

Ghrelin Stimulates Gastric Emptying and Hunger in Normal-Weight Humans

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Context: Ghrelin is produced primarily by enteroendocrine cells in the gastric mucosa and increases gastric emptying in patients with gastroparesis.

Main Objective: The objective of the study was to evaluate the effect of ghrelin on gastric emptying, appetite, and postprandial hormone secretion in normal volunteers.

Design: This was a randomized, double-blind, crossover study.

Subjects: Subjects included normal human volunteers and patients with GH deficiency.

Intervention: Intervention included saline or ghrelin (10 pmol/kg-min) infusion for 180 min after intake of a radioactively labeled omelette (310 kcal) or GH substitution in GH-deficient patients.

Main Outcome Measures: Measures consisted of gastric empty-

ing parameters and postprandial plasma levels of ghrelin, cholecystokinin, glucagon-like peptide-1, peptide YY, and motilin.

Results: The emptying rate was significantly faster for ghrelin ($1.26 \pm 0.1\%$ per minute), compared with saline (0.83% per minute) ($P < 0.001$). The lag phase (16.2 ± 2.2 and 26.5 ± 3.8 min) and half-emptying time (49.4 ± 3.9 and 75.6 ± 4.9 min) of solid gastric emptying were shorter during ghrelin infusion, compared with infusion of saline ($P < 0.001$). The postprandial peak in plasma concentration for cholecystokinin and glucagon-like peptide-1 occurred earlier and was higher during ghrelin infusion. There was no significant effect of ghrelin on plasma motilin or peptide YY. There was no difference in gastric emptying before and after GH substitution.

Conclusion: Our results demonstrate that ghrelin increases the gastric emptying rate in normal humans. The effect does not seem to be mediated via GH or motilin but may be mediated by the vagal nerve or directly on ghrelin receptors in the stomach. Ghrelin receptor agonists may have a role as prokinetic agents. (*J Clin Endocrinol Metab* 91: 3296–3302, 2006)

GHRELIN WAS DISCOVERED as the endogenous ligand to the orphan G protein-coupled GH secretagogue receptor (GHS-R) and demonstrated to specifically stimulate GH release from rat pituitary cells *in vitro* as well as *in vivo* (1, 2). From the GHS-R gene, two mRNAs are produced, which are translated into two distinct proteins that have been named GHS-R1a and GHS-R1b. The GHS-R1a, now named the ghrelin receptor, is a typical G protein-coupled seven-transmembrane domain receptor. The GHS-R1b is a truncated receptor with only five transmembrane domains and is pharmacologically inactive (3). *In situ* hybridization indicated that ghrelin was elaborated in and released from enteroendocrine X/A-like cells in the gastric mucosa (4) to circulate in human blood at a considerable concentration (1). However, in addition, ghrelin-containing neural cells are localized in the arcuate nucleus of the hypothalamus, which is a well-known center for feeding reg-

ulation, suggesting involvement in the regulation of feeding (5, 6).

Peripheral administration of ghrelin causes weight gain by reducing fat use and stimulating food intake in rats (2), and serum ghrelin concentrations are increased by fasting and reduced by refeeding in rats and humans (7). Serum ghrelin rises sharply before and falls within 1 h of a meal (8). Several studies on rats and humans confirm that ghrelin initiates food intake (9, 10), and circulating ghrelin levels are increased by up to 3-fold in states of negative energy balance, such as anorexia nervosa, starvation, cachexia, and also after weight loss in obesity (11) and are, conversely, decreased in conditions such as obesity, hyperglycemia, and feeding (12, 13), suggesting that ghrelin plays a central role in the short- and long-term energy homeostasis (14).

Additional data indicate that ghrelin also plays a role in the regulation of gastrointestinal motility and acid secretion. Thus, *iv* administration of ghrelin stimulates gastric motility as well as acid secretion in rats, and the effect is abolished by pretreatment with atropine or bilateral cervical vagotomy (15–18). Several studies also show a significant acceleration of solid gastric emptying in rats (18–20) and mice (21, 22). However, the results in dogs are not unequivocal as stimulation (23) and recently lack of effect have been reported (24). Because gastric emptying rate and the sensation of hunger

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Abbreviations: CCK, Cholecystokinin; GHD, GH-deficient; GHS-R, GH secretagogue receptor; GLP, glucagon-like peptide; NPY, neuropeptide Y; PYY, peptide YY; T_{50} , half-emptying time; VAS, visual analog scores.

