The effects of gastric emptying on the postprandial ghrelin response

Wendy A.M. Blom$^{1,3}$, Anne Lluch$^4$, Sophie Vinoy$^4$, Annette Stafleu$^1$, Robin van den Berg$^2$, Jens J. Holst$^5$, Frans J. Kok$^3$, Henk F.J. Hendriks$^2$

TNO* Quality of Life, Departments of $^1$Food and Chemical Risk Analysis and $^2$Physiological Sciences, Zeist, the Netherlands

$^3$Wageningen University, Department of Human Nutrition, Wageningen, the Netherlands

$^4$Danone Vitapole, Department of Nutrition, Palaiseau Cedex, France

$^5$The Panum Institute, Department of Medical Physiology, Copenhagen, Denmark

* Acronym for Netherlands Organization for Applied Scientific Research

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Correspondence: Henk F.J. Hendriks

TNO Quality of Life, Department of Physiological Sciences, P.O. Box 360, 3700 AJ Zeist, The Netherlands. Phone: +31 30 6944294; Fax: +31 30 6944928; E-mail: Hendriks@voeding.tno.nl

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Abstract

Distension and chemo sensitization of the stomach are insufficient to induce a ghrelin response, suggesting that post gastric feedback is required. This post gastric 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 ± 1.9 kg/m², age: 20.5 ± 2.5 y) were studied in a single blind, crossover design. Subjects received two treatments separated by one week; 1) a dairy breakfast in combination with a 3-h intravenous infusion of GLP-1, which delays gastric emptying 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 as compared to 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% C.I. = -0.90, -0.49, GLP-1: r = -0.47; 95% C.I. = -0.76, -0.04). Ghrelin concentrations were only weakly correlated with insulin concentrations (saline: r = -0.36; 95% C.I. = -0.69, 0.09; GLP-1: r = -0.42; 95% C.I. = -0.73, 0.03), but strongly inversely correlated with GIP concentrations (saline: r = -0.74; 95% C.I. = -0.89, -0.45; GLP-1: r = -0.63; 95% C.I. = -0.84, -0.27).

In conclusion, our results support the hypothesis that ghrelin requires post gastric feedback, which may not be regulated through insulin. Conversely, our data suggest a role of GIP in ghrelin secretion.

Key words: Post gastric feedback, GIP, GLP-1, insulin, acetaminophen absorption test
Introduction

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 and colleagues (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 post gastric processes are required. These post gastric 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, independent of glucose (11; 17; 20; 28).

Gastric emptying is regulated by several post gastric 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 post gastric feedback, and if so, whether insulin or other post gastric processes provide this feedback. If the postprandial ghrelin response indeed requires post gastric 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 insulinoactive polypeptide (GIP)).

**Materials and Methods**

**Subjects**

The study was conducted at TNO Quality of Life, Zeist, the Netherlands, where subjects were recruited from a pool of volunteers. Each subject gave his written informed consent after being informed about the study, both verbally and in writing. All subjects filled out a questionnaire on life-style, 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 at least 1 month prior to the study. Smokers, restrained eaters, as assessed with the Dutch Eating Behavior Questionnaire (31) (score of restriction > 2.5), subjects with hemoglobin concentrations below 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 may have influenced appetite and sensory functioning or who reported metabolic or endocrine disease, gastro-intestinal disorders or a history of medical or surgical events that may have affected study outcome were not included.
Fifteen healthy, lean young men with a mean body mass index (BMI) of 21.6 ± 1.9 kg/m² (range: 19.0 - 25.0) and a mean age of 20.5 ± 2.5 y (range: 18 - 26) completed the study (Table 1).

