Reciprocal changes in endogenous ghrelin and growth hormone during fasting in healthy women

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Koutkia, Polyxeni, Sunita Schurgin, Jacqueline Berry, Jeff Breu, B. S. Hang Lee, Anne Klibanski, and Steven Grinspoon. Reciprocal changes in endogenous ghrelin and growth hormone during fasting in healthy women. Am J Physiol Endocrinol Metab 289: E814–E822, 2005. First published June 21, 2005; doi:10.1152/ajpendo.00093.2005.—Ghrelin stimulates growth hormone (GH) secretion, but it is unknown whether there is a feedback of GH on ghrelin secretion. In this study, we characterized the relatedness of GH and ghrelin in a model of acute caloric deprivation in 10 healthy women (age 26.7 ± 1.6 yr) during a 4-day fast in the early follicular phase. GH, ghrelin, and cortisol were assessed every hour over 24 h during an isocaloric diet and after a 4-day complete fast. Sampling during a normal diet at baseline demonstrated that ghrelin decreased 17.9% within 1 h after meals (P < 0.0001), but there was no meal effect on GH. BMI (22.3 ± 0.4 vs. 21.5 ± 0.4 kg/m2, P < 0.0001) and IGF-I (312 ± 28 vs. 124 ± 22 ng/ml, P < 0.0001) decreased during fasting. Mean 24-h GH increased (2.6 ± 0.5 vs. 5.6 ± 0.5 ng/ml, P < 0.001), but ghrelin decreased (441.3 ± 59.7 vs. 359.8 ± 54.2 pg/ml, P = 0.012). The peak ghrelin level decreased from 483.5 to 375.6 pg/ml (P < 0.0001), and the time of the peak ghrelin changed from 0415 to 1715. In contrast, the diurnal pattern of GH was maintained, with increases in the nadir (1.1 to 3.4 ng/ml) and peak GH concentrations (4.1 to 7.9 ng/ml) from the fed to fasted state (P < 0.0001). The change in morning GH concentrations was inversely related to the change in ghrelin (r = -0.79, P = 0.012). During complete short-term caloric deprivation in healthy women, ghrelin decreases, even as GH rises, and these processes appear to be reciprocal, suggesting that GH exhibits feedback inhibition on ghrelin. Our data provide new evidence of the physiological relationship of GH and ghrelin in response to changes in protein-energy metabolism.

GHRELIN IS AN ENDogenous LIGAND for the growth hormone (GH) secretagogue receptor (18). Ghrelin levels are significantly lower in obese than in lean subjects (5, 13, 33). Prior studies have reported that ghrelin increases preprandially (4) and in response to chronic caloric deprivation in patients with anorexia nervosa (1, 25) and is suppressed by food intake (4). In a previous study (19), we demonstrated ghrelin pulsatility and relatedness to GH during fasting in healthy men. Feedback inhibition of GH on ghrelin was suggested in response to testing with growth hormone-releasing hormone (GHRH)/arginine (19). In addition to potential effects of arginine and somatostatin on ghrelin, another explanation of our prior data is that increasing GH during arginine/GHRH testing contributes to decreased ghrelin by feedback inhibition. GH and ghrelin exhibited mirror image responses during GH stimulation testing in our prior paradigm, suggesting a classical neuroendocrine feedback loop (19). Coordinated regulation of ghrelin and GH may be an important component in nutrient signaling to the brain but has not previously been assessed. In this study, we sought to characterize the relatedness of GH and ghrelin in a model of acute caloric deprivation in women, recognizing that the physiology in men and women with respect to ghrelin may differ and that prior conclusions regarding ghrelin physiology in men cannot necessarily be extrapolated to women. We evaluated the 24-h secretion of ghrelin and GH during normal feeding and again after fasting, using cosinor analysis and regression modeling. We also evaluated the relatedness of 24-h cortisol secretion to ghrelin and investigated changes in ghrelin among healthy young women randomized to short-term prednisone and GH. Our study suggests reciprocal changes in GH and ghrelin during acute caloric deprivation and reductions in ghrelin during GH administration, providing new data on the physiological regulation of these hormones in response to changes in protein-energy metabolism in women.

