TRANSLATIONAL PHYSIOLOGY

Secretory dynamics of ghrelin in adolescent girls with anorexia nervosa and healthy adolescents

Madhusmita Misra,1,2 Karen K. Miller,1 Kelly Kuo,1 Kathryn Griffin,1 Victoria Stewart,1 Emily Hunter,1 David B. Herzog,3 and Anne Klibanski1

1Neuroendocrine Unit, 2Pediatric Endocrine Unit, and 3Eating Disorders Unit, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts

Submitted 30 December 2004; accepted in final form 7 March 2005

Ghrelin, a 28-amino acid peptide, is a growth hormone (GH) secretagogue (2, 4, 25, 26, 50–53) that is also orexigenic (40, 56). It is unknown whether elevated ghrelin values are an important contributing factor to the elevated GH levels that occur in anorexia nervosa (AN), a unique model of chronic undernutrition. We have demonstrated higher fasting ghrelin levels in adolescent girls with AN compared with healthy adolescents and higher nadir ghrelin levels following an oral glucose load (36). Girls with AN also have higher GH concentrations, a consequence of increased basal GH secretion and secretory burst frequency (33). Similarly, elevated ghrelin (21, 39) and GH concentrations (47, 49) have been reported in adult women with AN. Negative feedback from low IGF-I levels has been hypothesized to cause increased GH secretion, and the relationship between ghrelin and GH secretion in AN has not been explored.

Administration of a single dose of intravenous ghrelin causes an increase in GH secretion in both rodent models (51, 66) and healthy adults (4, 50, 52). However, the relation between endogenous ghrelin concentrations and endogenous GH levels is unclear (57). Both ghrelin (15, 28) and GH (3, 33, 47, 49) exhibit pulsatility in their secretory patterns, and the precise physiological relationship between ghrelin and GH may be better demonstrated using secretion and concentration parameters obtained by frequent sampling over a several-hour time period rather than by single fasting samples. This is particularly relevant for ghrelin because ghrelin levels rise in the fasting state (40), likely as an adaptive mechanism to increase food intake, and may not be representative of net ghrelin secretion over the day or night. Rather, basal secretion and spontaneous peaks of ghrelin may better predict GH levels.

Secretony dynamics of ghrelin in adolescent girls and in AN have not been described.

In addition to effects on GH, rodent and human data demonstrate that, after ghrelin administration, adrenocorticotropic hormone (ACTH) and cortisol secretions increase (52, 66), and TSH secretion (66) and luteinizing hormone (LH) pulse amplitude (63) decrease. Cortisol concentrations are elevated in girls with AN as a function of increased burst frequency of cortisol, and girls with AN also have low total triiodothyronine (T3) values (35). In contrast to the increase in ACTH with exogenous ghrelin administration in healthy adults, endogenous fasting ghrelin has been observed to be either unrelated or inversely related to cortisol concentration in adults (13, 45). However, the relation between integrated concentration and secretory parameters of ghrelin and cortisol over time has not been examined, particularly in adolescents. In addition, the relation between endogenous ghrelin secretion and other hormones affected by undernu-
trition, including total T₃ and gonadotropins, has also not been investigated.

To determine the effects of undernutrition on ghrelin parameters and the relation between ghrelin and other nutritionally dependent hormones, we examined ghrelin secretory dynamics overnight in adolescent girls with AN compared with healthy adolescents and the relation between concentration and secretory parameters of ghrelin, GH, and cortisol and other hormone variables. We hypothesized that girls with AN would have increased ghrelin concentrations measured over a 12-h period of nocturnal sampling, compared with healthy adolescents, as a consequence of increased basal and pulsatile ghrelin secretion, and that endogenous ghrelin secretory and concentration parameters would independently predict GH, cortisol, total T₃, and gonadotropin levels.

SUBJECTS AND METHODS

Subject selection. We studied 22 adolescent girls meeting the Diagnostic and Statistical Manual of Mental Disorders (4th edition) (DSM-IV) criteria for AN and 18 healthy adolescents of comparable maturity (chronological age: 16.2 ± 1.7 vs. 15.1 ± 1.8 yr, P not significant; bone age: 15.8 ± 1.6 vs. 15.5 ± 2.2 yr, P not significant). The mean duration since diagnosis of AN was 8.2 ± 10.6 mo. Clinical characteristics and hormonal data (but not ghrelin data from frequent sampling) have been previously reported (33–36). Subjects with AN were recruited through referrals from eating disorder units and providers in the New England region, and healthy adolescents were recruited through advertisements in local newspapers, within the Partners HealthCare system, and mass mailings to primary care providers in New England. None of the healthy controls had a present or past history of eating disorders. Use of medications and presence of disorders (other than AN) that might affect GH, cortisol, or ghrelin levels were exclusion criteria for the study. We also excluded subjects with hematocrit <30%, hypokalemia (potassium <3 mmol/l), and hypoglycemia (blood sugar <50 mg/dl) and girls with high levels of gonadotropins or abnormal TSH levels from the study.

