Ghrelin response to carbohydrate-enriched breakfast is related to insulin¹–³

Wendy AM Blom, Annette Stafleu, Cees de Graaf, Frans J Kok, Gertjan Schaafsma, and Henk FJ Hendriks

ABSTRACT

Background: Ghrelin plays an important role in the regulation of food intake. Little is known about how ghrelin concentrations are modified by dietary factors.

Objective: We examined the effects of both amount and type of carbohydrate on ghrelin concentrations and all correlations among the variables ghrelin, glucose, insulin, leptin, and all 4 subjective measures of appetite.

Design: Twenty healthy nonobese men were studied in a double-blind, randomized, crossover design. Subjective measures of appetite and concentrations of ghrelin, glucose, insulin, and leptin were frequently assessed for 4 h after liquid breakfast meals differing in energy content and carbohydrate structure—ie, water, low-calorie (LC) meal, high-calorie simple carbohydrate (HC-SC) meal, and high-calorie complex carbohydrate (HC-CC) meal.

Results: Ghrelin concentrations decreased after the HC-SC breakfast by 41%, after the HC-CC breakfast by 33%, and after the LC breakfast by 24%. No significant differences in ghrelin concentration among the 3 breakfasts were observed until 120 min. Ghrelin concentrations were correlated with subjective measures of hunger (r = 0.51) and fullness (r = −0.44). The percentage decrease in ghrelin between 0 and 30 min was inversely correlated with the percentage increases in insulin (r = −0.76) and glucose (r = −0.79) but not with changes in leptin (r = 0.10). The percentage changes in ghrelin concentrations between 30 and 180 min were correlated with the percentage changes in insulin (r = −0.53) and leptin (r = −0.47) but not with changes in glucose (r = 0.22).

Conclusion: The results support the hypothesis that ghrelin requires postgastric feedback, which may be regulated through insulin.

INTRODUCTION

The gastric peptide ghrelin (1) appears to play a pivotal role in the regulation of food intake. Ghrelin concentrations in plasma rise gradually before a meal and decrease immediately after a meal (2–5). In addition, intravenous infusion of ghrelin increases food intake and enhances appetite (6, 7), and these effects suggest that ghrelin plays a role in feelings of hunger and in meal initiation. Ghrelin is suggested to be involved not only in meal initiation but also in body weight control, because body mass index (BMI; in kg/m²) is negatively correlated with fasting plasma ghrelin concentrations (3, 4, 7–10).

Leptin and insulin are 2 other hormones involved in the regulation of energy balance and food intake (11–17). Ghrelin, leptin, and insulin are secreted in peripheral tissues, and they act on the central nervous system. Ghrelin stimulates the expression of neuropeptideY and agouti-related protein in the hypothalamus, and that expression stimulates food intake (18–21). Leptin and insulin both suppress food intake partly through the suppression of neuropeptideY and agouti-related protein (22, 23) and partly through the activation of the hypothalamic melanocortin system (24).

Although the central mechanisms of action have been and are still being characterized, little is known about the effects of dietary factors (eg, structure and energy content) on plasma ghrelin concentrations, ghrelin’s interactions with leptin and insulin, and the correlation between ghrelin and appetite (ie, feelings of hunger and satiety). Plasma ghrelin concentrations are known to decrease after oral and intravenous administration of glucose (4, 25–27), whereas lipids or high-fat diets suppress the postprandial ghrelin concentrations less effectively (27–29). This suggests that the postprandial ghrelin response may be modulated by glucose and insulin. Carbohydrate structure is one of the important factors determining the glucose and insulin concentrations after carbohydrate consumption. Complex carbohydrates and fibers are known to decrease feelings of hunger and to increase fullness (12, 30, 31). We hypothesized that the amount and type of carbohydrate may influence the ghrelin response. Therefore the relations among ghrelin, glucose, insulin, and leptin concentrations and subjective measures of appetite were studied by analyzing the postprandial responses to water and to 3 liquid breakfasts that differed in the amount and type of carbohydrate.