METHODS

Study A

Normal-weight, healthy female subjects had participated in a prior study of leptin administration during short-term fasting (28). In that prior study, no effects on leptin or ghrelin were seen, and the relationship of ghrelin and GH was not assessed. In the current study, we sought to characterize the relatedness of GH and ghrelin during acute caloric deprivation in the subset of 10 patients not receiving leptin. Eligible subjects were eumenorrheic women between 18 and 35 yr of age with a BMI between 20.0–26.0 kg/m2 and normal puberty and development. All subjects underwent pregnancy testing at screening and immediately before the start of the protocol. Subjects with current or prior history of medical problems known to affect thyroid, GH, reproductive, or adrenal function were excluded. Subjects with a prior history of eating disorder, hypothalamic amenorrhea, or menstrual irregularities were excluded. Other exclusion criteria included abnormal creatinine, FSH, TSH, or prolactin levels, or hemoglobin of ≤12.0 g/dl. Participants were studied in the early to midfollicular phase (cycle days 1–8) of their menstrual cycle. All subjects gave written informed consent, and the study was approved by the Human Research Committee of the Massachusetts General Hospital and the Committee on the Research of Human Subjects at the Massachusetts Institute of Technology.

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Study B

In a second protocol, 18 normal-weight, healthy females participated in a study to determine the effects of exogenous GH and prednisone on ghrelin. Participants received prednisone (30 mg po bid) and were simultaneously randomized to receive GH (20 µg·kg⁻¹·day⁻¹) vs. placebo, for 5 days. Subjects were eumenorrheic, between 20 and 40 yr of age, and had normal puberty and development. None of the subjects participating in study B had participated in study A. All subjects underwent pregnancy testing immediately before the start of the protocol. Subjects with diabetes mellitus, alcoholism, or hypertension or heart, renal, or liver disease were excluded from the study. Subjects with a history of any disease or medication known to affect GH were also excluded. Other exclusion criteria included active peptic ulcer disease, acute superficial herpes simplex infection, depression, psychosis, use of anticoagulants, and extensive physical training. Participants were studied in the early follicular phase of their menstrual cycle. The study was approved by the Human Research Committee of the Massachusetts General Hospital, and all subjects gave written informed consent.

Study Design

Study A. Before baseline testing, calorie, protein, and fat intakes were determined from 4-day outpatient food records (Minnesota Nutrition Data Systems, version 8A/2.6, Minneapolis, MN). Subjects returned for baseline testing after an overnight fast, and weight and body composition were determined. Fasting blood was drawn for IGF-I, glucose, leptin, ghrelin, and insulin. Subjects were then maintained for 24 h on an isocaloric diet with similar proportions of protein, fat, and carbohydrate to the prebaseline diet. Frequent sampling of blood was performed every hour for GH, ghrelin, and cortisol over 24 h during normal dietary intake.

After baseline testing was complete, subjects underwent a complete fast for 4 days. Water intake was ad libitum, and subjects received a daily multivitamin and no medications (28). Blood was drawn daily at 0800 for ghrelin and electrolytes. On the final day of fasting, subjects again underwent frequent sampling every hour for GH, ghrelin, and cortisol. On the final morning of the protocol, while still fasting, repeat hormone evaluations for ghrelin, IGF-I, glucose, leptin, and insulin were performed and weight and body composition again determined.

Body composition was determined by dual-energy X-ray absorptiometry (Hologic, Waltham, MA) at baseline and after 4 days of fasting. A visual analog scale (VAS) evaluating hunger was administered by a bionutritionist to each subject in the morning, after awakening, before the initiation of the fast and again just before the end of the 4-day fasting period. Subjects were asked to rate their level of hunger on a 10-cm scale, with 0 cm being “not at all hungry” and 10 cm being “extremely hungry”. Subjects were asked to mark the VAS at the point on the scale that they considered to be appropriate to their hunger sensation at the time of the testing (30). After all testing was completed, the fast was broken, and the subjects were allowed to eat breakfast ad libitum.

Study B. Subjects completed 4-day outpatient food records prior to baseline to determine their calorie, protein, and fat intake and were instructed to maintain a meat-free diet with protein substitution for 3 days prior to baseline. Subjects returned for baseline after an overnight fast, and weight was determined. Fasting blood was drawn for IGF-I and ghrelin, and all subjects completed a fasting oral glucose tolerance test (OGTT). Subjects were then administered prednisone (30 mg po bid) and either GH (20 µg·kg⁻¹·day⁻¹) or matching placebo subcutaneously for 5 days. Fasting blood was drawn each morning of the protocol for IGF-I and glucose. On the final morning of the study, repeat evaluations for IGF-I and ghrelin as well as a fasting OGTT were performed. Weight was again determined.

