Effect of a high-protein breakfast on the postprandial ghrelin response¹⁻³

Wendy AM Blom, Anne Lluch, Annette Stafleu, Sophie Vinoy, Jens J Holst, Gertjan Schaafsma, and Henk FJ Hendriks

ABSTRACT
Background: The most satiating macronutrient appears to be dietary protein. Few studies have investigated the effects of dietary protein on ghrelin secretion in humans.

Objective: This study was designed to investigate whether a high-protein (HP) breakfast is more satiating than a high-carbohydrate breakfast (HC) through suppression of postprandial ghrelin concentrations or through other physiologic processes.

Design: Fifteen healthy men were studied in a single-blind, crossover design. Blood samples and subjective measures of satiety were assessed frequently for 3 h after the consumption of 2 isocaloric breakfasts that differed in their protein and carbohydrate content (58.1% of energy from protein and 14.1% of energy from carbohydrate compared with 19.3% of energy from protein and 47.3% of energy from carbohydrate). The gastric emptying rate was indirectly assessed with the acetaminophen absorption test.

Results: The HP breakfast decreased postprandial ghrelin secretion more than did the HC breakfast (P < 0.01). Ghrelin concentrations were correlated with glucose-dependent insulinoctropin polypeptide (r = −0.65; 95% CI: −0.85, −0.29) and glucagon concentrations (r = −0.47; 95% CI: −0.75, −0.03). Compared with the HC breakfast, the HP breakfast increased glucagon (P < 0.0001) and cholecystokinin (P < 0.01), tended to increase glucose-dependent insulinoctropin polypeptide (P = 0.07) and glucagon-like peptide 1 (P = 0.10), and decreased the gastric emptying rate (P < 0.0001). Appetite ratings were not significantly different between the 2 treatments, and the HP breakfast did not significantly affect ad libitum energy intake.

Conclusions: The HP breakfast decreased postprandial ghrelin concentrations more strongly over time than did the HC breakfast. High associations between ghrelin and glucose-dependent insulinoctropin polypeptide and glucagon suggest that stimulation of these peptides may mediate the postprandial ghrelin response. The HP breakfast also reduced gastric emptying, probably through increased secretion of cholecystokinin and glucagon-like peptide 1. Am J Clin Nutr 2006;83:211–20.

KEY WORDS Dietary protein, gut hormones, gastric emptying, satiety

INTRODUCTION
The most satiating macronutrient appears to be dietary protein. In most cases, high-protein meals increase feelings of satiety and decrease subsequent energy intake compared with high-carbohydrate or high-fat meals (1, 2). A few possible mechanisms exist by which protein induces satiety; these include thermic effects and physiologic processes related to metabolic factors, gut hormones, and gastrointestinal function. Proteins have a greater thermic effect than do carbohydrates and fats (2–5). This effect may be larger because proteins, which cannot be stored in the body, need to be metabolized immediately. Increased amino acid concentrations may also contribute to satiety through the stimulation of gluconeogenesis, thereby preventing a decrease in glycemia (6). Another physiologic process through which proteins appear to induce satiety is the stimulation of secretion of the gut peptides cholecystokinin and glucagon-like peptide 1 (GLP-1) (7–9). Cholecystokinin and GLP-1 are known to enhance satiety and to decrease gastric emptying (10–15). In the present study, we tested whether dietary protein affects satiety through other physiologic effects and specifically through postprandial ghrelin secretion. Ghrelin is a peptide secreted from the stomach that exists in two major molecular forms: acylated ghrelin, which has an n-octanoylation at serine 3, and unacylated ghrelin (16). Until recently, only the acylated form of ghrelin was thought to be biologically active. The current perspective is that unacylated ghrelin also exerts some biological activities (17–20). Ghrelin appears to be a hunger signal (21, 22); intravenous infusion of ghrelin increases food intake and enhances appetite (21, 23), which suggests a role of ghrelin in meal initiation. In addition, plasma ghrelin concentrations rise gradually before a meal and decrease immediately after eating (24–27). This postprandial decrease in ghrelin secretion is independent of the volume of the meal, because intake of water does not decrease ghrelin concentrations (26, 28). The relation between carbohydrate intake and ghrelin concentrations has been investigated extensively. Both oral and intravenous administration of glucose strongly and dose-dependently decrease ghrelin concentrations.

