Effects of gastric emptying on the postprandial ghrelin response

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Departments of 1Food and Chemical Risk Analysis and 2Physiological Sciences, Netherlands Organization for Applied Scientific Research Quality of Life, Zeist; 3Department of Human Nutrition, Wageningen University, Wageningen, the Netherlands; 4Department of Nutrition, Danone Vitapole, Palaiseau Cedex, France; and 5Department of Medical Physiology, The Panum Institute, Copenhagen, Denmark

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Blom, Wendy A. M., Anne Lluch, Sophie Vinoy, Annette Staffeul, Robin van den Berg, Jens J. Holst, Frans J. Kok, and Henk F. J. Hendriks. Effects of gastric emptying on the postprandial ghrelin response. Am J Physiol Endocrinol Metab 290: E389–E395, 2006. First published September 27, 2005; doi:10.1152/ajpendo.00238.2005.—Distension and chemosensitization of the stomach are insufficient to induce a ghrelin response, suggesting that postgastric feedback is required. This postgastric feedback may be regulated through insulin. We investigated the relation between gastric emptying rate and the postprandial ghrelin response as well as the role of insulin and other hormones possibly mediating this response. Fifteen healthy men [BMI 21.6 kg/m2 (SD 1.9), age 20.5 yr (SD 2.5)] were studied in a single-blind, crossover design. Subjects received two treatments separated by 1 wk: 1) a dairy breakfast in combination with a 3-h intravenous infusion of glucagon-like peptide-1 (GLP-1), which delays gastric emptying, and 2) a dairy breakfast in combination with a 3-h intravenous infusion of saline. Blood samples were drawn before breakfast and during the infusion. Postprandial ghrelin (total) responses were lower following the saline infusion compared with the GLP-1 infusion (P < 0.05). Acetaminophen concentrations, an indirect measurement of gastric emptying rate, were inversely correlated with total ghrelin concentrations (saline r = −0.76; 95% CI = −0.90, −0.49, GLP-1 r = −0.47; 95% CI = −0.76, −0.04). Ghrelin concentrations were only weakly correlated with insulin concentrations (saline r = −0.36; 95% CI = −0.69, 0.09; GLP-1 r = −0.42; 95% CI = −0.73, 0.03), but strongly inversely correlated with GIP concentrations (saline r = −0.74; 95% CI = −0.89, −0.45; GLP-1 r = −0.63; 95% CI = −0.84, −0.27). In conclusion, our results support the hypothesis that ghrelin requires postgastric feedback, which may not be regulated through insulin. Conversely, our data suggest a role of glucose-dependent insulino tropic polypeptide (GIP) in ghrelin secretion.

postgastric feedback; glucose-dependent insulino tropic polypeptide; glucagon-like peptide-1; insulin; acetaminophen absorption test

Ghrelin is a peptide that is predominantly secreted by the oxyntic glands of the stomach (2, 7, 15) and is involved in the regulation of food intake (21, 34). Ghrelin concentrations decrease rapidly following nutrient intake (2, 4, 9, 13) but not after intake of water (4, 30). Williams et al. (33) have shown that, when gastric emptying was prevented in rats, neither glucose nor water administration affected ghrelin concentrations. These observations suggest that distension and chemosensitization of the stomach are insufficient to induce a ghrelin response and that postgastric processes are required. These postgastric processes may involve insulin concentrations, because postprandial changes in ghrelin concentrations are associated with postprandial changes in insulin concentrations (4, 10). This association is supported by clamp studies, which provided some evidence that insulin decreases ghrelin concentrations independently of glucose (11, 17, 20, 28).

Gastric emptying is regulated by several postgastric hormones such as cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1), which both decrease the gastric emptying rate (14, 18, 22, 23), but also by ghrelin, which appears to increase the gastric emptying rate (8, 26).

The objective of this study was to investigate whether the postprandial ghrelin response requires postgastric feedback and, if so, whether insulin or other postprandial processes provide this feedback. If the postprandial ghrelin response indeed requires postgastric feedback, ghrelin concentrations should be dependent on the gastric emptying rate.