SUBJECTS AND METHODS

Subjects

The study was conducted at the Netherlands Organization for Applied Scientific Research (TNO) Nutrition and Food Research (TNO) Nutrition and Food Research, Zeist, Netherlands, and the University of Wageningen, Wageningen, Netherlands. 

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(Zeist, Netherlands), where subjects were recruited from a pool of volunteers. All subjects completed a questionnaire on lifestyle, medical history, and dietary habits. The medical investigator physically examined each of the subjects. Blood and urine were collected after an overnight fast for routine analysis. Each subject reported a Western lifestyle, regular Dutch dietary habits, and a stable body weight for ≥1 mo before the study. Smokers, restrained eaters [a score > 2.5 on the Dutch Restrained Eating Questionnaire (32)], and subjects who reported that they were following either a weight-reduction diet or a medically prescribed diet were excluded from participation. Subjects who were taking medication that may have influenced appetite and sensory functioning or who reported metabolic or endocrine disorders, gastrointestinal disorders, or a history of medical or surgical events that may have affected study outcome were also excluded. A total of 20 healthy nonobese men with a mean BMI of 22.6 ± 1.5 (range: 19.9–25.4) and a mean age of 36.1 ± 13.4 y (range: 19–57 y) completed the study (Table 1).

The study was performed according to the ICH Guidelines for Good Clinical Practice (33), and the protocol was approved by the independent Medical Ethics Committee of TNO. Each subject gave written informed consent after being informed about the study, both orally and in writing.

Study design

The experiment had a randomized crossover design. Each subject received 4 treatments on separate days, with a washout period of 1 wk preceding each subsequent treatment. Subjects were randomly assigned to 1 of the 4 treatment orders. With the assignment of treatment order, we ensured that the average body weight and age of the subjects in all 4 groups were more or less the same. Treatment orders were balanced according to a Latin square design. Subjects and personnel were blinded to the treatment order except the water condition. The study had a staggered start: 5 subjects started per day.

Liquid breakfasts

Liquid breakfasts were prepared for each person according to the estimated daily energy requirement (MJ/24 h) of that person, which was estimated by calculating the basal metabolic rate according to Schofield’s equations (34) and multiplying that value by a correction factor for physical activity level. All subjects were considered moderately active, and therefore the same correction factor of 1.79 was applied for all subjects (35).

The 4 liquid breakfasts (volume: 578 ± 5 mL) consisted of 1) noncarbonated mineral water (Spa Reine; Spadel Nederland BV, Maarssen, Netherlands); 2) a low-calorie (LC; 128 kJ/100 mL) yogurt drink flavored with red fruit (eg, strawberry, cherry, raspberry, cherry, and blackberry) and containing intensive (ie, non-energy-containing) sweeteners only (Fristi; Friesland Dairy & Drinks Group, Ede, Netherlands), which is the LC breakfast; 3) an LC yogurt drink with Maltodextrin (Avebe, Veendam, Netherlands), a carbohydrate with a simple structure, which is the high-calorie simple carbohydrate (HC-SC) breakfast; and 4) an LC yogurt drink with both the exopolysaccharide Reuteran (TNQ Nutrition and Food Research, Zeist, Netherlands; 36–38), a carbohydrate with a complex structure, and Maltodextrin, which is the HC complex carbohydrate (HC-CC) breakfast. The ratio of Reuteran to Maltodextrin was fixed at 4:21. Relatively low amounts of Reuteran were added to the HC-CC breakfast to ensure that the viscosities for the 3 yogurt-based treatments (LC, HC-SC, and HC-CC) were similar. Both the HC-SC and HC-CC breakfasts provided a total of 20% of the estimated daily energy requirement. The LC breakfast provided ≈6% of the estimated daily energy requirement.

Reuteran and Maltodextrin were added to 500 mL of the yogurt drink. Adding carbohydrates to beverages (or liquid breakfasts) resulted in a volume increase (71–88 mL, depending on the energy requirement of the subject). We corrected for this volume increase by adjusting the volumes of water and of the LC yogurt drink. The average volume and energy content of the 4 liquid breakfasts are shown in Table 2.

We also made sure that the taste of the 3 yogurt drinks was very similar by choosing a sweet yogurt drink with a distinct, red fruit flavor and by adding 2 carbohydrates that are almost tasteless.