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usually are correlated in humans (25), one could expect that ghrelin increases gastric emptying in humans. Previous data have shown that ghrelin infused iv at a dose of 5 pmol/kg·min did not alter the rate of gastric emptying using an acetaminophen test in normal human volunteers (10). However, iv ghrelin was recently shown to accelerate gastric emptying in patients suffering from gastroparesis (26–29).

The main objective of this study was to study the effect of ghrelin on gastric emptying in normal humans. In addition, the effects of ghrelin on hunger, satiety, and the gut hormones cholecystokinin (CCK), glucagon-like peptide (GLP)-1, peptide YY (PYY), and motilin as well as ghrelin were studied. A secondary aim was to study gastric emptying in a group of GH-deficient (GHD) patients before and after 6 months of GH substitution to assess whether GH substitution alters gastric emptying in light of the fact that ghrelin increases plasma GH concentrations. The distribution of GHS-R was studied in different regions of the human stomach because an effect of ghrelin on gastric emptying may be mediated by ghrelin receptors in the stomach.

Subjects and Methods

Subjects

Solid scintigraphic gastric emptying and plasma concentrations of gut peptides were studied in eight healthy, nonsmoking volunteers (five men, three women, 26.5 ± 1.6 yr), with a mean body mass index of 24.0 ± 1.1 kg/m².

Solid scintigraphic gastric emptying was also studied in six GHD patients (three men, 58.3 ± 3.0 yr) before and after 6 months of GH substitution therapy. All patients had GHD of adult onset. They all had a medical history of pituitary adenomas treated by transphenoidal surgery and/or pituitary irradiation. The diagnosis of GHD was confirmed by provocation test (insulin-induced hypoglycemia or arginine test) with a low GH response (<3 mg/liter). The GH substitution dose used increased the serum IGF-I values to levels within the age-matched range.

RT-PCR for ghrelin and the two subtypes of the ghrelin receptor was performed in human stomach (fundus, n = 3; corpus, n = 3; antrum, n = 6) obtained from patients undergoing gastric resection for cancer (n = 6).

The study protocol was approved by the Ethics Committee of the Karolinska Institutet North, and all subjects gave written informed consent.

Solid gastric emptying

The study was performed in a randomized, double-blind, placebo-controlled crossover fashion on two occasions with a washout interval of at least 1 wk. The subjects were studied after an overnight fast at 0800 h in the morning. An indwelling catheter was placed in each antecubital vein for administration of ghrelin and plasma sampling. The scintigraphic gastric emptying test of a solid meal has been described in detail elsewhere (30). In short, concomitantly with the intake of a 310-kcal omelette labeled with 12–15 MBq ^{99m}Tc-macroaggregated albumin (Pulmonate plc; Amersham International, Little Chalfont, UK) and a glass of fruit punch, either saline or ghrelin (10 pmol/kg⁻¹·min⁻¹; NeoMPS, Strasbourg, France) dissolved in 0.9% saline containing 1% albumin (Albumin Kabi, 200 g/liter⁻¹; Kabi, Stockholm, Sweden), subjected to sterile filtration, and stored at -70 C until use, was started in one of the iv catheters and continued for 180 min.

Anterior and posterior 1-min acquisitions were performed with the subject in standing position. Acquisitions were then obtained every 5 min during the first 50 min and thereafter every 10 min during 70 min and finally one acquisition at 180 min. Imaging data were collected using a γ -camera (General Electric Maxicamera 400 T; General Electric, Milwaukee, WI). The following parameters were calculated: lag phase, defined as the time period from termination of the meal until 90% radioactivity remained in the stomach; gastric emptying rate, defined as

percentage of radioactivity decreasing per minute during the linear slope after termination of the lag phase; and half-emptying time (T₅₀), defined as the time for 50% emptying of gastric radioactivity after termination of the meal. Time 0 was defined as the time of the first acquisition (10 min after beginning the meal and the infusion of ghrelin or saline).

The GH-deficient subjects were studied with the same protocol before and after 6 months of GH substitution with the exception that only saline was administered and no blood samples were obtained.

RIA for ghrelin, GLP-1, PYY, CCK, and motilin

Blood samples were collected in prechilled EDTA tubes every 10 min from -20 until 60 min and at 90, 120, and 180 min for measurements of plasma concentrations of the different gut hormones. Samples were centrifuged at 4 C for 10 min at 3000 rpm. Plasma was collected and stored at -20 C until analysis in the same assay run.