Biochemical Assays

GH was measured by two-site radioimmunometric assay (IRMA) with an intra-assay coefficient of variation (CV) of 2.7–4.2% (Nichols Institute Diagnostics, San Juan Capistrano, CA). The sensitivity of the assay was determined to be 0.01 µg/l, and linearity of the assay was confirmed to a GH concentration of 0.05 µg/l. IGF-I was measured by two-site IRMA (intra-assay CV 4.9%; DSL, Webster, TX).

Total serum ghrelin was measured by RIA (Phoenix Pharmaceuticals, Belmont, CA). The RIA uses 125I-labeled bioactive ghrelin as a tracer and a polyclonal antibody raised against full-length, octanoylated human ghrelin that recognizes both the octanoyl and des-octanoyl forms of the hormone. The intra-assay CV was 7.4%. Fresh, previously unfrozen ghrelin samples were used. Serum leptin levels were determined using an RIA kit (Linco Research, St. Charles, MO; intra-assay CV of 3.4%, interassay CV of 3.0–6.2%, sensitivity of 0.5 ng/ml). Serum insulin levels were run using RIA (Diagnostic Products, intra-assay CV of 3.1–9.3%). Serum cortisol levels were measured using two-site RIA (DiaSorin, Stillwater, MN; intra-assay CV 5.1–6.1%, interassay CV 8.8–9.8%). Glucose levels were assessed by standard technique.

Statistical Analysis

Study A. Change from baseline was determined by a paired t-test. The diurnal patterns of GH, ghrelin, and cortisol during fed and fasted states were examined by cosinor analysis (23), by which the circadian phases and amplitudes of GH and ghrelin were estimated and compared between the fed and fasted states. We employed the longitudinal mixed-effects models approach to the modeling. The following model, involving a single cosine function of time, describes the diurnal pattern of the longitudinal measurement for subject i at time t over a 24-h period on each day, Yi(t) = Mi(t) + AMP cos(2πt/24 + φi) + ei, where Mi(t) is a subject’s random intercept (mesor), AMP is the random amplitude (difference between the subject’s peak and nadir levels), φi is the random acrophase that determines the subject’s time of nadir and peak, and ei is the residual random error term. Noting that for τ = 2πt/24, Cos(τ) = Cos(τ)Cos(φi) = Sin(φi)Sin(φi) = Sin(φi)Cos(φi), the mixed-effects longitudinal regression equation Yi(t) = Mi(t) + β1Cos(2πt/24) + β2Sin(2πt/24) + ei is equivalent to the above single cosine function mixed-effects model. The estimates of the fixed mean effects, b1, and b2, for M, β1, and β2, respectively, were estimated by the maximum likelihood method wherein the longitudinal correlation structure of the random error terms was assumed as exchangeable. The amplitude and acrophase in hours were then determined as AMP = [(b1² + b2²)¹/²] and φ = (24πτ) × Tan⁻¹[(−b2/b1)] respectively. The meal effects on GH and ghrelin during baseline testing were examined by including a fixed-effect dummy variable in each baseline cosinor regression model to estimate the magnitude of immediate change after any meal (i.e., breakfast, lunch, or dinner). The comparison of the models between fed and fasted states was performed by testing for Cos(2πt/24) × fasted dummy variable and Sin(2πt/24) × fasted dummy variable interactions and testing for the change in mesor based on the model which was fitted to the combined observations in fed and fasted states.

Mean GH, ghrelin, and cortisol levels were also determined from the 24-h sampling. Univariate regression analysis was performed to examine the linear relationship of the change in ghrelin to changes in weight, GH, IGF-I, leptin, estradiol, cortisol, insulin, and glucose over the course of fasting. In addition, forward stepwise regression analysis was performed (P < 0.1 to enter the model) to assess the independent effects of these variables on the change in ghrelin. Weight, GH, IGF-I, leptin, estradiol, cortisol, insulin, and glucose were tested for entry into the model, and a final model was constructed with variables that entered the model. A multivariable regression model was also constructed to determine the independent contributions of changes in ghrelin and leptin to change in hunger over the course of fasting. A standard least squares model was constructed, as ghrelin did not enter
a stepwise model at $P = 0.1$ but was significant in a least squares regression model with all variables (leptin, glucose, ghrelin) entered into the model (see RESULTS).