Study protocol

After an overnight fast (nothing to eat or drink except water after 2200), subjects arrived at the research center, filled out a questionnaire on their current general well-being, and were weighed. A cannula was placed in the antecubital vein, and a blood sample was taken. After ≈30 min, subjects drank one of the liquid breakfasts within 5 min. Thereafter, subjects were not allowed to eat or drink anything for 4 h. Blood was collected at 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, and 240 min. Immediately after each blood sample was taken, subjects filled out visual analogue scales (VASs) to measure subjective feelings of hunger, fullness, desire to eat, and prospective food consumption.

### Table 1

Subject characteristics at the beginning of the study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>36.1 ± 13.4 (19–57)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.4 ± 6.3 (65.1–90.7)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>183.7 ± 6.5 (167.4–191.2)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.6 ± 1.5 (19.9–25.4)</td>
</tr>
<tr>
<td>Waist:hip ratio</td>
<td>0.86 ± 0.05 (0.77–0.97)</td>
</tr>
<tr>
<td>DEBQ²</td>
<td>1.6 ± 0.5 (1.0–2.5)</td>
</tr>
</tbody>
</table>

¹ All values are x ± SD; range in parentheses. n = 20. DEBQ, Dutch Eating Behavior Questionnaire.

² Score on the restrained-eating scale of the DEBQ. Range of possible scores on the restrained-eating scale, 1.0–5.0.

### Table 2

Energy and macronutrient composition of the liquid breakfasts

<table>
<thead>
<tr>
<th>Breakfast</th>
<th>Water</th>
<th>LC</th>
<th>HC-SC</th>
<th>HC-CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (mL)</td>
<td>578 ± 5²</td>
<td>578 ± 5</td>
<td>578 ± 5</td>
<td>578 ± 5</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>578 ± 5</td>
<td>601 ± 5</td>
<td>641 ± 8</td>
<td>641 ± 8</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>0 736 ± 7</td>
<td>2674 ± 137</td>
<td>2674 ± 137</td>
<td>2674 ± 137</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>0 15²</td>
<td>13</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>0 0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>0 29</td>
<td>146 ± 8</td>
<td>146 ± 8</td>
<td>146 ± 8</td>
</tr>
<tr>
<td>Maltodextrin (g)</td>
<td>0 0</td>
<td>121</td>
<td>121</td>
<td>121</td>
</tr>
<tr>
<td>Reuteran (g)</td>
<td>0 0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>0 14</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

¹ LC, low-calorie breakfast; HC-SC, high-calorie, simple carbohydrate breakfast; HC-CC, high-calorie, complex carbohydrate breakfast.

² x ± SD (all such values).

³ x (all such values).
**Blood samples**

For plasma, blood was collected in evacuated tubes containing K$_2$EDTA as coagulant and put in ice water immediately. For serum, blood was collected in evacuated tubes containing clot activator. All tubes were centrifuged for 15 min at 2000 × g at 4 °C. Plasma and serum were removed and stored at −70 °C and −18 °C, respectively, until they were analyzed.

Serum glucose concentrations were measured by using a commercial test kit (Boehringer, Mannheim, Germany) on a Hitachi 911 automatic analyzer (Hitachi Instrument Division, Ibaraki, Japan), with intraassay CVs that ranged between 0.7% and 0.9%, depending on the concentration. Serum insulin concentrations were measured by using an AIA-600 Immunoassay Analyzer (Tosoh Corporation, Toyama, Japan), with intraassay CVs that ranged between 4.3% and 5.8%, depending on the concentration. Plasma concentrations of leptin and ghrelin were analyzed by using a radioimmunoassay. Leptin was measured in duplicate with the use of a commercial Sensitive Human Leptin kit (Linco Research Inc, St Charles, MO) with intraassay CVs that ranged between 4.9% and 6.4%, depending on the concentration. Ghrelin was measured in duplicate at 0, 30, 45, 60, 90, 120, 180, and 240 min in 18 subjects with the use of a commercial human radioimmunoassay kit (Phoenix Pharmaceuticals, Belmont, CA). The mean intraassay CV was 1.8% at a concentration of 0.40 μg/L and 4.5% at a concentration of 0.10–0.20 μg/L.