Therefore, subjects received either an intravenous infusion of GLP-1, which delays gastric emptying, or saline. Gastric emptying was indirectly measured by acetaminophen absorption (29, 32). We measured the postprandial ghrelin (total and active) and insulin responses, as well as other factors involved in the regulation of food intake [e.g., glucose, glucagon, and glucose-dependent insulino tropic polypeptide (GIP)].

MATERIALS AND METHODS

Subjects

The study was conducted at Netherlands Organization for Applied Scientific Research (TNO) Quality of Life, Zeist, the Netherlands, where subjects were recruited from a pool of volunteers. Each subject gave written informed consent after being informed about the study both verbally and in writing. 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 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, as assessed with the Dutch Eating Behavior Questionnaire (31) (score of restriction >2.5), subjects with hemoglobin concentrations <8.4 mmol/L, and subjects who reported slimming or who were on a medically prescribed diet were excluded from participation. Also, subjects who were on medication that might have influenced appetite and sensory functioning or who reported metabolic or endocrine disease, gastrointestinal disorders, or a history of medical or surgical events that might have affected study outcome were not included.

Fifteen healthy, lean young men with a mean body mass index (BMI) of 21.6 kg/m2 (SD 1.9) (range 19.0–25.0) and a mean age of 20.5 yr (SD 2.5) (range 18–26) completed the study (Table 1).
were removed. Blood was collected at 15, 30, 45, 60, 90, 120, and 180 min. Subjects received an ad libitum buffet-style lunch, at the same time, an intravenous infusion of saline was infused for 180 min. Thereafter, subjects received an ad libitum buffet-style lunch, consisting of an isocaloric high-protein peanut butter was provided in a jar of 500 g). The third treatment, not allowed to eat or drink anything for 3 h. Blood was collected at 15, 30, 45, 60, 90, 120, and 180 min. Subjects received an ad libitum lunch after 180 min, when the infusion was stopped and the cannulas were removed.

**Study Design**

The study had a crossover design. Each subject received three treatments on separate days, with a washout period of one wk. For practical reasons, all subjects received the same treatment order. Subjects were blinded for treatment order and were informed that the treatment order was randomized. The study had a staggered start, with 5 subjects starting per day. Subjects were successfully randomized for body weight and age. The study was designed to investigate two separate hypotheses. In this paper, only one hypothesis is presented, i.e., that the postprandial ghrelin response requires postgastric feedback. The second hypothesis, i.e., that protein exerts its satiating effects partly through suppression of postprandial ghrelin concentrations, will be described in a separate paper (4a). We only describe the outcome of the two treatments used to investigate the hypothesis presented in this paper. All three treatments are mentioned, because the statistical plan was based on these three treatments.

**Study Treatments**

At breakfast, subjects consumed 400 g of plain yogurt to which 20 g of saccharose and 1.5 g of aceticaminophen were thoroughly mixed for both treatments. Table 2 presents the energy and macronutrient contents of the breakfast. At the same time, an intravenous infusion of saline was infused for 180 min. Thereafter, subjects received an ad libitum buffet-style lunch, which consisted of standard Dutch food items, e.g., bread, milk, orange juice, cheese, ham, yogurt, and fruit. Subjects ate their lunch in separate rooms within 30 min. They were instructed to eat until they were satiated. To prevent habitual intake, foods were provided in unusual portion sizes (e.g., slices of bread were cut in 4 pieces, and peanut butter was provided in a jar of 500 g). The third treatment, not further presented in this paper, consisted of an isocaloric high-protein breakfast in combination with an intravenous infusion of saline.

**Study Protocol**

Subjects were instructed to eat and drink the same food items the evening before each of the two test days by recording this in a diary. After an overnight fast (nothing to eat or drink except for water after 2000), subjects handed in their diaries, filled out a well-being questionnaire, and were weighed. The subjects were seated in a semisupine position for the rest of the treatment to prevent effects of position on gastric emptying. An indwelling cannula was placed in the antecubital vein of each forearm, the first for the infusion of saline or GLP-1-(7–36) and the second for blood sampling. A preingestion blood sample was collected. After breakfast, consumed within 10 min, subjects were not allowed to eat or drink anything for 3 h. Blood was collected at 15, 30, 45, 60, 90, 120, and 180 min. Subjects received an ad libitum lunch after 180 min, when the infusion was stopped and the cannulas were removed.