Study B. The change in ghrelin between the two treatment groups (prednisone + GH vs. prednisone alone) was compared using ANCOVA, in which the ghrelin level on day 5 was the dependent variable and randomization and baseline ghrelin were tested in the model. Changes in weight and IGF-I between the groups were similarly analyzed.

All values in both studies are expressed as mean values ± SE. All comparisons were performed by two-sided tests and resulting $P$ values <0.05 were considered statistically significant. All statistical analyses were made using SAS (version 8; SAS Institute, Cary, NC).

RESULTS

Study A

Ten healthy women (BMI 22.3 ± 0.4 kg/m², age 26.7 ± 1.6 yr) were studied during a 4-day fast in the early follicular phase. Nine completed the study. Weight (22.3 ± 0.4 vs. 21.5 ± 0.4 kg/m², $P < 0.0001$), IGF-I (312 ± 28 vs.124 ± 22 ng/ml, $P < 0.0001$), leptin (11.9 ± 1.9 vs. 3.5 ± 0.2 ng/ml, $P = 0.002$), glucose (81 ± 1 vs. 51 ± 3 mg/dl, $P < 0.0001$), and insulin (5.5 ± 1.1 vs. 2.8 ± 0 μIU/ml, $P = 0.048$) decreased (baseline vs. day 4 of fasting respectively; Table 1).

Mean 24-h GH increased (2.6 ± 0.5 vs. 5.6 ± 0.5 ng/ml, $P < 0.001$), but ghrelin decreased, as measured by serial change in daily AM levels from baseline [−59.4 ± 78.8 pg/ml (after day 1 of fasting), −102 ± 47.3 pg/ml (after day 2 of fasting), −115.6 ± 35.9 pg/ml (after day 3 of fasting, $P = 0.012$), −117.9 ± 37.1 pg/ml (after day 4 of fasting, $P = 0.013$), and by comparison of baseline with end-of-study mean 24-h values (441.3 ± 59.7 vs. 359.8 ± 54.2 pg/ml, $P = 0.012$)]. Mean 24-h cortisol also increased significantly (7.7 ± 0.4 vs. 12.1 ± 0.6 μg/dl, $P < 0.0001$; Fig. 1). Hunger rating increased significantly from 2.8 to 5.4 ($P = 0.013$).

Ghrelin levels demonstrated an overall diurnal pattern during isocaloric feeding. Ghrelin levels decreased on average 17.9% within 1 h after meals at 0800, 1230, and 1730 ($P < 0.0001$). Meal effects were abolished during fasting. GH dem-

Table 1. Change from baseline in hormonal and metabolic parameters during 4 days of fasting in study A

| Age, yr | 26.7 ± 1.6 |
| Cycle day | 3.9 ± 0.7 |

Baseline Characteristics

<table>
<thead>
<tr>
<th>Hormonal and Metabolic Parameters</th>
<th>Baseline</th>
<th>After 4 days of Fasting</th>
<th>Change from Baseline with Fasting</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>59.4 ± 1.9</td>
<td>56.9 ± 2.1</td>
<td>−2.7 ± 0.2 (−4.2%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.3 ± 0.4</td>
<td>21.5 ± 0.4</td>
<td>−1.0 ± 0.1 (−3.6%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ghrelin, pg/ml</td>
<td>467.9 ± 68.3</td>
<td>344.4 ± 57.7</td>
<td>−117.9 ± 37.1 (−26.4%)</td>
<td>0.013</td>
</tr>
<tr>
<td>24-h Mean ghrelin, pg/ml</td>
<td>441.3 ± 59.7</td>
<td>359.8 ± 54.2</td>
<td>−81.5 ± 18.5 (−18.5%)</td>
<td>0.012</td>
</tr>
<tr>
<td>24-h Mean GH, ng/ml</td>
<td>2.0 ± 0.5</td>
<td>5.6 ± 0.5</td>
<td>3.3 ± 0.5 (115.4%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>24-h Mean cortisol, μg/dl</td>
<td>7.7 ± 0.4</td>
<td>12.1 ± 0.6</td>
<td>4.7 ± 0.4 (57.1%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Estradiol, pg/ml</td>
<td>39.0 ± 5.6</td>
<td>104.8 ± 21.5</td>
<td>63.6 ± 20.8 (+168.7%)</td>
<td>0.016</td>
</tr>
<tr>
<td>IGF-I, ng/ml</td>
<td>312 ± 28</td>
<td>124 ± 22</td>
<td>−178 ± 19 (−60.3%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>81 ± 1</td>
<td>51 ± 3</td>
<td>−30 ± 3 (−37.0%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin, μIU/ml</td>
<td>5.5 ± 1.1</td>
<td>2.8 ± 0</td>
<td>−2.9 ± 1.3 (−49.1%)</td>
<td>0.048</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>11.9 ± 1.9</td>
<td>3.5 ± 0.2</td>
<td>−9.0 ± 2.0 (−72.3%)</td>
<td>0.002</td>
</tr>
<tr>
<td>VAS hunger</td>
<td>2.8 ± 0.6</td>
<td>5.4 ± 0.9</td>
<td>−3.0 ± 0.9 (92.9%)</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Results are means ± SE. GH, growth hormone; VAS, visual analog scale.