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SUBJECTS AND METHODS

Subjects

The study was conducted at TNO Quality of Life, Zeist, Netherlands, where subjects were recruited from a pool of volunteers. All subjects gave their informed consent, both verbally and in writing, after being informed about the study. All subjects filled out a questionnaire on lifestyle, medical history, and dietary habits. The medical investigator physically examined each of the subjects. Blood and urine were collected from the subjects after an overnight fast for routine analysis. Each subject reported a weight for height for age and sex consistent with normal Dutch dietary habits, and a stable body weight for age and sex consistent with normal Dutch dietary habits.

The objective of the present study was to investigate whether a high-protein (HP) breakfast is more satiating than is a high-carbohydrate (HC) breakfast through suppression of postprandial ghrelin concentrations or through other physiologic processes [e.g., GLP-1, cholecystokinin, and glucose-dependent insulinotropic polypeptide (GIP)].

Dairy breakfasts

The subjects received 2 isocaloric dairy breakfasts that differed in the protein and carbohydrate contents. These 2 breakfasts (weight: 400 g) consisted of the following: 1) plain yogurt, in which 20 g saccharose and 1.5 g acetaminophen were mixed, and the final product had a HC content (47.3% of energy) and a moderate protein content (19.3% of energy); and 2) a dairy product enriched with a whey protein isolate, in which 1.5 g acetaminophen was thoroughly mixed and in which sweeteners [aspartame and Acesulfame K (Ajinomoto Switzerland AG, Zug, Switzerland)] were added to obtain a sweetness comparable with the other breakfast, and the final product had HP content (58.1% of energy) and a low carbohydrate content (14.1% of energy). The subjects were blinded for treatment order, because breakfasts were kept constant in weight, volume, fat and energy content, viscosity, and taste. The energy and macronutrient contents of the breakfasts are presented in Table 2.

The 2 treatments described in the present study consisted of either the HC or the HP breakfast in combination with a 3-h intravenous infusion of saline. The third treatment, which will not be mentioned again, consisted of the HC breakfast in combination with a 3-h intravenous infusion of GLP-1.

Study protocol

The subjects were instructed to eat and drink the same foods the evening before a test day and to record this in a diary. After an overnight fast (nothing to eat or drink except for water after...
Blood samples

Blood was collected as previously described (28). Plasma acetaminophen was analyzed with the use of a commercially available enzyme-linked immunosorbent assay kit (Immunalysis Corporation, Pomona, CA) with intraassay CVs of 3.7% at a concentration of 5 μg/mL and 0.9% at a concentration of 25 μg/mL. Total plasma GLP-1 concentrations were measured by radioimmunoassay after extraction of plasma with 70% ethanol (by vol, final concentration). The carboxyl-terminal GLP-1 immunoreactivity was measured with the use of antisemur 89390, which has an absolute requirement for the intact amidated carboxyl terminus of GLP-1 7–36 amide and cross reacts <0.01% with the carboxyl-terminally truncated fragments and 89% with the GLP-1 9–36 amide (36). The sensitivity was <5 pmol/L and the intraassay CV was <10%. Serum glucose was measured with a commercially available test kit (Roche Diagnostics GmbH, Mannheim, Germany) on a Hitachi 911 automatic analyser (Hitachi Instrument Division, Ibaraki-ken, Japan), with intraassay CVs ranging from 0.7% to 0.9%, depending on the concentration. Serum insulin was measured as previously described (28). Total and active plasma ghrelin concentrations were measured with commercially available human radioimmunoassay (RIA) kits (Linco Research Inc, St Charles, MO). The intraassay CV of the total ghrelin RIA kit was 10% at a concentration of 1000 ng/L and 3.3% at a concentration of 1500 ng/L. The intraassay CV of the active ghrelin RIA kit was 6.7% at a concentration of 139 ng/L and 9.5% at a concentration of 237 ng/L. Plasma glucagon concentrations were measured with a commercially available human RIA kit (Linco Research Inc) with an intraassay CV of 6.8% at a concentration of 60 ng/L and 4.0% at a concentration of 220 ng/L. Plasma GIP concentrations were measured with a commercial human RIA kit (Phoenix Peptide, Belmont, CA) with an intraassay CV of 3.3% at a concentration of GIP of 0.40 μg/L and 2.5% at a concentration of 0.80 μg/L. Plasma cholecystokinin-8 (cholecystokinin 26–33) concentrations were measured with an optimized and validated commercial human RIA kit (Euro-Diagnostica, Malmö, Sweden). This improved assay system has been optimized to reach a high sensitivity of 0.05 pmol/L and to have no cross-reactivity to gastrin-17 or sulfated gastrin. The intraassay CV was 8.9% at a concentration of 0.84 pmol/L and 4.9% at a concentration of 1.98 pmol/L.