The study was performed according to the ICH Guideline for Good Clinical Practice (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use topic E6, adopted 05/01/1996 and implemented 01/17/1997) and was approved by the independent Medical Ethics Committee of the Academic Hospital in Utrecht.

**Blood Samples**

Blood was collected as previously described (4). Plasma acetonidopen was analyzed using a commercially available ELISA kit (Immunoanalysis, Pomona, CA) with an intra-assay coefficient of variation (CV) of 3.7% at a concentration of 5 pg/ml and 0.9% at a concentration of 25 pg/ml. GLP-1 concentrations in plasma were measured by radioimmunoassay after extraction of plasma with 70% ethanol (vol/vol, final concentration). Carboxy-terminal GLP-1 immunoreactivity was determined using antisera GLP-1290 (24), which has an absolute requirement for the intact amidated carboxy terminus of GLP-1-(7–36) amide and cross-reacts less than 0.01% with carboxy-terminally truncated fragments and 89% with GLP-1-(9–36) amide (24). Sensitivity was below 5 pmol/l and intra-assay CV below 10%. Serum glucose was determined using a commercially available test kit (Roche Diagnostics, Mannheim, Germany) on a Hitachi 911 automatic analyzer (Hitachi Instrument Division, Ibaraki-ken, Japan), with intra-assay CVs ranging from 0.7 to 0.9% depending on the concentration. Serum insulin was determined as previously described (4). Plasma ghrelin (total and active) concentrations were measured using commercially available human RIA kits (Linco Research, St. Charles, MO). The intra-assay CV of the total ghrelin RIA kit was 10% at a concentration of 1.00 pg/ml, and 3.3% at a concentration of 1.500 pg/ml. The intra-assay CV of the active ghrelin RIA kit was 6.7% at a concentration of 139 pg/ml and 9.5% at a concentration of 237 pg/ml. Plasma glucagon concentrations were measured using a commercially available human RIA kit (Linco Research) with an intra-assay CV of 6.8% at a concentration of 60 pg/ml and 4.0% at a concentration of 220 pg/ml. Plasma GIP concentrations were measured using a commercially available human RIA kit (Phoenix Pep- tide, Belmont, CA) with an intra-assay CV of GIP of 3.3% at a concentration of 0.40 μg/l and 2.5% at a concentration of 0.80 μg/l. Plasma CCK-8 [cholecystokinin-(26–33)] concentrations were measured using an optimized and validated commercial human RIA kit (Euro-Diagnostica, Malmo, Sweden). This improved assay system has been optimized to reach a very high sensitivity of 0.05 pmol/l and no cross-reactivity toward gastrin-17 and sulfated gastrin. The intra-assay CV was 8.9% at a concentration of 0.84 pmol/l and 4.9% at a concentration of 1.98 pmol/l.

**Statistical Analyses**

With analysis of variance (ANOVA) for repeated measures, the response curves of ghrelin, GLP-1, CCK, GIP, glucose, insulin, and glucagon after the three treatments were compared, testing for time × treatment interactions. Tests comparing the GLP-1 and saline treatments were only performed in case an overall treatment effect was observed. With mixed-model analysis of variance, differences in

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**Table 1. ** *Subject characteristics*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>20.5 ± 2.5</td>
<td>18.0–26.0</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.85 ± 0.06</td>
<td>1.72–1.94</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>73.8 ± 7.4</td>
<td>62.5–85.3</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>21.6 ± 1.9</td>
<td>19.0–25.0</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.86 ± 0.06</td>
<td>0.77–0.97</td>
</tr>
<tr>
<td>DEBO*</td>
<td>1.3 ± 0.4</td>
<td>1.0–2.3</td>
</tr>
</tbody>
</table>

Values represent measurements taken at the beginning of the study (n = 15).

*Score on the restrained eating scale of the Dutch Eating Behavior Questionnaire; range of possible scores on the restrained eating scale, 1.0–5.0.