Study design
The study had a crossover design. Each subject received three treatments on separate days, with a washout period of one week. 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 manuscript, only one hypothesis will be 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, is described in a separate paper. We only described 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 grams of plain yogurt to which 20 grams of saccharose and 1.5 grams of acetaminophen was thoroughly mixed for both treatments. Table 2 presents the energy and macronutrient content of the breakfast. At the same time, an intravenous infusion of either saline (0.9% NaCl) (2.5 ml/min) or 0.75 pmol/kg body
weight/min GLP-1 (7-36) amide (Clinalfa, Merck Biosciences Ag, Läufelfingen, Switzerland) dissolved in saline was infused during 180 minutes. Thereafter, subjects received an ad libitum, buffet-style lunch, which consisted of standard Dutch food items. Subjects ate their lunch in separate rooms within 30 minutes. They were instructed to eat until they were satiated. In order 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 grams). 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 20.00 h), subjects handed in their diary, filled out a well-being questionnaire and were weighed. The subjects were seated in a semi-supine 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 pre-ingestion blood sample was collected. After breakfast, consumed within 10 minutes, subjects were not allowed to eat or drink anything during three hours. Blood was collected at 15, 30, 45, 60, 90, 120, and 180 minutes. Subjects received an ad libitum lunch, after 180 minutes, when the infusion was stopped and the cannulas were removed.
The study was performed according to the ICH Guideline for Good Clinical Practice (ICH topic E6, adopted 01-05-1996 and implemented 17-01-1997) 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 acetaminophen was analyzed using a commercially available ELISA kit (Immunalysis Corporation, Pomona, CA, USA) with an intra-assay CV of 3.7% at a concentration of 5 µg/ml, and 0.9% at a concentration of 25 µg/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 antiserum 89390 (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 coefficient of variation below 10%. Serum glucose was determined using a commercially available test kit (Roche Diagnostics GmbH, Mannheim, Germany) on a Hitachi 911 automatic analyser (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 Inc., St. Charles, MO). The intra-assay CV of the total ghrelin RIA kit was 10% at a concentration of 1000 pg/ml, and 3.3% at a concentration of 1500 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 Inc., St. Charles, MO) 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 Peptide, Belmont, California, USA) with an intra-assay CV of GIP was 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, Malmö, Sweden). This improved assay system has been optimized to reach a very high sensitivity of 0.05 pmol/L and no cross-reactivity towards to gastrin-17, and sulphated 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 3 treatments were compared, testing for time X 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 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”. With the 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, based on 8 (8 time points) pairs of data. On these individual correlations, a Fisher’s z-transformation was applied, in order 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% C.I.) 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 <0.05 (two-sided) was considered statistically significant in all analyses. Results are given as mean ± 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 ± 4.0 µg/ml at 120 minutes. Acetaminophen concentrations after the GLP-1 infusion reached maximum concentrations of only 12.9 ± 3.2 µg/ml at 180 minutes. The acetaminophen responses showed an overall time X 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 (about 32%) after GLP-1 infusion as compared to saline infusion
(P<0.0001).

**Blood parameters**

*Glucagon-Like Peptide 1 (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 about 50% after the saline
infusion, and about 150% after the GLP-1 infusion, reaching peak values at 90 and 45
minutes, respectively (see figure 1). The GLP-1 responses showed an overall interaction
between time X 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 (about 207%) after GLP-1 infusion compared to infusion of saline
(P<0.0001).

*Ghrelin*

**Total ghrelin (ghrelin)**

Ghrelin responses and AUCs are presented in figure 2. Ghrelin concentrations decreased
after both the saline infusion (-18%) and the GLP-1 infusion (-15%), reaching lowest
values at 60 and 120 minutes, respectively. The ghrelin responses showed an overall
interaction between time X treatment (P<0.0001). Partial tests showed that the ghrelin
responses after GLP-1 and saline were different (P<0.05). The AUC’s 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 minutes after the saline infusion
as compared to 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 minutes, respectively (figure 2). There was no overall treatment effect.

Glucose

Serum glucose responses and AUCs are presented in figure 2. Glucose concentrations increased about 24% after the saline infusion, reaching peak values at 30 minutes. In contrast, during GLP-1 infusion, glucose concentrations decreased by about 11%. The glucose responses showed an overall interaction between time X 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 (about 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 as compared to saline infusion (2.5 fold increase compared to 8 fold increase after saline). ANOVA for repeated measures showed an overall interaction between time X 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 (about 45%) after the GLP-1 infusion as compared to the saline infusion (P<0.0001).

Glucagon

Glucagon concentrations increased about 31% after the saline infusion, and reached peak values at 30 minutes, but were hardly affected by the GLP-1 infusion (+ 8%) (figure 2).
The glucagon responses showed an overall time X treatment effect (p<0.001). Partial tests showed that the glucagon responses were different between the two treatments (P<0.05). The AUC of glucagon was smaller (about 67%) after the GLP-1 infusion than after the saline infusion (P<0.05).