**Subjective appetite**

Subjective appetite was evaluated by using VASs for hunger, fullness, desire to eat, and prospective food consumption (39). VASs consisted of 150-mm horizontal lines with phrases in Dutch anchored at each end that expressed the most positive or most negative sensation (ie, I have never been more hungry/I am not hungry at all). Subjects drew a vertical line at the point on the horizontal line that corresponded to their hunger sensation. VASs were automatically processed with the use of TELEFORM ELITE software (version 6.1; Cardiff Software Inc, Sunnyvale, CA). Distances on the VASs were converted into scores between 0 and 100.

**Statistical analysis**

With analysis of variance (ANOVA) for repeated measures, the response curves of ghrelin, leptin, glucose, and insulin and the VAS scores after the 4 liquid breakfasts were compared, and we tested for time × treatment interactions and the effect of time separately. Incremental areas under or over the baseline were calculated. In this report, we use the term *area under the curve* (AUC) to refer to both values, delineated as negative AUC and positive AUC (the latter for the area over the curve). Evaluation of the residual plots showed that the negative or positive AUC of all variables except ghrelin and glucose could not be used for the analysis, and therefore we 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. If there was a treatment effect, partial tests were performed to compare treatments pairwise, and Tukey’s adjustments were used for multiple comparisons. Correlation coefficients were calculated to evaluate the relation among subjective measures of appetite and blood variables. Pearson’s correlation coefficient was calculated for each subject, based on 52 (13 time points, 4 treatments) or 32 (8 time points, 4 treatments for ghrelin) pairs of data. On these individual correlations a Fisher’s $z$ transformation was applied, to correct for deviations from the normal distribution. The mean of these 20 (18 in case of ghrelin) coefficients was calculated, the inverse of the Fisher transformation was performed, and the 95% CI for each correlation coefficient was calculated. Relations among changes in blood concentrations over different intervals were investigated. The percentage change in concentration between 2 time points [(30–0)/0 × 100, (180–0)/0 × 100, and (180–30)/30 × 100] was calculated for each subject. Pearson’s correlation coefficients were calculated per subject on the basis of 4 pairs of data. After correction with Fisher’s $z$ transformation, the average and 95% CIs were calculated. To test whether the correlation coefficients are significantly different from each other, a paired $t$ test of the $z$ scores was performed (Bonferroni corrected). In addition, the percentage change from baseline to the highest (ie, glucose, insulin, and fullness) or lowest (ie, ghrelin, leptin, and hunger) value was calculated.

Statistical analysis of the data were carried out with SAS/STAT statistical software (version 8.2; SAS Institute, Cary, NC). A $P$ value < 0.05 (two-sided) was considered significant in all analyses. Values are given as means ± SDs.

**RESULTS**

**Ghrelin**

Postprandial ghrelin responses are presented in Figure 1. Ghrelin concentrations decreased rapidly after the LC (−24%), HC-SC (−41%), and HC-CC (−33%) breakfasts, but less so after water (−2%). Until 90 min, ghrelin concentrations were the same after the LC and HC treatments. Ghrelin concentrations returned to their starting value between 90 and 120 min after the LC breakfast, but not until 240 min after the HC breakfasts. The repeated-measures ANOVA showed a time × treatment interaction for ghrelin ($P < 0.0001$). The negative AUC of the ghrelin response differed significantly ($P < 0.01$) between all of the treatments except between the water and LC treatment ($P = 0.09$).

**Glucose**

Postprandial glucose responses are shown in Figure 1. Blood glucose concentrations increased after the LC (12%), HC-SC (47%), and HC-CC (40%) breakfasts, reaching peak values at 30 min. By 45 min after the LC breakfast, blood glucose concentrations had returned to baseline values, and they dropped below baseline values and remained there until 120 min after the breakfast. Blood glucose concentrations after the HC breakfasts remained elevated until 120–150 min after breakfast. The glucose responses showed a significant time × treatment interaction ($P < 0.0001$). The positive AUC of glucose was significantly different between all of the treatments ($P < 0.0001$) except between the water and LC treatment and between the HC-SC and HC-CC treatment.