Ghrelin (total) was measured with a commercially available RIA kit (Linco Research, St. Charles, MO), which was semiautomated and thereby standardized in the laboratory. A lyophilized ghrelin standard stock at 6 μ g/liter (no. 8089-K) was used from which dilutions were made. The detection limit of the assay was 27 pmol/liter (100 μ l sample) and the coefficient of variation was 10% (300 pmol/liter) and 4.4 (900 pmol/liter). No cross-reactivity to ghrelin 1–10, motilin-related peptide, glucagon, GLP-1 (7–36), human leptin, or human insulin has been detected.

The plasma concentrations of GLP-1 were measured (31) against standards of synthetic GLP-1 (7–36) amide using antiserum code no. 89390, which is specific for the amidated C terminus of GLP-1 and therefore mainly reacts with GLP-1 of intestinal origin. The assay reacts equally with intact GLP-1 and GLP-1 (3–36) amide, the primary metabolite. Because of the rapid and intravascular conversion of GLP-1 to its primary metabolites, it is essential to determine both the intact hormone and metabolite for estimation of its rate of secretion. Sensitivity was less than 1 pmol/liter, intraassay coefficient of variation less than 6% at 20 pmol/liter, and recovery of standard, added to plasma before extraction, about 100% when corrected for losses inherent in the plasma extraction procedure (31).

RIAs of PYY in plasma were performed using antiserum code no. 8412-2II (32). It reacts equally with PYY1–36 and PYY3–36. Synthetic human PYY 1–36 (Peninsula Laboratories, St. Helens, UK) was used for standards. ¹²⁵I-PYY1–36 (code no. IM259) was from Amersham Biosciences (Buckinghamshire, UK). Assay buffer was 0.05 mol/liter sodium phosphate (pH 7.5), containing in addition 400 KIE/ml Trasylol-aprotinin, 0.1 mol/liter sodium chloride, 10 mmol/liter EDTA, 0.6 mmol/liter merthiolate; 150 μ l unknown plasma samples + 150 μ l assay buffer or 150 μ l charcoal-treated plasma + 150 μ l standards were preincubated with antiserum, 100 μ l, diluted 1:20,000 (final concentration) for 48 h at 4 C. Then 100 μ l tracer (5 fmol, specific activity 70 MBq/nmol) were added and the mixture incubated for 24 h before bound and free peptide moieties were separated by plasma-coated charcoal (33). Detection limit of the assay was less than 2.5 pmol/liter, and 50% inhibition was obtained with 23 pmol/liter PYY. Recovery of PYY added to plasma in concentrations between 5 and 50 pmol/liter deviated less than 15% from expected values. Intraassay coefficient of variation was less than 5%. The antiserum showed no cross-reaction with human neuropeptide Y (NPY) or human pancreatic polypeptide in concentrations up to 500 pmol/liter.

CCK was analyzed as previously described (34). Briefly, CCK was assayed using an antibody (92128) raised in rabbits against an O-sulfated human CCK-12 analog. Antibody 92128 binds all the bioactive forms of CCK with equimolar potency and displays no reactivity to gastrin. The tracer used was the Bolton-Hunter-labeled sulfated CCK-8 (¹²⁵I-CCK-8). Separation of antibody-bound and free tracer was achieved by using plasma-coated charcoal. The detection limit of the assay was 0.1 pmol/liter. The intraassay variation at different concentrations ranged between 5 and 15%.

Plasma concentrations of motilin were determined on EDTA plasma extracted with ethanol [70% (vol/vol), final concentration] against standards of human motilin as previously described (35). The antibody (code no. 8422) was raised against porcine motilin. ¹²⁵I-motilin was purchased from Phoenix Europe GmbH (Karlsruhe, Germany). Detection limit was 2 pmol/liter, and recovery of motilin added to plasma deviated less than

15% from expected values after correction for the recovery of ethanol extraction, which amounted to $70 \pm 6\%$ (mean \pm SD). Intraassay coefficient of variation was less than 10% (including extraction).