**Table 2. ** *Energy and macronutrient composition of the breakfast*

<table>
<thead>
<tr>
<th>Component</th>
<th>Weight, g</th>
<th>Energy, KJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>18.8</td>
<td>1,628</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>46.0</td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>14.4</td>
<td></td>
</tr>
<tr>
<td>Protein, En%</td>
<td>19.3</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate, En%</td>
<td>47.3</td>
<td></td>
</tr>
<tr>
<td>Fat, En%</td>
<td>33.3</td>
<td></td>
</tr>
</tbody>
</table>
concentrations were investigated per time point. Incremental areas under or over the baseline were calculated. The term area under the curve (AUC) refers 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 AUC of “ghrelin total” and “ghrelin active” could not be used for the analysis. We defined the total AUC as the sum of the areas under and over the baseline, in case of “ghrelin total” and “ghrelin active”. By use of a mixed-model ANOVA, the AUCs of the different variables were tested for an overall treatment effect. Correlation coefficients were calculated to evaluate the relation among blood parameters. Per treatment, the Pearson correlation coefficient was calculated for each subject on the basis of eight (8 time points) 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 15 coefficients was calculated, the inverse of the Fisher transformation was performed, and the 95% confidence interval (95% CI) for each correlation coefficient was calculated. Also the proportional change from baseline to the highest (glucose, insulin, glucagon, GIP, CCK, and GLP-1) or lowest (ghrelin) value was calculated.

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

**RESULTS**

**Gastric Emptying**

Gastric emptying was indirectly estimated using acetaminophen absorption. Figure 1 shows the postprandial acetaminophen concentrations and the AUCs of the acetaminophen response. After the saline infusion, acetaminophen concentrations in plasma increased rapidly, reaching maximum values of 16.2 µg/ml (SD 4.0) at 120 min. Acetaminophen concentrations after the GLP-1 infusion reached maximum concentrations of only 12.9 µg/ml (SD 3.2) at 180 min. The acetaminophen responses showed an overall time × treatment interaction (*P* < 0.0001). Partial tests showed that the acetaminophen responses after GLP-1 and saline were different (*P* < 0.0001); namely, the AUC was smaller (~32%) after GLP-1 infusion compared with saline infusion (*P* < 0.0001).

**Blood Parameters**

**GLP-1.** Total GLP-1 concentrations following infusion of GLP-1-(7–36) amide were within the physiological range (19, 25). GLP-1 concentrations increased ~50% after the saline infusion, and ~150% after the GLP-1 infusion, reaching peak values

Fig. 1. Three-hour postprandial responses of acetaminophen and glucagon-like peptide-1 (GLP-1, total; *n* = 15) after infusion of saline (□) or GLP-1 (active, ●). By ANOVA, there was a significant time × treatment effect for acetaminophen (*P* < 0.0001) and GLP-1 (*P* < 0.001). Different letters indicate level of statistical significance: *a* *P* < 0.01, *b* *P* < 0.001, *c* *P* < 0.0001. **Insets:** means ± SD area under the curve (AUC) of the acetaminophen and GLP-1 responses. By ANOVA, there was a significant treatment effect of the AUCs for GLP-1 and acetaminophen (both *P* < 0.0001).
at 90 and 45 min, respectively (Fig. 1). The GLP-1 responses showed an overall interaction between time × treatment (P < 0.0005). Partial tests showed that GLP-1 responses after GLP-1 and saline infusion were different (P < 0.0001) as well. In addition, the AUCs of GLP-1 were larger (~207%) after GLP-1 infusion compared with infusion of saline (P < 0.0001).

**Ghrelin.** **TOTAL GHRELIN.** Ghrelin responses and AUCs are presented in Fig. 2. Ghrelin concentrations decreased after both the saline infusion (~18%) and the GLP-1 infusion (~15%), reaching lowest values at 60 and 120 min, respectively. The ghrelin responses showed an overall interaction between time × treatment (P < 0.0001). Partial tests showed that the ghrelin responses after GLP-1 and saline were different (P < 0.05). The AUCs of the ghrelin responses were not different between the GLP-1 and saline infusions. Ghrelin concentrations tended to be lower (P < 0.10) at 90 and 120 min after the saline infusion compared with the GLP-1 infusion.

**ACTIVE GHRELIN.** Active ghrelin concentrations decreased after the saline (~18%) and the GLP-1 (~33%) infusions, reaching lowest values at 45 and 60 min, respectively (Fig. 2). There was no overall treatment effect.