**Insulin**

Serum insulin responses are shown in Figure 1. Insulin concentrations increased ∼6-fold after the LC breakfast and ∼12- and 10-fold after the HC-SC and HC-CC breakfasts, respectively, reaching peak values at 30 (LC) and 45 (HC) min. Serum insulin concentrations had returned to baseline values by 105 min after consumption of the LC breakfast and by 210–240 min after...
FIGURE 1. Mean (±SEM) responses of ghrelin (n = 18), glucose (n = 20), insulin (n = 20), and leptin (n = 20) 4 h after the intake of 4 liquid breakfasts. Total AUC, total area under and over the curve (or baseline); negative AUC, area under the curve; positive AUC, area over the curve. Left: ○, water; □, low-calorie (LC) meal; ●, high-calorie simple carbohydrate (HC-SC) meal; ■, HC complex carbohydrate (HC-CC) meal. There was a significant time × treatment interaction for ghrelin, glucose, insulin (all: P < 0.0001), and leptin (P < 0.05) and a significant time effect (all: P < 0.0001). Right: bars in the same panel with different letters are significantly different, P < 0.05 (Tukey’s adjustment).
the consumption of the HC treatments. The insulin responses showed a significant time × treatment interaction (P < 0.0001). The total AUCs of insulin differed significantly between all 4 treatments (P < 0.05) except between the HC-SC and HC-CC treatments (P = 0.08) (Figure 1).

### Leptin

The plasma leptin responses during the 4 h after the 4 liquid breakfasts are shown in Figure 1. Plasma leptin concentrations decreased after breakfast by ≈20%, independent of the liquid breakfast consumed. The lowest leptin concentrations were observed at 180 (water and LC), 120 (HC-SC), and 90 (HC-CC) min. The repeated-measures ANOVA showed a significant time × treatment interaction (P < 0.05). The AUCs of leptin did not show a significant overall treatment effect of leptin (P = 0.125).

### Subjective appetite

VAS scores are shown in Figure 2. Analysis of total AUC showed a significant overall treatment effect on hunger (P < 0.05), fullness (P < 0.01), desire to eat (P < 0.001), and prospective food consumption (P < 0.01). There was also a significant time × treatment interaction (P < 0.01) for all 4 postprandial appetite responses. Subjective measures of hunger decreased by ≈30% after the LC, HC-SC, and HC-CC breakfasts, reaching the lowest values at 15 min. No decrease in hunger scores was observed after consumption of water. The total AUC of the hunger response was significantly smaller after water consumption than after the HC-SC (P < 0.01) or HC-CC (P < 0.05) breakfast. Subjective measures of fullness increased ≈14% after water, ≈67% after LC, 68% after HC-SC, and 91% after HC-CC breakfast, reaching peak values at 15 (LC and HC-CC) and 30 (HC-CC) min (Figure 2). Fullness scores with the LC and HC-CC treatments differed significantly (P < 0.05) between 90 and 180 min. The total AUC of the fullness response was significantly smaller after water consumption than after the HC-SC and HC-CC breakfasts (both: P < 0.01). The scores for the subjective measures desire to eat and prospective food consumption were essentially similar to those for hunger, although the decreases were generally smaller (Figure 2). The total AUC of desire to eat was significantly smaller after water consumption than after the HC-SC (P < 0.001) and HC-CC (P < 0.05) breakfasts and borderline significantly (P = 0.06) lower after the LC breakfast than after the HC-SC breakfast. The total AUC of prospective food consumption was significantly smaller after water consumption than after the HC-SC (P < 0.001) and HC-CC (P < 0.01) breakfasts and significantly smaller after the LC breakfast than after both HC treatments (both: P < 0.05).

### Correlations

#### Ghrelin and appetite

Ghrelin concentrations were positively correlated with hunger (r = 0.51; 95% CI: 0.09, 0.78), desire to eat (r = 0.51; 95% CI: 0.09, 0.78), and prospective food consumption (r = 0.52; 95% CI: 0.09, 0.78) and negatively correlated with fullness (r = −0.44; 95% CI: 0.00, −0.74), as shown in Table 3.