Determination of ghrelin and GHS-R distribution in human stomach

During surgery segments from corpus and antrum were quickly removed and placed in RNA later. About 50 mg of each tissue was homogenized and total RNA purified using RNeasy minikit and RNase-free DNase set (QIAGEN, Hilden, Germany). The quality and concentration of the RNA was controlled by 1% agarose-gel electrophoresis and spectrophotometry ($\lambda 260:\lambda 280$), respectively. Total RNA was reverse transcribed to cDNA. Total RNA (1 μ g) and random primer (0.25 μ g) were dissolved in 10 μ l sterile water. After incubation for 10 min at 70 C, the volume was increased to 20 μ l with reaction mix (50 mM Tris-HCl, 75 mM KCl, 3 mM MgCl₂, 10 mM dithiothreitol, 0.5 M deoxynucleotide triphosphate, 60 U RNase inhibitor, and 300 U Superscript II; Invitrogen Ltd., Paisley, UK) and incubated for 1 h at 37 C. For amplification, 2 μ l of the resulting reverse transcription reaction was increased to 20 μ l with distilled water, 0.5 pmol of forward and reverse primers, 1.5 mM MgCl₂, 0.2 mM deoxynucleotide triphosphates, 2 μ l 10 \times PCR buffer, and 0.5 U Taq DNA polymerase (QIAGEN). The thermal profile and primers were selected from Carraro *et al.* (53) or with the software Primer-3 (Whitehead Institute, Massachusetts Institute of Technology Center for Genome Research, Boston, MA). In a thermal cycler (Eppendorf, Hamburg, Germany) after an initial step at 95 C for 3 min, we used a denaturation step at 95 C for 30 sec, an annealing step for 30 sec, and an extension step at 72 C for 30 sec for a total of 40 cycles. An additional extension step at 72 C for 5 min was then performed. Primer sequences, annealing temperature, and size of PCR products are given in Table 1. Detection of the PCR amplification products was carried out by size fractionation on 2% agarose gel electrophoresis.

Hunger and satiety

Measures of hunger, desire to eat, fullness, and prospective food consumption were assessed with visual analog scores (VAS) at -20, 10, 30, 60, 120, and 180 min in the healthy volunteers.

Statistics

Data are shown as mean \pm SEM. Data were analyzed with the Wilcoxon signed rank test for matched pairs [gastric emptying data (emptying rate, T₅₀, and lag phase) and VAS], and ANOVA for repeated measurements was used to analyze the data with time (12 time points) and treatment (saline or ghrelin) as dependent factors (peptide data and gastric emptying plot). All tests were two sided, and $P < 0.05$ was considered as statistically significant. Planned comparisons were used to identify differences between conditions at specific time points when a significant interaction effect was found for each peptide. With regard to the VAS, data changes in intermeal (10–180 min) rating were compared.

Results

Gastric emptying normal human volunteers

Infusion of ghrelin resulted in a marked increase in the emptying rate (Fig. 1) (1.26 ± 0.1 and $0.83 \pm 0.04\%$ per

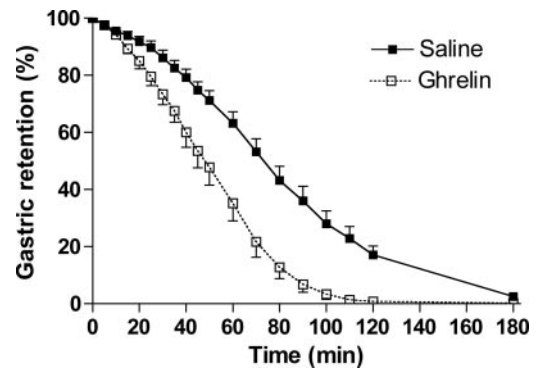


FIG. 1. Scintigraphic gastric emptying of a solid meal (310 kcal) in eight healthy volunteers during infusion of saline or ghrelin (10 pmol/kg-min) for 180 min. Mean \pm SEM, $P < 0.001$, ANOVA repeated measures time, treatment, and time \times treatment.

minute), compared with saline ($P < 0.001$). Both the lag phase (16.2 ± 2.2 and 26.5 ± 3.8 min) and T₅₀ (49.4 ± 3.9 and 75.6 ± 4.9 min) of solid gastric emptying were shorter during ghrelin infusion, compared with infusion of saline ($P < 0.001$).

Gastric emptying after GH substitution

The rate of gastric emptying (0.81 ± 1.13 and $0.79 \pm 0.07\%$ per minute), lag phase (21.5 ± 3.4 and 20.9 ± 4.1 min), and T₅₀ (77.2 ± 7.4 and 74.3 ± 5.0 min) did not differ significantly before and after GH substitution in the GHD subjects.