Subjective satiety

Subjective satiety was evaluated with the use of VAS for hunger, fullness, desire to eat, and prospective food consumption (37). In addition, the subjects also filled out VASs 30 min after breakfast to evaluate the taste, texture, and enjoyment of the meals. VASs consisted of 150 mm horizontal lines with Dutch wordings anchored at each end that expressed the most positive or negative sensation (ie, I have never been more hungry or I am not hungry at all). The subjects drew a vertical line on the horizontal line corresponding to their hunger sensation. VASs were automatically processed with TELEFORM Elite software (version 6.1; Cardiff Software Inc, Sunnyvale, CA). Distances on the VAS were converted into scores between 0 and 100.

Statistical analyses

An analysis of variance (ANOVA) for repeated measures was used to compare the response curves of ghrelin, GLP-1, cholecystokinin, GIP, glucose, insulin, glucagon, and the VAS scores after the 3 treatments by testing for time × treatment interactions. When there was a significant overall time × treatment effect, partial tests were performed to compare the HP and HC breakfasts. Incremental areas under or over the baseline were calculated; we used the term area under the curve (AUC) to refer to both values, which were delineated as negative AUC and positive AUC, respectively. Evaluation of the residual plots showed that the negative AUC of total ghrelin, active ghrelin, and desire to eat could not be used for the analysis; we therefore used the total AUC, which we defined as the sum of the areas under and over the baseline. With the use of a mixed-model ANOVA, the AUCs of the different variables were tested for an overall treatment effect. Partial tests were performed to compare the HC and HP breakfasts when there was a treatment effect. A mixed-model ANOVA was also used to test whether the taste, texture, and enjoyment of the breakfasts differed. Correlation coefficients were calculated to evaluate the relation between subjective measures of satiety and blood variables. The Pearson correlation coefficient was calculated for each subject, with basis on 16 pairs of data (8 time points, 2 treatments). A Fisher’s z transformation was applied on these individual correlations to correct for deviations from the normal distribution. The mean of these 15 coefficients was calculated, the inverse of the Fisher transformation was performed, and the 95% CIs for each correlation coefficient were calculated. In addition, correlation coefficients were calculated to evaluate the relation between energy and macronutrient intake during lunch and the AUC of the different blood variables. The correlation coefficient was calculated by treatment, with basis on 15 observations (15 subjects). To test whether the correlation coefficients were significantly different between treatments, a Bonferroni corrected paired t test of the z scores was performed. Also, the proportional change from mean baseline concentration to the highest (glucose, insulin, glucagon, GIP, cholecystokinin, GLP-1 and fullness) or lowest (ghrelin,
hunger, desire to eat and prospective food consumption) value was calculated.

Statistical analysis of the data were carried out with the SAS statistical software package (SAS-STAT version 8.2; SAS Institute, Cary, NC). A P value < 0.05 (2-sided) was considered statistically significant in all analyses. Results are given as means ± SDs unless stated otherwise.

RESULTS

Gastric emptying

Gastric emptying was indirectly estimated by acetaminophen absorption. The postprandial acetaminophen concentrations and the AUCs are shown in Figure 1. After the HC treatment, acetaminophen concentrations in plasma increased rapidly, reaching a maximum mean (±SD) value of 16.2 ± 4.0 µg/mL at 120 min. Acetaminophen concentrations after the HP treatment rose more slowly, reaching a maximum mean (±SD) concentration of 13.0 ± 2.7 µg/mL at 120 min. The acetaminophen responses showed a time × treatment interaction (P < 0.0001). Compared with the HC breakfast, the AUC of the acetaminophen response was smaller (∼18%) after the HP breakfast (P < 0.0001), which suggests that the HP breakfast reduced the rate of gastric emptying.

Blood variables

The 3-h postprandial responses and the AUCs of the different blood variables are presented in Figure 2.

Ghrelin

Total ghrelin. Ghrelin concentrations decreased after both the HC treatment (−18%) and the HP treatment (−25%) and reached the lowest values at 60 and 120 min, respectively. The ghrelin responses showed an overall time × treatment interaction (P < 0.0001). Partial tests showed that the ghrelin responses after the HP and HC breakfasts were different (P < 0.0001). The total AUC of the ghrelin response was larger (∼45%) after the HP breakfast than after the HC breakfast (P < 0.01).

Active ghrelin. Active ghrelin concentrations decreased after both the HC and the HP treatments and reached the lowest values at 45 and 120 min, respectively. ANOVA for repeated measures showed no significant overall time × treatment interaction. The total AUC of the active ghrelin response also did not differ significantly between the 2 breakfasts.