AJP-Endocrinol Metab • VOL 289 • NOVEMBER 2005 • www.ajpendo.org
to the change in glucose ($r = 0.59, P = 0.097$) in univariate regression analysis. In a stepwise regression analysis, only the change in 24-h mean GH (parameter estimate $61.5, P = 0.012$), but not leptin ($P = 0.197$), weight ($P = 0.336$), IGF-I ($P = 0.679$), insulin ($P = 0.815$), glucose ($P = 0.121$), estradiol ($P = 0.133$), or 24-h mean cortisol ($P = 0.932$) was significantly associated with the change in ghrelin and entered the model (overall $R^2$ for model = 0.62).

In a multivariate regression analysis, the change in ghrelin (parameter estimate = 0.027, $P = 0.039$) was related to the change in hunger, controlling for the changes in leptin ($P = 0.176$) and glucose ($P = 0.232$) (overall $R^2$ for model = 0.61). The directionality of this relationship suggested that the more ghrelin dropped the more hunger was blunted.

**Study B**

Subjects were age 25.8 ± 1.2 yr with BMI 23.3 ± 1.0 kg/m². Weight did not change significantly between the groups over the 5-day course of the study ($-0.5 ± 0.3$ vs. $-0.3 ± 0.5$ kg, $P = 0.810$, GH + prednisone vs. prednisone alone). IGF-I levels increased significantly more in the GH + prednisone group compared with prednisone alone ($131 ± 30$ vs. $31 ± 17$ ng/ml, $P = 0.007$). Ghrelin levels decreased significantly in both groups, but the decrease was significantly greater in women receiving prednisone + GH than prednisone alone during the 5-day treatment period; e.g., ghrelin levels fell from $309.3 ± 20.8$ to $164.7 ± 12.1$ pg/ml in the GH + prednisone group compared with a change from $330.8 ± 45.1$ to $226.9 ± 37.5$ pg/ml in the group receiving prednisone alone ($P < 0.01$ for difference by ANCOVA).

**DISCUSSION**

Previous studies suggest that ghrelin is increased during prolonged starvation and in conditions of undernutrition and may act to simulate appetite. However, little is known regarding more acute changes in endogenous ghrelin with caloric
restriction in healthy subjects and whether changes in GH and endogenous ghrelin are related during short-term fasting. In this study, we sought to characterize the changes in GH and ghrelin among healthy women undergoing a 4-day complete fast. In contrast to increased levels of ghrelin associated with chronic undernutrition, our data demonstrate a steady decrease in ghrelin concentrations with short-term fasting. A significant inverse relationship between the increase in GH and decrease in ghrelin was observed.

![Fig. 2. Fitted cosinor curves for ghrelin (A), GH (B), and cortisol (C) during isocaloric feeding (dotted line) and fasting (solid line).](image)

**A**

Baseline and fasting ghrelin concentrations over 24 hours. The peak ghrelin level decreased from 483.5 to 375.6 pg/ml, and the time of peak ghrelin changed from 0415 to 1715 (P < 0.001 for testing difference in \( \beta_1 \), and \( P < 0.001 \) for testing a nonzero common \( \beta_2 \)). However, both the GH nadir (1.1 to 3.4 ng/ml) and peak concentrations (4.1 to 7.9 ng/ml) increased significantly from fed to fasted state (\( P < 0.0001 \)).