Plasma concentrations of ghrelin, GLP-1, PYY, CCK, and motilin

During infusion of ghrelin, plasma concentrations increased 5-fold from a preinfusion concentration of 300–1500 pmol/liter (time, treatment, and time \times treatment interaction effect was $P < 0.001$, respectively; Fig. 2).

Plasma concentrations of GLP-1 [time ($P < 0.001$), treatment ($P = 0.02$), and time \times treatment interaction effect was $P < 0.001$] and CCK [time ($P < 0.001$), treatment ($P = 0.2$), and time \times treatment interaction effect was $P < 0.001$] increased more rapidly after intake of the solid meal, and there was a greater total amount of each peptide secreted in the postprandial period during ghrelin infusion (Fig. 3). In contrast, motilin [time ($P = 0.1$), treatment ($P = 0.2$), and time \times treatment interaction effect was $P = 0.1$] and PYY [time ($P < 0.001$), treatment ($P < 0.01$), and time \times treatment interaction effect was $P = 0.3$] were not changed significantly by ghrelin infusion (Fig. 3).

TABLE 1. RT-PCR primers and PCR products

Primer	Sequence (5'–3')	Annealing temperature (C)	Product size (bp)
Ghrelin FORW	AGCCTCCTGCTCCTCGGCAT	62	339
Ghrelin REV	TGTGGGCGATCACTTGTCGGCT		
GHS-R1a FORW	CTGGACCTCGTTCGCCTCT	58	520
GHS-R1a REV	CAAACACCACTACAGCCAGCAT		
GHS-R1b FORW	TGGAGCACGAGAACGGCA	58	304
GHS-R1b REV	AGGCACAGGGAGAGGATAGGA		
GAPDH FORW	CCTTAAAGGGTCATCATCTCT	59	489
GAPDH REV	CCTGCTTCACCCTTCTTG		

FORW, Forward; REV, reverse.

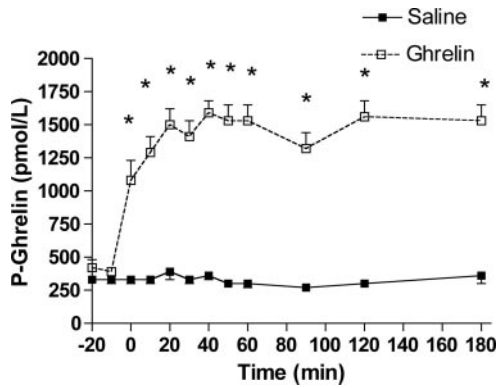


FIG. 2. Mean \pm SEM plasma concentrations of total ghrelin during infusion of saline or ghrelin (10 pmol/kg-min) after intake of a solid meal (310 kcal) in eight healthy human volunteers (time, treatment, and time \times treatment interaction effect all $P < 0.001$, ANOVA repeated measures, *, $P < 0.05$ for planned comparisons were used to identify differences between condition at specific time points when a significant interaction effect was found for each peptide).

Hunger and fullness ratings

As expected, hunger and desire to eat were significantly higher and fullness ratings lower during ghrelin infusion, compared with saline ($P < 0.05$ for all). Prospective food consumption tended to increase but did not reach significance ($P = 0.06$; Fig. 4).

Localization of ghrelin and GHS-R in the human stomach

Expression of the ghrelin gene was found in both the antrum and corpus of the stomach, and mRNA of the GHS-1a and GHS-1b was found in both the antrum and corpus (Fig. 5).

Discussion

This study shows a clear-cut and consistent stimulation of the gastric emptying of solids by ghrelin in healthy humans. Indeed, all subjects exhibited a clear increase in gastric emptying rate when given ghrelin iv. Similar findings have been made in experimental animals (19–21) and recently in patients with idiopathic and diabetic gastroparesis (26, 29) but never before in healthy humans. Although gastric T_{50} as measured by the noninvasive ^{13}C -octanoic acid breath test was proved to correlate with fasting plasma ghrelin levels in one study (7), fasting ghrelin concentrations were inversely correlated with gastric T_{50} in another study using the same breath test but a slightly different standardized meal (36). In a previous study on the effects of exogenous ghrelin in healthy subjects, there was no difference in comparison with placebo. This might be due to lower ghrelin concentrations. Thus, Wren *et al.* (10) infused 5.0 pmol/kg-min and evaluated gastric emptying with the paracetamol absorption test after a meal from a free choice buffet without any difference in gastric emptying *vs.* control. Another gastric-emptying study