Glucose-dependent insulinotropic polypeptide

Plasma GIP responses and AUCs are presented in figure 2. GIP concentrations increased about 150% after the saline infusion and about 100% after the GLP-1 infusion, reaching peak values at 30 and 90 minutes, respectively. The GIP responses showed an overall time X treatment interaction (P<0.0001). Partial tests showed that also 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 minutes (P<0.05).

Cholecystokinin

CCK concentrations increased about 3 fold after the saline infusion reaching peak values at 15 minutes (figure 2). CCK concentrations showed a biphasic response following GLP-1 infusion. Initially CCK concentrations increased about 4.5 fold, but then dropped at 30 minutes, followed by a steady increase, reaching peak values at 90 minutes (6.5 fold increase compared to baseline values). The CCK responses showed an overall time X 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 (about 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 with gastric emptying.

Acetaminophen concentrations were inversely correlated with total ghrelin concentrations both during saline infusion (r = -0.76; 95% C.I. = -0.90, -0.49) and during GLP-1 infusion (r = -0.47; 95% C.I. = -0.76, -0.04) (see table 3). Also GIP concentrations were positively associated with acetaminophen concentrations (saline: r = 0.54; 95% C.I. = 0.13, 0.79; GLP-1: r = 0.59; 95% C.I. = 0.19, 0.82). CCK concentrations were only positively associated with acetaminophen concentrations during GLP-1 infusion (r = 0.55; 95% C.I. = 0.14, 0.80), but not during infusion of saline (r = 0.36; 95% C.I. = -0.10, 0.69). In contrast, insulin concentrations were not correlated at all with acetaminophen concentrations (saline: r = 0.15; 95% C.I. = -0.32, 0.55, GLP-1: r = 0.12; 95% C.I. = -0.34, 0.54). There was no association between acetaminophen and active ghrelin concentrations, as well (saline: r = -0.10; 95% C.I. = -0.52, 0.35, GLP-1: r = -0.08; 95% C.I. = -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% C.I. = 0.15, 0.80), but not during saline infusion (r = 0.35; 95% C.I. = -0.11, 0.69). Total ghrelin concentrations were strongly
inversely correlated with concentrations of the insulinotropic peptide GIP (saline: \( r = -0.74; 95\% \text{ C.I.} = -0.89, -0.45; \) GLP-1: \( r = -0.63; 95\% \text{ C.I.} = -0.84, -0.27 \)) and were also inversely correlated with concentrations of CCK (saline: \( r = -0.54; 95\% \text{ C.I.} = -0.80, -0.13; \) GLP-1: \( r = -0.50; 95\% \text{ C.I.} = -0.77, -0.08 \)). Ghrelin concentrations were also inversely associated with concentrations of the other insulinotropic peptide, GLP-1, during GLP-1 infusion (\( r = -0.58; 95\% \text{ C.I.} = -0.81, -0.18 \)), but this association was not present during infusion of saline (\( r = -0.16; 95\% \text{ C.I.} = -0.56, 0.31 \)). Conversely, glucagon concentrations were inversely associated with ghrelin concentrations during saline infusion \( r = -0.52; 95\% \text{ C.I.} = -0.78, -0.10 \), but not after infusion of GLP-1 (\( r = -0.16; 95\% \text{ C.I.} = -0.56, 0.30 \)). In contrast with our hypothesis, total ghrelin concentrations were not associated with insulin concentrations (saline: \( r = -0.36; 95\% \text{ C.I.} = -0.69, 0.09; \) GLP-1: \( r = -0.42; 95\% \text{ C.I.} = -0.73, 0.03 \)). There were no associations between active ghrelin concentrations and other physiological parameters than total ghrelin (see table 3).

**Discussion**

Animal studies show that distension and chemo sensitization of the stomach are insufficient to induce a ghrelin response (33), suggesting that post gastric feedback is required. In this study, we investigated the association between gastric emptying rate and the postprandial ghrelin response, and whether insulin or other post gastric 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 post gastric 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 association between the different measures were investigated by correlational analysis. Although correlations do provide more insight into the associations between different measures, 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 post gastric feedback. However, ghrelin concentrations were only significantly higher between 90 and 120 minutes, despite 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 post-gastric feedback for postprandial ghrelin secretion.