#### Ghrelin and other variables

Fasting ghrelin concentrations were not significantly correlated with age or BMI. The percentage decrease in ghrelin concentrations over the first 30 min correlated with the percentage increases in insulin (r = −0.76; 95% CI: −0.48, −0.90) and glucose (r = −0.79; 95% CI: −0.53, −0.91) but not with the percentage decreases in leptin (r = 0.10; 95% CI: −0.36, 0.52), as shown in Table 4. Moreover, the percentage increase in ghrelin concentrations between 30 and 180 min was correlated with the percentage decreases in insulin concentrations (r = −0.53; 95% CI: −0.11, −0.79) and leptin (r = −0.47; 95% CI: −0.03, −0.75) but not with the percentage decreases in glucose (r = 0.22; 95% CI: −0.24, 0.61) (see Table 4). The percentage decrease in ghrelin concentrations between 0 and 180 min was correlated with the percentage increases in insulin concentrations (r = −0.89; 95% CI: −0.73, −0.95). No such correlations were found between ghrelin and glucose (r = 0.06; 95% CI: −0.39, 0.49) or between ghrelin and leptin (r = −0.38; 95% CI: −0.70, 0.08), as shown in Table 5. The correlation coefficient of the percentage changes in ghrelin and insulin concentrations between 0 and 30 min did not differ significantly from the correlation coefficient of the percentage changes in ghrelin and glucose concentrations between 0 and 30 min (P = 0.69). However, the correlation coefficient of the percentage changes in ghrelin and insulin between 30 and 180 min (P < 0.0001) and between 0 and 180 min (P < 0.0001) was different from the correlation coefficients of the percentage changes in ghrelin and glucose within these time periods.

### Appetite and other blood variables

Glucose concentrations were negatively correlated with hunger scores (r = −0.38; 95% CI: −0.70, 0.08) and positively correlated with fullness scores (r = 0.31; 95% CI: −0.16, 0.66). Insulin concentrations also were negatively correlated with hunger scores (r = −0.51; 95% CI: −0.09, −0.78) and positively correlated with fullness scores (r = 0.46; 95% CI: 0.03, 0.75). Leptin concentrations and sensations of hunger and fullness were less correlated with hunger (r = −0.15; 95% CI: −0.56, 0.31) and fullness (r = 0.13; 95% CI: −0.33, 0.54).

### DISCUSSION

Postprandial ghrelin responses have been investigated, but the response of ghrelin after the intake of different amounts of carbohydrates was not studied until now. In this study, we show that ghrelin responds according to the amount of carbohydrate given, although ghrelin responses differed no sooner than at 120 min, which suggests that ghrelin requires postgastric feedback. The volume of intake itself did not appear to influence ghrelin secretion, because the consumption of water hardly affected ghrelin concentrations. The postprandial ghrelin concentrations are correlated with subjective measures of appetite and with insulin concentrations but less so with glucose concentrations, which suggests that ghrelin is directly or indirectly regulated by insulin. We found no evidence for such an involvement of leptin.