**Glucose.** Serum glucose responses and AUCs are presented in Fig. 2. Glucose concentrations increased ~24% after the saline infusion, reaching peak values at 30 min. In contrast, during GLP-1 infusion, glucose concentrations decreased by ~11%. The glucose responses showed an overall interaction between time × treatment (P < 0.0001), and partial tests showed that the GLP-1 and saline responses differed from each other (P < 0.0001). The AUC of glucose was smaller (~67%) after the GLP-1 infusion than after the saline infusion (P < 0.001).
Insulin. Figure 2 presents the serum insulin responses and AUCs. GLP-1 infusion reduced postprandial insulin concentrations compared with saline infusion (2.5-fold increase compared with 8-fold increase after saline). ANOVA for repeated measures showed an overall interaction between time × treatment (P < 0.0001). Partial tests showed that insulin responses after GLP-1 and saline were different (P < 0.0001); namely, the AUC of the insulin response was smaller (~45%) after the GLP-1 infusion compared with the saline infusion (P < 0.0001).

Glucagon. Glucagon concentrations increased ~31% after the saline infusion and reached peak values at 30 min but were hardly affected by the GLP-1 infusion (+8; Fig. 2). The glucagon responses showed an overall time × treatment effect (P < 0.0001). Partial tests showed that the glucagon responses were different between the two treatments (P < 0.05). The AUC of glucagon was smaller (~67%) after the GLP-1 infusion than after the saline infusion (P < 0.05).

GIP. Plasma GIP responses and AUCs are presented in Fig. 2. GIP concentrations increased ~150% after the saline infusion and ~100% after the GLP-1 infusion, reaching peak values at 30 and 90 min, respectively. The GIP responses showed an overall time × treatment interaction (P < 0.0001). Partial tests showed also that the GIP responses following GLP-1 and saline infusion were different (P < 0.0001). Although there was an overall treatment effect (P < 0.01) of the AUCs of the GIP responses, the AUCs of the GIP responses after saline and GLP-1 infusion were not different (P = 0.11). GIP concentrations were significantly higher after saline than after GLP-1 infusion between 30 and 90 min (P < 0.05).

CCK. CCK concentrations increased about threefold after the saline infusion reaching peak values at 15 min (Fig. 2). CCK concentrations showed a biphasic response following GLP-1 infusion. Initially CCK concentrations increased ~4.5-fold but then dropped at 30 min, followed by a steady increase, reaching peak values at 90 min (6.5-fold increase compared with baseline values). The CCK responses showed an overall time × treatment interaction (P < 0.01). Partial tests showed that the CCK responses following GLP-1 and saline infusion were different (P < 0.001). The AUC of the CCK response after GLP-1 infusion was larger (~37%) than after the saline infusion (P < 0.05).

Correlations

Acetaminophen concentrations, used as an indirect measurement of gastric emptying rate, were correlated with concentrations of the other blood parameters to assess the relation of these parameters to gastric emptying.

Acetaminophen concentrations were inversely correlated with total ghrelin concentrations both during saline infusion (r = −0.76; 95% CI = −0.90, −0.49) and during GLP-1 infusion (r = −0.47; 95% CI = −0.76, −0.49; Table 3). Also, GIP concentrations were positively associated with acetaminophen concentrations (saline r = 0.54; 95% CI = 0.13, 0.79; GLP-1 r = 0.59; 95% CI = 0.19, 0.82). CCK concentrations were positively associated with acetaminophen concentrations only during GLP-1 infusion (r = 0.55; 95% CI = 0.14, 0.80), but not during infusion of saline (r = 0.36; 95% CI = −0.10, 0.69). In contrast, insulin concentrations were not correlated at all with acetaminophen concentrations (saline r = 0.15; 95% CI = −0.32, 0.55; GLP-1 r = 0.12; 95% CI = −0.34, 0.54). There was no association between acetaminophen and active ghrelin concentrations as well (saline r = −0.10; 95% CI = −0.52, 0.35; GLP-1 r = −0.08; 95% CI = −0.50, 0.38).

Correlation coefficients between ghrelin concentrations and concentrations of other blood parameters were calculated to assess the relation of these blood parameters with ghrelin.