**B**

Baseline and fasting GH concentrations over 24 hours. The diurnal pattern of GH during feeding (baseline) and after 4 days of fasting were not statistically different (\( P = 0.539 \) for testing difference in \( \beta_1 \), and \( P = 0.261 \) for \( \beta_2 \) between the fed and fasted states), with peak GH maintained at midnight and nadir GH maintained at noon during fed and fasted states (\( P = 0.0028 \) for testing a nonzero common \( \beta_1 \), and \( P < 0.0001 \) for testing a nonzero common \( \beta_2 \)).

**C**

Mean cortisol concentrations over 24 hours. The diurnal pattern of cortisol during feeding and after 4 days of fasting were statistically different (\( P = 0.0105 \) for testing difference in \( \beta_1 \), and \( P = 0.0074 \) for \( \beta_2 \)). More importantly, however, the overall cortisol level increased by 60% after 4 days of fasting (\( P < 0.0001 \)). Times of peak and nadir cortisol levels were 45 min earlier after 4 days of fasting compared with those of baseline peak (1000) and nadir (2200) times.
in ghrelin was observed during fasting. Furthermore, we demonstrate that GH administration over 5 days reduces ghrelin in young healthy women on an ad libitum diet. Taken together, these data suggest that increased GH may feed back and decrease ghrelin during caloric restriction and exogenous GH administration in healthy women. These data help to define the physiological relationship between ghrelin and GH.

Ghrelin is produced in the stomach and is thought to have stimulatory effects on appetite and eating behavior. Ghrelin activates the GH secretagogue (GHS) receptor (GHS-R) in the hypothalamus (12), enhancing the activity of GHRH-secreting neurons and also acting as a functional somatostatin antagonist (21). Circulating ghrelin levels have been shown to rise before a meal and fall afterward, suggesting that anticipation of a meal
may stimulate secretion (4). Exogenous ghrelin has orexigenic effects in humans (34) and may play a role in meal initiation and regulation of appetite and energy balance (4). Ghrelin is reduced in obesity (7, 33) and increased in chronic undernutrition (29), for example in patients with anorexia nervosa (32). Despite the potentially important effects of ghrelin on appetite and eating behavior, little is known about the endogenous regulation of ghrelin during caloric restriction.

In this study, we investigated simultaneous endogenous ghrelin, GH, and cortisol concentrations in healthy young women before and after 4 days of fasting. During the baseline period, ghrelin demonstrated the anticipated postprandial decrease as demonstrated by Cummings et al. (4). This pattern was ablated with fasting in our study. Bagnasco et al. (2) demonstrated that ghrelin pulsatility was increased with fasting and decreased with feeding in rats. In a recent study, Espelund et al. (9) studied 33 healthy subjects and performed measurements of ghrelin every 3 h during 84 h of fasting. In accord with our data, Espelund et al. reported that ghrelin levels decreased during fasting in lean and obese females but remained unchanged in males. Furthermore, our study demonstrated a diurnal pattern in ghrelin, with a nadir in the afternoon and an evening peak. Although a diurnal pattern was maintained with fasting, consistent with the prior study by Espelund et al., we now demonstrate a significant shift in this pattern, with a change in peak amplitude and timing.

In this study, we further extend the results of Espelund et al. (9), to show reciprocal changes in ghrelin and GH during short-term caloric restriction. In chronic undernutrition, ghrelin and GH are increased (31). Exogenous ghrelin administration in humans stimulates GH release, and it is therefore possible that increased endogenous ghrelin may contribute to increased GH in chronic starvation. In contrast, we demonstrate a reciprocal relationship between changes in ghrelin and GH in response to short-term caloric restriction. Norrelund et al. (24) studied normal-weight, obese, and lean subjects and GH-deficient patients and reported no significant changes in ghrelin concentration during 36 h of fasting. The authors concluded that amplification of GH release during prolonged fasting is not caused by an increase in ghrelin (24) in accordance with our data. In contrast, Muller et al. measured plasma ghrelin levels every 6 h and responses to a growth hormone secretagogue before and after fasting. Muller et al. (22) reported a diurnal rhythm of ghrelin with low levels in the morning and subsequent elevations in the afternoon and midnight after 3 days of fasting and concluded that ghrelin contributes to enhanced GH secretion during fasting.