In the current study, subjective measures of appetite were correlated with ghrelin concentrations. This correlation was stronger than that between appetite and glucose and comparable with that between appetite and insulin. Our observation that ghrelin concentrations after the LC and HC treatments did not differ until 120 min after consumption is in accordance with the findings of Williams et al (40). They showed that, when gastric emptying was prevented in rats, neither glucose nor water administration affected ghrelin concentrations. However, when gastric emptying was not prevented, ghrelin was suppressed by...
FIGURE 2. Mean (±SEM) responses of hunger, fullness, desire to eat, and prospective food consumption in 20 men 4 h after the intake of 4 liquid breakfasts. Total AUC, total area under and over the curve (or baseline). Left: ○, water; □, low-calorie (LC) meal; ●, high-calorie simple carbohydrate (HC-SC) meal; ■, HC complex carbohydrate (HC-CC) meal. There was a significant time \times treatment interaction for hunger, prospective food consumption (both: $P < 0.0001$), fullness, and desire to eat (both: $P < 0.01$) and a significant time effect (all: $P < 0.0001$). Right: bars in the same panel with different letters are significantly different, $P < 0.05$ (Tukey's adjustment).
TABLE 3
The relation between ghrelin (n = 18 subjects) and measures of appetite (n = 20 subjects)\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>Fullness</th>
<th>Desire to eat</th>
<th>Prospective food consumption</th>
<th>Ghrelin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hunger</td>
<td>-0.66 (−0.31, −0.85)</td>
<td>0.90 (0.75, 0.96)</td>
<td>0.86 (0.68, 0.94)</td>
<td>0.51 (0.09, 0.78)</td>
</tr>
<tr>
<td>Fullness</td>
<td>-0.61 (−0.23, −0.83)</td>
<td>-0.59 (−0.21, −0.82)</td>
<td>-0.44 (0.00, −0.74)</td>
<td></td>
</tr>
<tr>
<td>Desire to eat</td>
<td>0.92 (0.81, 0.97)</td>
<td>0.51 (0.09, 0.78)</td>
<td>0.52 (0.09, 0.78)</td>
<td></td>
</tr>
<tr>
<td>Prospective food consumption</td>
<td>0.22 (0.64, 0.41)</td>
<td>-0.48 (−0.34, −0.86)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) All values are \(r\) (correlation coefficient); 95% CIs after Fisher’s \(z\) transformation in parentheses. Pearson’s correlation coefficients of the relation between subjective measures of appetite and ghrelin were calculated per subject.

TABLE 4
Correlations of percentage changes in concentrations between 0–30 and 30–180 min\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>0–30 min</th>
<th>30–180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghrelin-insulin</td>
<td>-0.76 (−0.48, −0.90)</td>
<td>-0.53 (−0.11, −0.79)</td>
</tr>
<tr>
<td>Ghrelin-glucose</td>
<td>-0.79 (−0.53, −0.91)</td>
<td>0.22 (−0.24, 0.61)</td>
</tr>
<tr>
<td>Ghrelin-leptin</td>
<td>0.10 (−0.36, 0.52)</td>
<td>-0.47 (−0.03, −0.75)</td>
</tr>
<tr>
<td>Insulin-glucose</td>
<td>0.82 (0.59, 0.93)</td>
<td>0.70 (0.37, 0.87)</td>
</tr>
<tr>
<td>Insulin-leptin</td>
<td>-0.12 (−0.53, 0.34)</td>
<td>-0.61 (−0.23, −0.83)</td>
</tr>
<tr>
<td>Glucose-leptin</td>
<td>-0.04 (−0.48, 0.41)</td>
<td>-0.68 (−0.34, −0.86)</td>
</tr>
</tbody>
</table>

\(^1\) All values are \(r\) (correlation coefficient); 95% CIs after Fisher’s \(z\) transformation in parentheses. Pearson’s correlation coefficients of the percentage changes in plasma and serum concentrations of ghrelin, insulin, glucose, and leptin between 0 and 30 min ([30–0]/0 × 100) and between 30 and 180 min ([180–30]/30 × 100) were calculated per subject. \(n = 20\) (except ghrelin, \(n = 18\)). Correlation coefficients were compared over the 2 time periods. The correlation coefficients of the correlation between percentage changes in ghrelin and insulin and of that between percentage changes in ghrelin and glucose were compared (paired \(t\) test of the \(z\) values, Bonferroni corrected) within a time period. Correlation coefficients with different superscript letters are significantly different from each other, \(P < 0.05\).

TABLE 5
Correlations of percentage changes in concentrations between 0 and 180 min

<table>
<thead>
<tr>
<th></th>
<th>Ghrelin</th>
<th>Insulin</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>-0.89*</td>
<td>−0.73</td>
<td>0.05*</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.06*</td>
<td>-0.39</td>
<td>-0.48</td>
</tr>
<tr>
<td>Leptin</td>
<td>0.38 (−0.70, 0.08)</td>
<td>0.49 (0.06, 0.77)</td>
<td>−0.36 (−0.69, 0.09)</td>
</tr>
</tbody>
</table>