Glucose

Serum glucose concentrations increased about 24% after the HC treatment, reaching peak values at 30 min. In contrast, glucose concentrations did not increase after the HP treatment, but decreased ∼10%, reaching the lowest values at 60 min. The glucose responses showed an overall time × treatment interaction (P < 0.0001), and partial tests showed that the glucose responses after the 2 breakfasts differed from each other (P < 0.0001). In addition, the AUC of glucose was smaller (∼76%) after the HP breakfast than after the HC breakfast (P = 0.0001).

Insulin

Serum insulin concentrations increased ∼8-fold after the HC treatment and ∼5.5-fold after the HP treatment, reaching peak values at 30 min for both. The insulin responses showed an overall time × treatment interaction (P < 0.0001). Partial tests showed that insulin responses differed after the HC and HP breakfasts (P < 0.0001). Insulin concentrations were lower after the HP breakfast than after the HC breakfast at 30 and 45 min. However, the AUCs were not significantly different.

Glucagon

Glucagon concentrations increased ∼31% after the HC treatment and reached peak values at 30 min. Glucagon concentrations increased ∼130% after the HP treatment and reached peak values at 60 min. The glucagon responses showed a significant time × treatment effect (P < 0.0001), and partial tests showed that the glucagon responses after the 2 breakfasts differed from each other (P < 0.0001). The AUC of the glucagon response was larger (∼380%) after the HP breakfast than after the HC breakfast (P < 0.0001).

Glucose-dependent insulinotropic polypeptide

Plasma GIP concentrations increased ∼150% after both the HC treatment and the HP treatment, reaching peak values at 30

FIGURE 1. Mean (±SEM) responses of acetaminophen (n = 15) during 3 h after the intake of the 2 breakfasts: ◆, high-carbohydrate (HC) breakfast; ◊, high-protein (HP) breakfast. There was a significant time × treatment interaction for acetaminophen (ANOVA), P < 0.0001. Inset: mean (±SEM) positive area under the curve (AUC) of acetaminophen (n = 15). There was a significant treatment effect of the AUC of the acetaminophen response (ANOVA), P < 0.0001. a,b,cSignificantly different from HP: a P < 0.05, b P < 0.001, c P < 0.0001.
FIGURE 2. Mean (±SEM) responses of total ghrelin, active ghrelin, glucose, insulin, glucagon, glucose-dependent insulinotropic polypeptide (GIP), cholecystokinin (CCK), and glucagon-like peptide 1 (GLP-1) during the 3 h after the intake of the 2 breakfasts; *n* = 15. AUC, area under the curve; AUCt, total area under and over the baseline curve; AUC+, positive AUC (ie, area over the baseline curve); □, high-carbohydrate (HC) breakfast; ○, high-protein (HP) breakfast. There was a significant time × treatment interaction (ANOVA) for total ghrelin, insulin, glucose, glucagon, and GIP (*P* < 0.0001 for all); for CCK (*P* < 0.01); and for GLP-1 (*P* < 0.001). Inset: mean (±SEM) AUC of the different responses (*n* = 15). There was a borderline significant treatment effect (ANOVA) of the AUCs of the GIP and GLP-1 responses (*P* = 0.10) and a significant treatment effect of the AUCs of the total ghrelin and CCK responses (*P* < 0.01 for both) and of the glucose and glucagon responses (*P* ≤ 0.0001 for both). a,b,c,dSignificantly different from HP: a *P* < 0.05, b *P* < 0.01, c *P* < 0.001, d *P* < 0.0001.
and 45 min, respectively. The GIP responses showed a significant time × treatment interaction (P < 0.0001). The partial test showed that the GIP responses after the HC and HP breakfast were different (P < 0.0001). The AUC of the GIP response tended to be larger (≈21%) after the HP breakfast than after the HC breakfast (P = 0.07) (Figure 2). GIP concentrations at 120 and 180 min were higher after the HP breakfast than after the HC breakfast.

**Cholecystokinin**

Plasma cholecystokinin concentrations increased ≈3-fold after the HC treatment and reached peak values at 15 min. After the HP breakfast, cholecystokinin concentrations showed a biphasic response. Cholecystokinin concentrations initially increased ≈6.5-fold, dropped ≈40% at 30 min, then steadily increased after 45 min to reach peak values at 60 min (6.5-fold increase compared with baseline values). The cholecystokinin responses showed a significant time × treatment interaction (P < 0.01), and partial testing showed that the cholecystokinin response differed between the 2 treatments (P < 0.05). The AUC of the cholecystokinin response was higher (≈54%) after the HP breakfast than after the HC breakfast (P < 0.01).