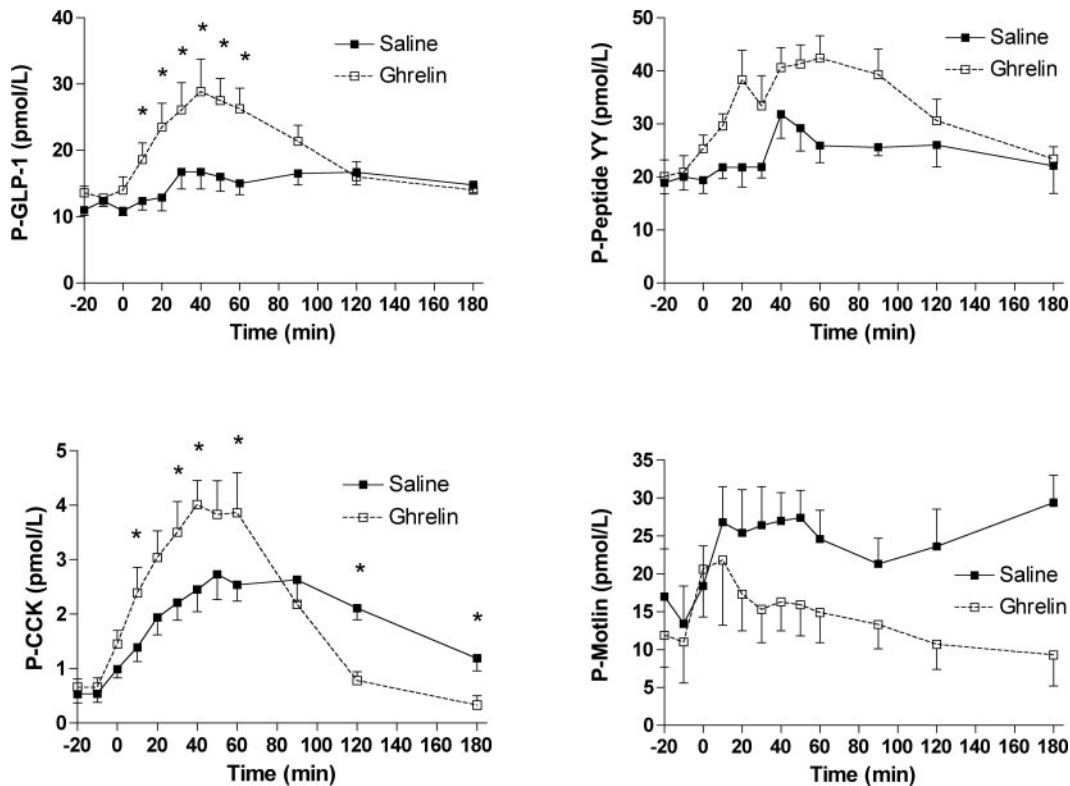


FIG. 3. Mean \pm SEM plasma concentrations of GLP-1 [time ($P < 0.001$), treatment ($P = 0.02$), time \times treatment ($P < 0.001$)], PYY [time ($P < 0.001$), treatment ($P < 0.01$), time \times treatment ($P = 0.3$)], CCK [time ($P < 0.001$), treatment ($P = 0.2$), time \times treatment ($P < 0.001$)], and motilin [time ($P = 0.1$), treatment ($P = 0.2$), time \times treatment ($P = 0.2$)] after intake of a solid meal (310 kcal) during saline or ghrelin (10 pmol/kg-min) infusion in healthy human volunteers. ANOVA repeated measures, *, $P < 0.05$ for planned comparisons were used to identify differences between condition at specific time points when a significant interaction effect was found for each peptide.