\(^1\) All values are \(r\) (correlation coefficient); 95% CIs after Fisher’s \(z\) transformation in parentheses. Pearson’s correlation coefficient of the percentage changes in plasma and serum concentrations of ghrelin, insulin, glucose, and leptin between 0 and 180 min ([180–0]/0 × 100) were calculated per subject. \(n = 20\) (except ghrelin, \(n = 18\)). The correlation coefficients of the correlation between percentage changes in ghrelin and insulin and of that between percentage changes in ghrelin and glucose were compared (paired \(t\) test of the \(z\) values, Bonferroni corrected). Correlation coefficients with different superscript letters are significantly different from each other, \(P < 0.05\).

glucose only. This suggests that gastric distension and gastric chemosensitization are insufficient to induce a ghrelin response. It is possible that these postgastric processes involve insulin secretion either directly or indirectly by stimulating the incretin hormones glucagon-like peptide 1 and gastric inhibitory peptide. Our observation that the postprandial change in ghrelin concentrations is highly and inversely correlated with the postprandial change in insulin concentrations supports this. Although postprandial changes in ghrelin during the first 30 min were correlated with both glucose and insulin, changes in ghrelin concentrations between 30 and 180 min were highly correlated with changes in insulin but not with changes in glucose.

Using clamp studies, several research groups have investigated the relation among ghrelin, insulin, and glucose. Most researchers found that insulin decreases ghrelin concentrations, independent of glucose (41–44). The mechanism by which insulin has this inhibitory effect on ghrelin concentrations has not yet been ascertained. The effect of insulin may be mediated by direct effects on ghrelin-secreting cells or by indirect effects on other humoral or central mechanisms.

The data retrieved from clamp studies together with our observations suggest that the postprandial ghrelin response is dependent on insulin. Because fasting ghrelin concentrations are negatively associated with BMI and insulin resistance (3, 45–47), postprandial ghrelin responses may also be associated with insulin sensitivity. Such a correlation was confirmed in a study of Lucidi et al (42), who found a strong positive correlation between insulin sensitivity and the percentage decrease in ghrelin after insulin infusion. This observation may be relevant in view of the obesity epidemic. People consuming meals with a high glycemic load may have higher insulin concentrations during the postprandial phase and consequently may be temporarily less insulin sensitive. This insulin insensitivity may blunt the postprandial ghrelin response and decrease satiety.

Our results with respect to leptin are in line with the observation that leptin concentrations do not change acutely (ie, within 3–4 h) in response to meals (48–50). Although leptin does not seem to play an important role in the short-term regulation of food intake when subjects are in energy balance, plasma leptin is negatively correlated with appetite and food intake when the energy balance is disturbed (13). Leptin therefore seems to have a role in the regulation of food intake when energy stores change.

In summary, ghrelin responds rapidly and dose-dependently to carbohydrate intake and is correlated with subjective measures of appetite, which suggests that ghrelin plays an important role in the regulation of food intake. The mechanism is not clear yet, although our results support the previous finding that ghrelin requires postgastric feedback, and ghrelin concentrations seem to be associated with insulin more than with glucose or leptin. However, these results are based only on a carbohydrate-rich liquid breakfast in studies of healthy nonobese men. There is some evidence that liquid meals are less satiating than are solid meals, independent of the energy density of the meals (51). The effects of BMI, sex, insulin sensitivity, and different macronutrients on the postprandial ghrelin response should also be investigated. To clarify whether ghrelin regulates meal initiation (satiety) or meal termination (satiation), the interval between meals and ad libitum food intake should be investigated. The current results support the hypothesis that ghrelin requires postgastric feedback, which may be regulated through insulin.
We express our gratitude to all those involved in the conduct of the study. We also thank the volunteers who participated in the study.

WAMB was involved in the design of the protocol, collection and analysis of the data, and writing of the manuscript. AS and CG were involved in the design of the protocol and provided significant advice during the writing of the manuscript. FJK and GS provided significant advice during the writing of the manuscript. HFJH was involved in the design of the protocol (Principal Investigator according to Good Clinical Practice guidelines) and writing of the manuscript and data analysis. None of the authors had personal or financial conflicts of interest.

REFERENCES