**Glucagon-like peptide 1**

GLP-1 concentrations increased ≈50% after the HC breakfast and ≈80% after the HP breakfast, reaching peak values at 90 and 120 min, respectively. The GLP-1 responses showed a significant overall time × treatment interaction (P < 0.0005). Partial tests showed that GLP-1 responses after the HP breakfast were not significantly different from the responses after the HC breakfast. In contrast, the AUC of GLP-1 tended to be higher (≈66%) after the HP breakfast than after the HC breakfast (P = 0.10).

**Questionnaires**

Subjective measures of satiety and the AUCs are presented in **Figure 3**. Fasting scores of the 4 satiety scales did not significantly differ between treatments. Subjective measures of hunger, desire to eat, and prospective food consumption decreased after both treatments, reaching the lowest values at 15 min. Subjective measures of fullness increased after both breakfasts, reaching peak values at 15 min. An analysis of the total AUC showed no significant treatment effect on hunger, fullness, desire to eat, or prospective food consumption. An overall time × treatment interaction for prospective food consumption tended toward significance (P = 0.08), but the responses of the 2 treatments did not differ significantly. No significant overall time × treatment interaction was found for hunger, fullness, and the desire to eat.

**Palatability of the test meals**

The subjects rated the palatability of the 2 test meals 30 min after the start of consumption on VASs (**Figure 4**). The ANOVA
showed no significant treatment effects on taste, texture, or enjoyment of the 2 meals.

**Energy and macronutrient intake**

Energy and macronutrient intake during the ad libitum lunch are presented in Table 3. Compared with the HC treatment, the HP treatment reduced fat intake \( (P = 0.05) \) during the subsequent ad libitum lunch. No significant differences in energy, carbohydrate, or protein intake were observed during lunch.

**Correlations**

**Ghrelin and other blood variables**

Total ghrelin concentrations were correlated with concentrations of GIP \( (r = -0.65; 95\% \text{ CI}: -0.85, -0.29) \), glucagon \( (r = -0.47; 95\% \text{ CI}: -0.75, -0.03) \) and acetaminophen \( (r = -0.73; 95\% \text{ CI}: -0.89, -0.42) \). No additional significant associations between total ghrelin concentrations and other physiologic variables were observed (Table 4).

**Subjective satiety and blood variables**

Correlations between blood variables and measures of satiety are presented in Table 5. Neither total nor active ghrelin concentrations were correlated with measures of satiety. Insulin concentrations were correlated with measures of fullness \( (r = 0.45; 95\% \text{ CI}: 0.00, 0.74) \).

**TABLE 3**

<table>
<thead>
<tr>
<th></th>
<th>HC</th>
<th>HP</th>
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<tbody>
<tr>
<td>Energy (kJ)</td>
<td>5136 ± 1205</td>
<td>4697 ± 1784</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>41 ± 12</td>
<td>33 ± 15</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>48 ± 14</td>
<td>43 ± 20</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>166 ± 58</td>
<td>161 ± 61</td>
</tr>
</tbody>
</table>

\( ^1 \) All values are \( \bar{x} \pm SD \).

\( ^2 \) Significantly different from HC, \( P = 0.05 \) (mixed-model ANOVA).

**Food intake and blood variables**

No significant associations were observed between the AUC of the ghrelin response and subsequent energy intake during the ad libitum lunch (HC: \( r = -0.15, P = 0.59 \); HP: \( r = 0.03, P = 0.93 \)). The AUC of the ghrelin response was also not significantly associated with fat intake during lunch after the HC breakfast \( (r = 0.41, P = 0.13) \) or the HP breakfast \( (r = 0.30, P = 0.28) \). During lunch, the AUC of the glucagon response was inversely associated with energy intake \( (r = -0.74, P < 0.01) \), protein intake \( (r = -0.82, P < 0.001) \), carbohydrate intake \( (r = -0.59, P < 0.05) \), and fat intake \( (r = -0.75, P < 0.01) \) after the HP breakfast. No significant association was observed between the AUC of the glucagon response and energy \( (r = -0.24, P = 0.39) \) or macronutrient intake after the HC breakfast. The AUC of the insulin response tended to be positively correlated with protein intake during lunch after the HC breakfast \( (r = 0.47, P = 0.08) \) but not after the HP breakfast \( (r = 0.10, P = 0.72) \). A correlation between the positive AUC of the cholecystokinin response and fat intake during lunch tended toward significance after the HC breakfast \( (r = 0.50, P = 0.06) \).