In contrast to previous studies, we measured simultaneously 1-h ghrelin and GH before and after 4 days of fasting. Ghrelin demonstrated meal-related changes and an overall diurnal pattern during the baseline period in response to meal intake. We now show that endogenous ghrelin diurnal rhythm persisted, but the peak was shifted during fasting. GH diurnal rhythm remained intact with similar times for nadir and peak GH. However, mean nadir and peak concentrations increased, consistent with data from prior studies showing increased GH during short-term caloric restriction (16).

Why would ghrelin decrease instead of rise after short caloric restriction? One possible explanation in this regard is a feedback suppression of GH on ghrelin. In support of this hypothesis, we compared changes in ghrelin and GH and showed a highly significant inverse relationship. Because it is known that exogenous ghrelin stimulates GH, the observation of increased GH in inverse relationship to decreased endogenous ghrelin would appear to rule out an effect of endogenous ghrelin to increase GH in short-term fasting. More likely, the increase in GH (14, 15) during short-term caloric deprivation results from depression of the GH-IGF-I axis due to GH resistance and decreased hepatic production of IGF-I (3, 20).

The possibility of acute GH feedback suppression on endogenous ghrelin was suggested in a prior study from our group (19), in which reciprocal changes in GH and ghrelin were seen in response to GHRH/arginine. In addition, ghrelin levels are decreased in acromegaly and increased after surgical cure (10).

A reciprocal relationship between GH and ghrelin is further supported by our data from a short-term interventional study in which healthy women were randomized to receive prednisone alone or prednisone and GH. Women in the interventional study were of similar age and weight to those in the fasting study. Furthermore, the interval of GH administration in study B, 5 days, was similar to the period of caloric deprivation in study A, 4 days. Prednisone dosing was pharmacological but
identical in each of the two treatment groups. In contrast, mean IGF-I levels increased in the GH group but remained within the normal range. Ghrelin levels decreased in both groups, but the decrease was significantly greater in the GH group. Although these data from a model in which prednisone was administered do not prove GH feedback inhibition of ghrelin, the decrease we observed in ghrelin was significant and remained so, even when we subtracted off the change due to prednisone alone.

Our data from a short-term study of GH administration are in agreement with some, but not all, studies in investigating this question. For example, Engstrom et al. (8) demonstrated that long-term GH administration in GH-deficient patients decreased ghrelin but that these changes were associated with changes in weight and body composition. In contrast, weight did not change in our short-term study, and therefore changes in ghrelin could not be attributed to this factor. Giavoli et al. (11) demonstrated decreased ghrelin in response to short-term GH administration but increases in ghrelin in response to long-term GH administration in association with reductions in body fat. Giavoli et al. also speculated as to the existence of a short-term feedback effect of GH on ghrelin. Dall et al. (6) demonstrated no change in ghrelin levels after acute infusion of GH in GH-deficient patients but did show lower ghrelin levels in GH-deficient patients receiving GH long-term compared with patients not receiving GH replacement. In contrast, Janssen et al. (17) did not demonstrate reductions in ghrelin over 1 yr of GH treatment. In animal studies, exogenous GH decreased stomach ghrelin mRNA over 3 days (26).

In this regard, it is unclear why ghrelin levels should be increased in chronic undernutrition and decreased in chronic overnutrition, states in which GH levels are increased and reduced, respectively. One potential explanation of the apparent discrepancy between our results, in which we postulate a short-term feedback of GH on ghrelin and data from chronic conditions in which there is no apparent inverse relationship, may be due to the overriding effect of body composition in the long term, as suggested by Giavoli et al. (11). In contrast, over the short term, direct effects of GH on ghrelin, either endogenous through changes in diet or in response to short-term administration, may be manifested. Further studies are necessary to determine the effects of exogenous GH alone on ghrelin in both men and women, as well as in both healthy controls and other populations with varying levels of GH sufficiency and obesity.