In the second hypothesis, we tested whether insulin is the post gastric 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 few studies investigated the relation between GIP and ghrelin secretion and showed contradictive results (1; 27). The strong inverse association between GIP and ghrelin concentrations, which were observed in this study, suggests that GIP, instead of insulin, might act as the post gastric feedback signal for the postprandial ghrelin response. There were also indications for a role of CCK herein. These results are
however correlative and do therefore not prove a causal role of 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 a 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 also unacylated (desacyl) ghrelin 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 as well as 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.

**Conclusions**

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 post gastric 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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**Disclosures**

This study is also partially financially supported by Danone Vitapole.

None of the authors had a conflict of interest.
Reference List


17. **Lucidi P, Murdolo G, Di Loreto C, De Cicco A, Parlanti N, Fanelli C, Santeusanio F, Bolli GB and De Feo P.** Ghrelin is not necessary for adequate


Legend

Figure 1: Three hour postprandial responses of acetaminophen and GLP-1 (total) (n=15) after infusion of saline or GLP-1 (active). -☐-: saline, -♦-: GLP-1. By ANOVA, there was a significant time X treatment effect for acetaminophen (P<0.0001) and GLP-1 (P<0.001). The different letters indicate the level of statistical significance: a: P<0.01, b: P<0.001, c: P<0.0001. Inserted graphs: mean ± SD 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).

Figure 2: Three hour postprandial responses of 1) ghrelin (total), 2) ghrelin (active), 3) glucose, 4) insulin, 5) glucagon, 6) GIP and 7) CCK-8 in 15 men after infusion of saline or GLP-1 (active). -☐-: saline, -♦-: GLP-1. By ANOVA, there was a significant time X treatment effect for ghrelin total, insulin, glucose, glucagon, GIP (all P<0.0001) and CCK (P<0.01). The different letters indicate the level of statistical significance: a: P<0.10, b: P<0.05, c: P<0.01, d: P<0.001, e: P<0.0001. Inserted graphs: mean ± SD AUC of the responses. By ANOVA, there was a significant treatment effect of the AUCs for insulin (P<0.0001), glucose (P<0.001), CCK and glucagon (both P<0.05).
Figure 1

Acetaminophen

GLP-1 total
Figure 2

Ghrelin (total)

Ghrelin (active)

Glucose

Insulin

Glucagon

GIP

CCK-8

Ghrelin: total AUC (ng * h /L)

Ghrelin active: AUCt (ng *h /L)

CCK: positive AUC (pmol/L)

GIP: positive AUC (pg/mL)

Glucose: positive AUC (mmol/L)

Insulin: positive AUC (mU/L)

Glucagon: positive AUC (pg/mL)

CCK-8: positive AUC (pg/mL)
Table 1 Subject characteristics (n=15)\(^1\)

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<th>Mean ± SD</th>
<th>Range</th>
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<td>1.3 ± 0.4</td>
<td>1.0 - 2.3</td>
</tr>
</tbody>
</table>

\(^1\) Values represent measurements taken at the beginning of the study

\(^2\) Score on the restrained eating scale of the Dutch Eating Behavior Questionnaire. Range possible scores on the restrained eating scale: 1.0 - 5.0
Table 2 Energy and macronutrient composition of the breakfast

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>400</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>1628</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>18.8</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>46.0</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>14.4</td>
</tr>
<tr>
<td>Protein (En%)</td>
<td>19.3</td>
</tr>
<tr>
<td>Carbohydrate (En%)</td>
<td>47.3</td>
</tr>
<tr>
<td>Fat (En%)</td>
<td>33.3</td>
</tr>
</tbody>
</table>
**Table 3:** Mean correlation coefficient (r) with 95% confidence intervals of the relation between physiological parameters by treatment (n=15)

<table>
<thead>
<tr>
<th></th>
<th>Saline ghrelin total</th>
<th>Saline GIP</th>
<th>GLP-1 ghrelin total</th>
<th>GLP-1 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>

The Pearson correlation coefficients of the relation between ghrelin and other physiological parameters were calculated per subject. The mean correlation coefficients together with the 95% confidence intervals after Fisher Z-transformation are presented.