**DISCUSSION**

In the present study, we investigated whether an HP meal is more satiating than an HC meal through suppression of the hunger peptide ghrelin or through other mechanisms. However, both subjective sensations of appetite and ad libitum energy intake during lunch did not differ significantly between the HP and HC breakfasts. The HP breakfast did suppress total ghrelin concentrations more than did the HC breakfast. The high association between total ghrelin concentrations and GIP and glucagon concentrations suggests that the postprandial decrease in ghrelin may be mediated through stimulation of GIP and glucagon secretion. The HP breakfast also reduced the rate of gastric emptying and stimulated cholecystokinin and GLP-1 secretion.

For practical reasons, the design of the study was not randomized for treatment order. Consequently, the period is entangled with the treatment, and period effects can therefore not be eliminated. However, we believe that the lack of randomization did not influence the results, because a washout period of 1 wk was sufficient to prevent any carry-over effects of the treatments and stress hormone concentrations were not significantly different between the treatments (data not shown). In addition, the baseline values of all variables did not differ significantly between the periods.

The present study was designed to compare the effects of meals that differed in the amount of protein and carbohydrate on subjective and physiologic measures of appetite. Therefore, other factors that could affect appetite were kept constant. A small difference in fat content of the meal was observed (a difference of 2.2 g/400 g portion which corresponds to a difference of 5.5% of energy from fat). We cannot exclude the possibility that this small difference in fat content may have affected the study outcomes to some extent. The subjects were blinded for the treatment order, and the hedonic aspects of the 2 breakfasts were similar based on the subjects’ ratings of the 2 breakfasts. Acetaminophen was added to the breakfast because its absorption is an indirect measure of the gastric emptying rate (38, 39); however, its bitter taste may explain the rather low taste scores for the breakfasts.
We expected that the HP breakfast would increase subjective satiety and possibly decrease energy intake compared with the HC breakfast. However, none of the appetite ratings were statistically different between the 2 treatments, and the HP breakfast did not affect ad libitum energy intake. We probably did not have sufficient statistical power to detect the small differences in appetite and energy intake between the 2 breakfasts (37). It is also possible that assessing subjective satiety during infusions and blood samplings may have decreased the amplitude of the results. Although not significant, we found that the HP breakfast reduced appetite and energy intake during the next meal by ≈439 kJ. Longer experiments would be useful to test whether these beneficial effects on the regulation of appetite can be maintained and have a clinical relevance.

The present study was also designed to investigate the effects of a HP meal on physiologic variables involved in the regulation of hunger and satiety, with special focus on postprandial ghrelin secretion. We observed a larger decrease in postprandial total ghrelin concentrations after the HP breakfast than after the HC breakfast. Also, active ghrelin concentrations decreased in the postprandial period, but these concentrations were not significantly different between the 2 treatments. The postprandial decrease in total ghrelin concentrations after protein intake was not apparent in studies performed by either Erdmann et al or by Greenman et al (32–34). This discrepancy may be explained by the type of protein used. Our HP meal consisted of a dairy product that was enriched with whey protein, whereas the HP meals in the other 3 studies consisted of meat (32–34).

Few studies have compared the effects of the type of dietary protein on satiety and subsequent food intake (7, 40–43). Among articles that showed differences between proteins, whey, for example, was found to be more satiating compared with casein (7) and had larger effects on food intake suppression than did egg and soy protein (40). The type of protein is reflected in the amino acid composition and differentially affects insulin, GIP, and glucagon secretion (9, 44, 45). In fact, plasma amino acid concentrations after the intake of a HP meal may almost completely account for the postprandial increase in insulin concentrations (46). Specifically, branched-chain amino acids, such as leucine, valine, and isoleucine, are insulinotropic (9, 44). Whey and casein both contain high concentrations of these amino acids, but intake of whey protein induces the largest insulin response (9). This may also indicate that the insulinotropic effect of amino acids is dependent on the bioavailability of amino acids, because in contrast to casein, which coagulates in the stomach, whey is a soluble milk protein (9, 44).

