations at any time point or when expressed as the area under the curve.

Discussion

A single HP meal in the postprandial phase increased feelings of satiety and tended to increase DIT. The observed effects on satiety did not occur simultaneously with effects on plasma ghrelin, GLP-1, and PYY concentrations. A single HP lunch, therefore, does not seem to exert its acute effect on satiety through concentrations of these hormones.

Most studies that observed effects on satiety used a higher percentage of energy from protein, e.g. 43–68%, in the HP meal compared with our study (2,9–11,33–36). Our study and that of Hill and Blundell (34) showed that when using mixed meals, satiety is already increased at 25% energy from protein compared with satiety at 10% of energy from protein. These findings are very relevant to the human diet, because 25% of energy from protein can easily be achieved using typically consumed foods. Barkeling et al. (36) asked subjects consume 43% of energy from protein using typically consumed foods in a mixed HP meal. This HP meal increased satiety compared with the mixed meal with 10% of energy from protein but also caused taste aversion for HP foods. The development of taste aversion may contribute more to satiation (terminating a meal) rather than to satiety (postponing the next meal). In this study, however, taste aversion cannot play a role, because the sausages used to manipulate the protein content of the treatment meals were exactly the same in taste, texture, and appearance. Here, the short-term effect of the

![FIGURE 1](image1.png) Changes in plasma GLP-1 (A), active ghrelin (B), and PYY (C) concentrations in subjects after they consumed the HP and AP lunches. Values are means ± SEM, n = 30. *Different between HP and AP at that time, P < 0.05.

![FIGURE 2](image2.png) Satiety ratings measured with the use of an anchored 100-mm VAS of subjects after they consumed the HP and AP lunches. Values are means ± SEM, n = 30. *Different between HP and AP at that time, P < 0.05.
single HP meal (after 30 min) on satiety may reflect satiation rather than satiety, because the subjects were aware that it was single fixed meal they had to finish and after which they would not receive any other foods. Strong feelings of satiety over a short period after ingestion of a meal may be interpreted as the wish to terminate the eating episode rather than the wish to postpone the next eating episode.

EE after the HP lunch tended to be higher than after the AP lunch. The effect of protein on 24-h thermogenesis has frequently been observed by others (2,4–7,37). The increased thermogenesis over 24 h is thought to be 1 of the mechanisms that increases feelings of satiety after a HP meal. The relationship between DIT and satiety, however, reflects a condition of a HP diet that the subjects experience (6) or appears after consuming extremely high contents of protein in the meal (2). For instance, Crovetti et al. (2) observed a correlation between DIT and fullness ratings over 7 h (HP meal contained 68% energy from protein). Lejeune et al. (6) did observe a relationship between DIT and satiety with 30% energy from protein in a respiration chamber study lasting 36 h. In these studies, DIT was measured over a longer period compared with our study, which may have contributed to a stronger relationship between appetite ratings and DIT as well.

Plasma ghrelin, GLP-1, and PYY did not differ in our study. In a recent study, Lejeune et al. (6) observed higher plasma GLP-1 responses following a HP dinner than after a AP dinner. The plasma GLP-1 response after the HP lunch in that study was, however, not different from the plasma GLP-1 response after the AP lunch. The exchange of percentage of energy from carbohydrate for protein in both the Lejeune study (6) and our study complicates the interpretation of the plasma GLP-1 responses. A larger availability of carbohydrates may have lead to increased contact of carbohydrates with the small intestine, which increases plasma GLP-1 responses to mixed meals (38). Although GLP-1 is often considered to be a satiety hormone, in our study, plasma GLP-1 responses were not correlated to satiety. This lack of relationship between the increased plasma concentrations of GLP-1 and satiety has been observed in several other studies, which suggests that the effect of peripheral GLP-1 on satiety may be influenced by the central sensitivity for GLP-1 or interactions with other hormones (19,38–40). In previous studies, effects of protein on plasma ghrelin responses have been conflicting (6,41–43). The amount of carbohydrate, through glucose and insulin, and the food form, through gastric emptying, may influence plasma ghrelin responses to a meal. In this study, plasma ghrelin concentrations did not contribute to the observed effects of the HP lunch on satiety. This is consistent with the results of Lejeune et al. (6), who observed no differences in plasma ghrelin responses following HP meals throughout the day compared with AP meals.

The HP and AP lunch did not affect plasma PYY responses. Plasma PYY responses are influenced by energy intake and meal composition. In a recent study, Battenham et al. (44) observed significantly higher plasma PYY responses to a HP meal in both lean and obese subjects. In that study, the size of the meals and the amount of protein were much larger than in our study. The size of the meals used in our study, which ranged from 2.8 to 4.5 MJ (35% of subject specific daily energy needs), may have been too small to evoke an acute response in plasma PYY in the postprandial state. The range of the measured plasma hormone responses was quite large at some time points. This variability in responses was due to, among other things, different responses between men and women (45) and between subjects who were normal weight or overweight according to their BMI (46).

Studying a heterogeneous group of subjects, however, makes the outcomes more applicable to the general population.

Apart from the hormones measured in this study, other hormones, which have been shown to be induced by protein ingestion, such as cholecystokinin, insulin, and gastric inhibitory polypeptide, may have contributed to the satiating effect of the HP meal (47–49).

Our results should be interpreted with caution, because this study was conducted at lunch in the postprandial state. Most studies of single HP meals have been conducted at breakfast in subjects in the postabsorptive state, which makes them difficult to compare with this study.

We conclude that a single HP meal of 25% of energy from protein rather than 10% of energy from protein, where protein was exchanged with carbohydrates and contained the same foods, has a greater effect on satiety. The effects of a single HP meal in the postprandial state are not mediated by increased plasma GLP-1 or PYY concentrations and decreased plasma ghrelin concentration. Over the longer term (meals or days), plasma GLP-1, PYY, and ghrelin responses most probably augment and, as a result, may contribute to the increased satiety observed for HP foods and diets. Obviously, there is a marked difference between the satiety effect due to a continuous HP diet and an acute HP lunch. The short-term effect of the single HP meal on satiety in this study may reflect satiation rather than satiety. Other factors, which may explain the HP effect on satiety, may be metabolites or amino acids. In a recent study, Veldhorst et al. (50) showed that the satiating effect of a HP breakfast was positively related to plasma concentrations of specific amino acids up to 4 h. In future studies, protein metabolites, plasma amino acids, and central effects of satiety-related hormones may give more insight into the acute effects of HP meals on satiety.

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Literature Cited