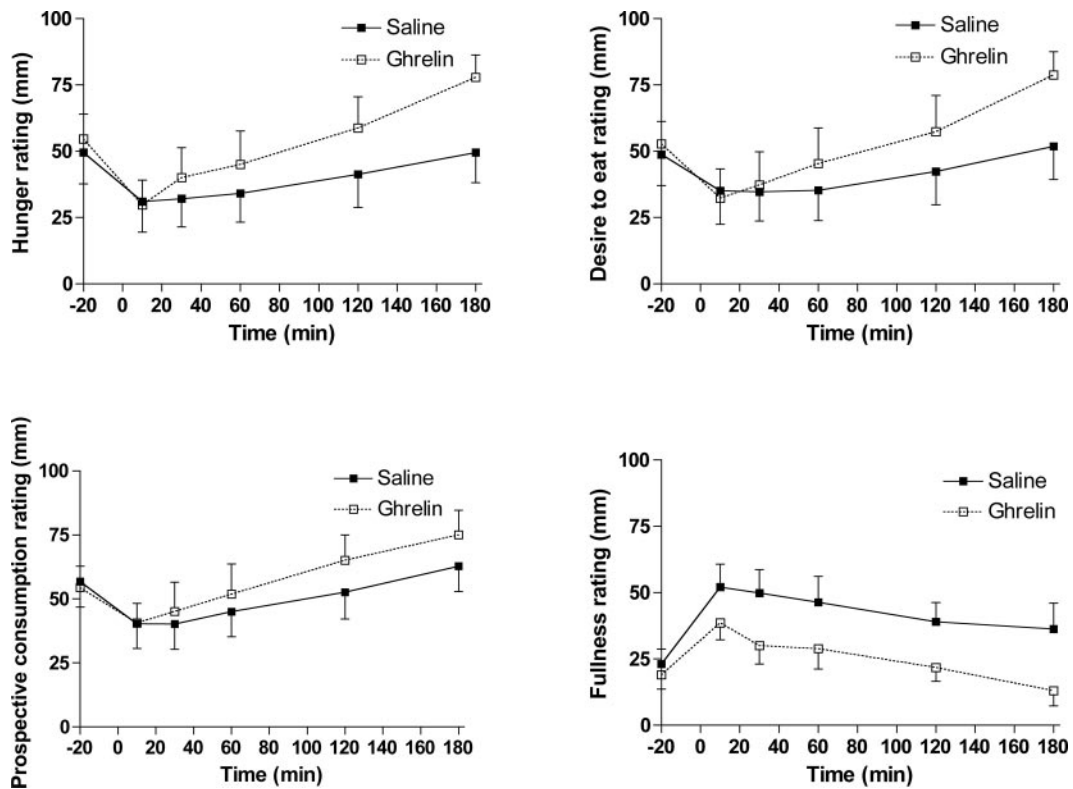


FIG. 4. Mean \pm SEM ratings for hunger, desire to eat, prospective consumption, and fullness in eight human volunteers during infusion of ghrelin (10 pmol/kg-min) or saline after eating a solid meal (310 kcal). $P < 0.05$ for change in rating between 10 and 180 min for all except prospective consumption ($P = 0.06$), Wilcoxon's signed rank test for matched pairs.

on dogs using the same method could not prove any difference in comparison with placebo; this might also be due to lower concentrations of ghrelin because only the highest concentration studied (10 μ g/kg) elicited a significant GH release (24). Moreover, the available techniques for assessing gastric emptying differ in their sensitivity in which scintigraphy has high reproducibility and is considered the golden standard (37).

For the time being, the therapeutic arsenal of gastrokinetic drugs is very limited. Metoclopramide, domperidone, cisapride, and erythromycin have all been studied, and the evidence for benefits is strongest for the latter two, although none of them is without problematic side effects (27, 38). Further evaluation of ghrelin as a prokinetic for the treatment of, for example, idiopathic and diabetic gastroparesis as well as functional dyspepsia, a condition characterized by de-

layed gastric emptying (39), would seem to be of great interest.

It is well known that ghrelin is a GH secretagogue (1, 4, 40, 41). Thus, one possible mechanism by which ghrelin could influence gastric emptying is by altering plasma GH concentrations. To test the hypothesis, we studied gastric emptying in GHD patients before and after GH substitution therapy. No significant effect was seen on any parameter of gastric emptying before and after substitution or when compared with the healthy volunteers, indicating that the ghrelin effect on gastric emptying is most likely not mediated by GH *per se*. However, this study design cannot explicitly exclude the possibility that the effect of ghrelin is mediated via the release of GH, as the normal gastric emptying seen in the GHD patients might be due to a compensation from other physiological mechanisms and tachyphylaxis might occur after prolonged GH substitution.