### TABLE 4
Pearson’s correlation coefficients of the relation between different physiologic variables

<table>
<thead>
<tr>
<th></th>
<th>Total ghrelin</th>
<th>Active ghrelin</th>
<th>Glucose-dependent insulinotropic polypeptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ghrelin</td>
<td>-</td>
<td>0.44 (0.01, 0.74)</td>
<td>-0.65 (0.85, -0.29)</td>
</tr>
<tr>
<td>Active ghrelin</td>
<td>0.44 (0.01, 0.74)</td>
<td>-0.28 (0.06, 0.18)</td>
<td>-0.21 (0.59, 0.26)</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>-0.73 (-0.89, -0.42)</td>
<td>-0.20 (-0.59, 0.27)</td>
<td>0.58 (0.19, 0.82)</td>
</tr>
<tr>
<td>Glucagon-like peptide 1</td>
<td>-0.26 (-0.63, 0.21)</td>
<td>-0.14 (-0.55, 0.32)</td>
<td>0.23 (-0.23, 0.61)</td>
</tr>
<tr>
<td>Insulin</td>
<td>-0.27 (-0.64, 0.19)</td>
<td>-0.12 (-0.54, 0.34)</td>
<td>0.74 (0.44, 0.89)</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.15 (-0.31, 0.55)</td>
<td>0.12 (-0.34, 0.54)</td>
<td>0.15 (-0.31, 0.56)</td>
</tr>
<tr>
<td>Glucagon</td>
<td>-0.47 (-0.75, -0.03)</td>
<td>-0.18 (-0.58, 0.28)</td>
<td>0.51 (0.09, 0.97)</td>
</tr>
<tr>
<td>Cholecystokinin</td>
<td>-0.34 (-0.68, 0.12)</td>
<td>0.20 (-0.59, 0.27)</td>
<td>0.52 (0.10, 0.78)</td>
</tr>
</tbody>
</table>

1 All values are mean correlation coefficients (r); 95% CIs in parentheses. Coefficients were calculated per subject after Fisher z transformation. Correlation coefficients were also calculated by treatment and compared with a paired t test of the z scores (Bonferroni corrected). None of the correlation coefficients were significantly different between the 2 treatments. n = 15. GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1.

### TABLE 5
Pearson’s correlation coefficients of the relation between different physiologic variables and measures of appetite

<table>
<thead>
<tr>
<th></th>
<th>Hunger</th>
<th>Fullness</th>
<th>Desire to eat</th>
<th>Prospective food consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ghrelin</td>
<td>0.04 (-0.41, 0.48)</td>
<td>-0.09 (-0.51, 0.37)</td>
<td>0.08 (-0.37, 0.51)</td>
<td>0.12 (-0.34, 0.53)</td>
</tr>
<tr>
<td>Active ghrelin</td>
<td>0.00 (-0.44, 0.44)</td>
<td>-0.12 (-0.53, 0.34)</td>
<td>0.08 (-0.38, 0.50)</td>
<td>0.11 (-0.35, 0.53)</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>0.02 (-0.42, 0.46)</td>
<td>-0.01 (-0.45, 0.43)</td>
<td>0.01 (-0.43, 0.45)</td>
<td>-0.02 (-0.46, 0.43)</td>
</tr>
<tr>
<td>GIP</td>
<td>-0.34 (-0.68, 0.12)</td>
<td>-0.40 (-0.05, 0.72)</td>
<td>-0.29 (-0.65, 0.17)</td>
<td>-0.30 (-0.66, 0.16)</td>
</tr>
<tr>
<td>GLP-1</td>
<td>-0.01 (-0.45, 0.43)</td>
<td>-0.03 (-0.47, 0.42)</td>
<td>-0.02 (-0.46, 0.43)</td>
<td>-0.02 (-0.46, 0.43)</td>
</tr>
<tr>
<td>Insulin</td>
<td>-0.40 (-0.71, 0.06)</td>
<td>0.45 (0.00, 0.74)</td>
<td>-0.33 (-0.67, 0.13)</td>
<td>-0.33 (-0.67, 0.13)</td>
</tr>
<tr>
<td>Glucose</td>
<td>-0.15 (-0.56, 0.31)</td>
<td>0.14 (-0.32, 0.55)</td>
<td>-0.07 (-0.49, 0.39)</td>
<td>-0.10 (-0.52, 0.36)</td>
</tr>
<tr>
<td>Glucagon</td>
<td>-0.16 (-0.57, 0.30)</td>
<td>0.27 (-0.20, 0.64)</td>
<td>-0.21 (-0.59, 0.26)</td>
<td>-0.20 (-0.59, 0.26)</td>
</tr>
<tr>
<td>Cholecystokinin</td>
<td>-0.30 (-0.66, 0.16)</td>
<td>0.42 (-0.03, 0.73)</td>
<td>-0.34 (-0.68, 0.13)</td>
<td>-0.32 (-0.67, 0.14)</td>
</tr>
</tbody>
</table>