Correlations between ghrelin concentrations and other parameters are presented in Table 3. The postprandial responses of total ghrelin and active ghrelin were positively correlated during GLP-1 infusion (r = 0.56; 95% CI = 0.15, 0.80) but not during saline infusion (r = 0.35; 95% CI = −0.11, 0.69). Total ghrelin concentrations were strongly inversely correlated with concentrations of the insulinotropic peptide GIP (saline r = −0.74; 95% CI = −0.89, −0.45; GLP-1 r = −0.63; 95% CI = −0.84, −0.27) and were also inversely correlated with concentrations of CCK (saline r = −0.54; 95% CI = −0.80, −0.13; GLP-1 r = −0.50; 95% CI = −0.77, −0.08). Ghrelin concentrations were also inversely associated with concentrations of the other insulinotropic peptide, GLP-1, during GLP-1 infusion (r = −0.58; 95% CI = −0.81, −0.18), but this association was not present during infusion of saline (r = −0.16; 95% CI = −0.56, 0.31). Conversely, glucagon concentrations were inversely associated with ghrelin concentrations during saline infusion (r = −0.52; 95% CI = −0.78, −0.10) but not after infusion of GLP-1 (r = −0.16; 95% CI = −0.56, 0.30). In contrast with our hypothesis, total ghrelin concentrations were not associated with insulin concentrations (saline r = −0.36; 95% CI = −0.69, 0.09; GLP-1 r = −0.42; 95% CI = −0.73, 0.03). There were no associations between active ghrelin concentrations and other physiological parameters than total ghrelin (Table 3).

Table 3. Mean correlation coefficient (r) with 95% CIs of the relation between physiological parameters by treatment (n = 15)

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>GIP</th>
<th>GLP-1</th>
<th>GIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>−0.76 (−0.90, −0.49)</td>
<td>0.54 (0.13, 0.79)</td>
<td>−0.47 (−0.76, −0.04)</td>
<td>0.59 (0.19, 0.82)</td>
</tr>
<tr>
<td>GLP-1</td>
<td>−0.16 (−0.56, 0.31)</td>
<td>0.19 (−0.27, 0.58)</td>
<td>−0.58 (−0.81, −0.18)</td>
<td>0.49 (0.06, 0.77)</td>
</tr>
<tr>
<td>Insulin</td>
<td>−0.36 (−0.69, 0.09)</td>
<td>0.81 (0.57, 0.92)</td>
<td>−0.42 (−0.73, 0.03)</td>
<td>0.64 (0.28, 0.84)</td>
</tr>
<tr>
<td>GIP</td>
<td>−0.74 (−0.89, −0.45)</td>
<td>0.63 (−0.84, −0.27)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCK</td>
<td>−0.54 (−0.80, −0.13)</td>
<td>0.71 (0.38, 0.87)</td>
<td>−0.50 (−0.77, −0.08)</td>
<td>0.61 (0.24, 0.83)</td>
</tr>
<tr>
<td>Ghrelin active</td>
<td>0.35 (−0.11, 0.69)</td>
<td>−0.28 (−0.64, 0.18)</td>
<td>0.56 (0.15, 0.80)</td>
<td>−0.18 (−0.58, 0.28)</td>
</tr>
</tbody>
</table>

Pearson correlation coefficients of the relation between ghrelin and other physiological parameters were calculated per subject. Mean correlation coefficients together with the 95% confidence intervals (CI) after Fisher z-transformation are presented.

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DISCUSSION

Animal studies show that distension and chemosensitization of the stomach are insufficient to induce a ghrelin response (33), suggesting that postgastric feedback is required. In this study, we investigated the association between gastric emptying rate and the postprandial ghrelin response and whether insulin or other postgastric processes are involved in the postprandial ghrelin response. The results of this study show that ghrelin responses are associated with the gastric emptying rate, supporting our hypothesis that ghrelin requires postgastric feedback. Our data did not support our hypothesis that insulin is involved in the postprandial regulation of ghrelin secretion. On the other hand, ghrelin concentrations were strongly associated with GIP and CCK concentrations.