Another mechanism by which ghrelin could influence gastric emptying is by altering the secretion of gastrointestinal peptides known to influence gastrointestinal motility. One obvious candidate is motilin with which ghrelin also shares a structural homology (21). However, as consistent with previous findings (42), we found no elevation of motilin during ghrelin infusion. Conversely, GLP-1 and CCK demonstrated an earlier postprandial rise of their plasma concentrations, although PYY did not demonstrate significant interaction effect. In addition, an overall greater release during ghrelin infusion was seen for GLP-1 and CCK. The most likely mechanism behind this finding is the increased gastric emptying

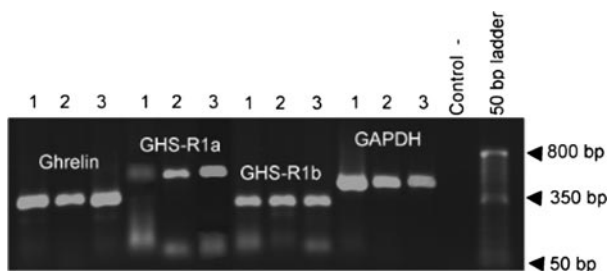


FIG. 5. Gene expression of ghrelin and the two subtypes of the GHS-R in the antrum (no. 1) and corpus (no. 2 and 3) of the stomach. GAPDH, Glyceraldehyde-3-phosphate dehydrogenase.

during ghrelin infusion, which results in an earlier entry of nutrients to the upper gut. These responses may be viewed as compensatory mechanisms serving to slow down transit of nutrients to facilitate uptake of nutrients because all three hormones are known to inhibit gastric emptying (30, 43). However, we cannot exclude a direct effect of ghrelin on the enteroendocrine cells producing these peptides or vagal afferent loops.

Previous studies have demonstrated by PCR that the GHS-1b receptor is present in the fundus of the stomach in man (44). We extend this observation to include the corpus and antrum of the stomach. These findings are supported by immunohistochemical studies of ghrelin receptor distribution (45) and the demonstration of ghrelin receptors in the enteric nervous system. Thus, it is possible that the effect of ghrelin on gastric emptying is a direct one on the stomach itself. Previous studies demonstrated that there are ghrelin receptors on the afferent vagus nerve (46), suggesting a third mechanism by which ghrelin can influence gastric emptying by altering signaling to the motor centers of the brain stem.

Consistent with previous findings, ghrelin significantly increased the appetite as measured by hunger, desire to eat, and prospective food consumption and significantly decreased satiety (2, 9, 10). Among the many gastrointestinal peptides, ghrelin is the only known appetite-stimulating hormone (47), and ghrelin is more potent than any of the other orexigenic peptides except NPY (48). Ghrelin given intracerebroventricularly exhibits gastroduodenal activity and potent orexigenic activity through an action on the hypothalamic NPY and Y₁ receptor, an effect that was lost after vagotomy in mice (21). NPY is an orexigenic neuropeptide that is abundant in the arcuate nucleus, a part of the lateral hypothalamus known for its involvement in the regulation of food intake.

Because ghrelin is produced mainly from the gut and it reaches ghrelin receptors in the anterior pituitary and potentially in the mediobasal and mediolateral hypothalamus through the general circulation to stimulate GH release and regulate energy homeostasis (49), ghrelin seems to be a physiological peripheral regulator of feeding behavior (50). Some ghrelin is produced also in the hypothalamus, however, suggesting that the brain GHS-R may be a target for locally produced ghrelin. Taken together, although a correlation between gastric emptying rate and sensation of hunger in humans is described (25), the orexigenic properties of ghrelin seem to be a result of the stimulation of NPY release in the hypothalamus, whereas the motility stimulating properties is rather a vagally mediated effect on the myenteric plexus. In previous studies, less consistent results regarding hunger and satiety have been obtained, and this could be due to lower infusion rates of ghrelin. For example, Arvat and co-workers as well as and Broglio *et al.* (41, 51, 52) infused 1.0 μg/kg as bolus doses, which corresponds to a dose of 300 pmol/kg, a dose that consistently yielded GH peaks within a physiological range. In our study, all of the subjects consistently scored higher for hunger, desire to eat, and prospective food consumption and less for satiety when given ghrelin. The most likely explanation seems to be somewhat higher infusion rate, 10 pmol/kg·min. No side effects or adverse events were observed during this study, in agree-

ment with previous trials in which ghrelin was reported to be well tolerated.

In conclusion, our study demonstrates that iv administration of ghrelin stimulates gastric emptying in normal human volunteers. This effect is likely direct and does not seem to be mediated via GH or motilin. The postprandial peak in plasma concentrations of CCK and GLP-1 is potentiated by ghrelin, possibly as a consequence to enhanced gastric emptying rate. Ghrelin receptor agonists may come to have a role as prokinetic agents.

Acknowledgments

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