1 All values are mean correlation coefficients (r); 95% CIs in parentheses. Coefficients were calculated per subject after Fisher z transformation. Correlation coefficients were also calculated by treatment and compared with a paired t test of the z scores (Bonferroni corrected). None of the correlation coefficients were significantly different between the 2 treatments. n = 15.
Apart from the insulin stimulating effects of amino acids, the type of protein may also affect GIP concentrations (9). GIP is secreted from the gut not only in response to carbohydrate and fat ingestion (47), but also in response to milk protein (7–9). An HP meal consisting of turkey does not stimulate GIP secretion (47), whereas the GIP response is pronounced after intake of whey protein (9). Because GIP is an insulinotropic peptide (48, 49), GIP may mediate the insulinotropic effect of milk proteins.

The postprandial increase in amino acid concentrations is also responsible for the rise in glucagon concentrations after protein intake (44). Whey proteins elicit the largest glucagon response because of the greater availability of amino acids after whey protein consumption (44). In the present study, we observed a strong increase in GIP and glucagon concentrations after the HP breakfast. Both GIP and glucagon concentrations were inversely associated with ghrelin concentrations. Possibly, the HP dairy product used in the present study specifically stimulates both GIP and glucagon, which may provide a strong stimulus to additionally decrease postprandial ghrelin concentrations. The interaction between ghrelin and GIP or glucagon has been investigated in a few studies. Thus far, there is no evidence that GIP suppresses ghrelin concentrations (50–52). In contrast, 2 studies have shown that intramuscular or intravenous glucagon suppresses ghrelin concentrations (53–55). Therefore, increased glucagon concentrations after protein intake may cause an additional decrease in postprandial ghrelin concentrations.

Consumption of the HP meal reduced acetaminophen absorption more than did consumption of the HC breakfast, which suggests that the HP breakfast reduced the gastric emptying rate (38, 39). This effect of protein on gastric emptying has been reported before (7) and may be one of the mechanisms by which protein induces satiety. The HP meal also increased concentrations of the gut peptides cholecystokinin and GLP-1. These peptides both potently reduce appetite and food intake, which is at least partly mediated by their ability to decrease gastric emptying (13–15). This suggests that the effects of protein on the gastric emptying rate are induced by the enhanced secretion of cholecystokinin and GLP-1.

We also hypothesized that protein exerts its satiating effects partly through the suppression of postprandial ghrelin concentrations. Although protein intake did indeed decrease ghrelin concentrations, we did not find an association between ghrelin concentrations and subjective satiety or energy intake. However, intake of a HP (milk) breakfast affected several other physiologic variables involved in the regulation of food intake that were associated with subjective satiety or energy intake. GIP and insulin concentrations rose after intake of the protein meal and were positively associated with satiety. In addition, glucagon concentrations, which were associated with decreased energy and macronutrient intake during the ad libitum lunch, were also increased after the protein meal. Besides these effects, the HP breakfast also increased concentrations of cholecystokinin and GLP-1 and decreased the rate of gastric emptying, but these factors were not associated with subjective satiety or energy intake in the present study.

In the present study, we compared a HP breakfast with a HC breakfast that also contained a moderate amount of protein. The difference in the protein quantity between the 2 breakfasts can also explain the observed effects. Similar to other studies that investigated the effects of protein on satiety, our HP treatment contained a large dose of protein. At the moment, the active dose of protein is still unknown (1, 40). Because several studies have shown that dietary protein can be helpful in weight management (56–58), studies that investigate the long-term effects of different amounts or types of proteins on physiologic variables and body-weight regulation should be initiated.

In conclusion, the HP breakfast decreased postprandial ghrelin concentrations more than did the HC breakfast, despite the lack of effect on satiety. Ghrelin concentrations were strongly associated with GIP and glucagon concentrations, which suggests that the postprandial decrease in ghrelin concentrations after the consumption of the HP breakfast may be mediated through the stimulation of these peptides. The HP breakfast also reduced the gastric emptying rate, probably through increased secretion of cholecystokinin and GLP-1.

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WAMB was involved in the design of the protocol, collection of the data, analysis of the data, and writing of the manuscript. AL, SV, and AS were involved in the design of the protocol and provided significant advice during the writing of the manuscript. JHJ was involved in the GLP-1 analyses and provided significant advice on the GLP-1 infusion. GS provided significant advice during the writing of the manuscript. HEJH was involved in the design of the protocol (Principal Investigator according to Good Clinical Practice guidelines), writing of the manuscript, and provided significant advice during the intervention study and data analysis. AL and SV are employees of Danone Vitapole. None of the other authors had any conflicts of interest.

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