Another possible mechanism for decreased ghrelin level after 4 days of fasting could be the interaction between ghrelin and insulin. In our study, the insulin level decreased, as was expected, during fasting. In contrast to feeding conditions, changes in insulin did not correlate with change in ghrelin during fasting in our study of healthy women. In addition, changes in ghrelin were not related to changes in IGF-I, leptin, estradiol, cortisol, weight, or body composition but tended to be related to changes in glucose; e.g., the more glucose decreased, the more ghrelin tended to decrease. Furthermore, in stepwise regression analysis, assessing the simultaneous contribution of GH, glucose, insulin, IGF-I, weight, leptin, cortisol, and estradiol, only the change in GH was significantly related to the change in ghrelin. The robust nature of the relationship between the change in endogenous ghrelin and GH, controlling for changes in other potentially confounding variables, adds further strength to the hypothesis that increased GH may indeed contribute to decreased endogenous ghrelin during short-term caloric deprivation. Different results might be obtained in men or obese subjects. For example, Espelund et al. (9) suggested differential effects of short-term fasting on ghrelin between men and women. In this study, we extend the results of Espelund et al. by investigating the relationship between ghrelin and GH during short-term fasting in women. Additional studies in men are critical to further define the relationship between ghrelin and GH but were beyond the scope of the current study. Furthermore, causality cannot be definitively determined in our study, in which we assessed the simultaneous changes in two variables.

Similar to ghrelin and GH, cortisol also showed a diurnal pattern that was maintained during fasting with a significant increase in mean levels. Although prednisone resulted in decreased ghrelin in the interventional study, this result may have been due to the pharmacological dose chosen. No relationship between endogenous cortisol and ghrelin secretion was seen in the fasting study. In contrast, Espelund et al. (9) did show an inverse relationship between ghrelin and cortisol, as seen in our interventional study.

The role of ghrelin on regulation of human eating behavior is complex. Ghrelin increases with caloric absence between meals and in chronic undernutrition, potentially as an orexigenic stimulus to increase appetite, and conversely decreases with a meal and in obesity to decrease appetite. In this study, we assessed changes in perceived hunger over the course of short-term fasting in relationship to changes in ghrelin and leptin. As anticipated, hunger increased with caloric deprivation. Of note, the change in endogenous ghrelin was significantly associated with the changes in hunger, controlling for changes in leptin and glucose. The more ghrelin decreased, the more hunger was blunted. Conversely, those in whom ghrelin dropped the least demonstrated the greatest increase in hunger.

Ghrelin administration is known to stimulate appetite (27), but the relationship between changes in endogenous ghrelin and hunger during fasting have not been investigated. In this study, we show that endogenous ghrelin paradoxically decreases during 4 days of fasting, potentially as a result of feedback suppression of GH. The decrease in ghrelin is, however, associated with decreases in hunger, consistent with the known relationship of ghrelin to hunger and appetite in other human models. Our data suggest that changes in endogenous ghrelin may modulate hunger during acute caloric deprivation. However, the mechanisms by which changes in caloric intake affect ghrelin and ultimately hunger and satiety require further study.

In summary, we have examined endogenous ghrelin, cortisol, and GH dynamics during acute caloric deprivation in normal-weight, healthy women and in response to a paradigm of exogenous GH administration. To our knowledge, this is the first study to simultaneously characterize endogenous ghrelin and GH pulse dynamics during short-term fasting in healthy women. Our data demonstrate that ghrelin does not increase, but actually decreases, in response to short-term caloric deprivation in a reciprocal relationship to the increase in GH. Our data argue against an effect of ghrelin to mediate increased GH and suggest, conversely, that GH exhibits feedback inhibition on ghrelin during complete short-term caloric deprivation in healthy women. These data are further supported by the decrease in ghrelin seen in response to GH administration. This
study therefore suggests, but does not definitively establish, a short-loop feedback system whereby increased GH decreases ghrelin during acute caloric deprivation in women. This situation contrasts with that observed in chronic caloric deprivation, in which both ghrelin and GH are increased. Further studies are necessary to determine the mechanism by which ghrelin is differentially regulated in acute and chronic undernutrition.

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REFERENCES

2. Bagnasco M, Kalaria RS, and Kalaria SP. Plasma ghrelin levels during acute caloric deprivation in women. This situation therefore suggests, but does not definitively establish, a short-loop feedback system whereby increased GH decreases ghrelin during acute caloric deprivation in women. This situation contrasts with that observed in chronic caloric deprivation, in which both ghrelin and GH are increased. Further studies are necessary to determine the mechanism by which ghrelin is differentially regulated in acute and chronic undernutrition.

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