In this study, the postprandial responses of different regulators of food intake were investigated. The associations between the different measurements were investigated by correlational analysis. Although correlations do provide more insight into the associations between different measurements, they do not provide a causal relationship. Therefore results should be confirmed by future experiments.

The design of the study involved infusion of GLP-1, which might have affected concentrations of the other variables. However, the relatively low dose of GLP-1 amide infused resulted in total GLP-1 concentrations that remained within the physiological range (19, 25). GLP-1 infusion reduced the insulin response following a meal despite the fact that GLP-1 is an insulinotropic hormone. This observation has also been reported by Nauck et al. (23) and suggests that the effect of GLP-1 infusion on gastric emptying outweighs the insulinotropic effects of GLP-1. Nevertheless, there are indications that GLP-1 infusion directly affected ghrelin concentrations. Our first hypothesis was that postprandial ghrelin response requires postgastric feedback. However, ghrelin concentrations were significantly higher only between 90 and 120 min, despite the fact that GLP-1 infusion did reduce the gastric emptying rate and ghrelin concentrations were correlated with acetaminophen absorption. Possibly, infusion of GLP-1 suppressed ghrelin secretion directly, since a study in the isolated rat stomach showed that GLP-1 decreases ghrelin secretion (16). This direct suppressive effect may have confounded the association between ghrelin and gastric emptying. Nevertheless, the association between ghrelin and GLP-1 has not been directly tested in humans yet. GLP-1 infusion in the absence of food intake may provide more insight into the direct effects of GLP-1 on ghrelin concentrations. The inverse association between ghrelin and acetaminophen concentrations during saline and GLP-1 infusion suggests that the postprandial ghrelin response is strongly related to the gastric emptying rate; however, other studies are needed to confirm this. Investigation of the effects of multiple different treatments that increase or decrease emptying rate through differing mechanisms, on ghrelin secretion, may provide more information about the importance of postgastric feedback for postprandial ghrelin secretion.

In the second hypothesis, we tested whether insulin is the postgastric factor that is involved in postprandial ghrelin secretion. To show that insulin provides feedback to ghrelin, we investigated the correlation between ghrelin and insulin concentrations. In contrast with our expectations, insulin concentrations were not significantly correlated with ghrelin concentrations (or with acetaminophen absorption). We do not believe that GLP-1 infusion may have confounded this relation, because a similar weak correlation was found after saline infusion. Ghrelin concentrations were inversely correlated with GIP and CCK concentrations. So far, little is known about the relation between GIP and ghrelin secretion. Only a few studies have investigated the relation between GIP and ghrelin secretion and they showed contradictory results (1, 27). The strong inverse association between GIP and ghrelin concentrations that were observed in this study suggests that GIP, instead of insulin, might act as the postgastric feedback signal for the postprandial ghrelin response. There were also indications of a role for CCK herein. These results are, however, correlative and therefore do not prove a causal role for GIP and CCK in ghrelin secretion. Future studies should directly investigate this causality. For example, the effect of GIP and CCK antagonists on postprandial ghrelin secretion may provide more information, as well as infusion of GIP and CCK.

There are two major molecular forms of ghrelin: acylated ghrelin, which has an n-octanoylation at serine 3, and unacylated ghrelin (15). Until recently, only the acylated form of ghrelin was thought to be biologically active. The current perspective is that unacylated (desacyl) ghrelin also exerts some biological activities (3, 5, 6, 12). To gain more insight into the postprandial responses of acylated (active) and unacylated ghrelin concentrations, we measured both active ghrelin and total ghrelin concentrations, which is the sum of acylated and unacylated ghrelin. Both active and total ghrelin concentrations decreased in the postprandial period. However, only total ghrelin concentrations were different between the two treatments. The effects observed for total ghrelin may be mediated by active ghrelin. However, due to the large variations in active ghrelin concentrations we may not have had sufficient statistical power to detect differences.

In conclusion, the results of this study show that postprandial ghrelin responses are inversely associated with the gastric emptying rate and support the hypothesis that ghrelin requires postgastric feedback. In these experimental conditions, our data did not support the hypothesis that insulin regulates the postprandial regulation of ghrelin secretion. Conversely, total ghrelin concentrations were associated with GIP and CCK concentrations, suggesting a role of GIP and CCK in postprandial ghrelin secretion.

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