Experimental protocol. After a screening visit to assess eligibility, subjects were admitted overnight to the Clinical Research Center of Massachusetts General Hospital. A history and physical examination were performed. Height was measured on a single stadiometer in triplicate and the average of three recordings read. Weight was measured on a single electronic scale. A bone age was obtained to determine maturity. Bone age was read by a single observer, a pediatric endocrinologist, using the methods of Greulich and Pyle (17). Percent body fat was determined using dual energy X-ray absorptiometry (DEXA; Hologic 4500, Waltham, MA) (24, 62).

Frequent sampling for ghrelin, GH, and cortisol was performed through a 20-gauge intravenous catheter in the antecubital region every half-hour for 12 h overnight (2000 to 0800). All subjects had supper before 1900 and were fasting thereafter. Fasting blood samples were obtained for IGF-I, glucose, insulin, ghrelin, leptin, and estradiol at 0800 the following morning. We also obtained levels of LH and TSH. All healthy adolescents and 17 girls with AN were then administered a 100-g oral glucose solution, and blood samples were obtained for ghrelin, insulin, and glucose at 30 and 60 min. One girl with AN declined the glucose load. Girls with AN were followed over a 1-yr period, and frequent sampling was repeated at weight recovery [defined as a 10% increase in body mass index (BMI); n = 9] (48).

Biochemical assessment. Levels of glucose, GH, and TSH were determined by the hospital laboratory using standard methods (23). Ghrelin was measured using a radioimmunoassay (RIA; Phoenix Pharmaceuticals, Belmont, CA; sensitivity 2 pg/ml, coefficient of variation 10%). We used an immunoradiometric assay (IRMA) to measure GH (Nichols Institute Diagnostics, San Juan Capistrano, CA; detection limit 0.05 ng/ml, coefficient of variation 2.4–9.4%) and IGF-I (Nichols Institute Diagnostics; detection limit 30 ng/ml, coefficient of variation 3.1–4.6%). RIA was used to measure serum cortisol (Diagnostic Products, Los Angeles, CA; limit of sensitivity 1.0 μg/dl, coefficient of variation 2.5–4.1%), insulin (Diagnostic Products; coefficient of variation 4.7–7.7%), leptin (Linco Diagnostics, St. Louis, MO; sensitivity 0.5 ng/ml, coefficient of variation 3.4–8.3%), and estradiol (Diagnostic Systems Laboratories, Webster, TX; limit of detection 2.2 pg/ml, coefficient of variation 6.5–8.9%). All samples were stored at −80°C until analysis, and all samples were run in duplicate.

Glucose levels can be converted to Système International (SI) units (mmol/l) by dividing by 18. Levels of GH, IGF-I, and leptin can be converted to SI units (μg/l) by multiplying by 1, and serum cortisol can be converted to SI units (nmol/l) by dividing by 0.0363.

The ratio of fasting glucose to fasting insulin and quantitative insulin sensitivity check index (QUICKI) were used as measures of insulin sensitivity (30, 67), homeostasis model assessment of insulin resistance (HOMA-IR) as a measure of insulin resistance (46), and pancreatic β-cell function was determined (64). QUICKI was calculated as the reciprocal of the sum of log fasting insulin (μIU/ml) and log fasting glucose (mg/dl) (67). HOMA-IR was calculated using the formula: [fasting glucose (mmol/l) × fasting insulin (μIU/ml)]/22.5. Pancreatic β-cell function was estimated using the following formula: [30-min insulin (μIU/ml) – fasting insulin (μIU/ml)]/30-min glucose (mmol/l) (64).

Analysis of ghrelin, GH, and cortisol concentrations obtained from frequent sampling overnight. We examined ghrelin, GH, and cortisol data from frequent sampling by both Cluster (1 × 2) and deconvolution analysis, using methods previously described (33, 35). Cluster characteristics reported include mean peak amplitude, mean peak area, nadir and valley mean levels, mean concentration, and integrated area under the curve (AUC). Nadir concentration refers to the lowest level of the hormone over the 12 h of sampling. Valley mean levels refer to the mean hormone concentrations during the troughs observed during frequent sampling. Nadir and valley mean concentrations reflect basal secretion of the hormone. The half-life for ghrelin applied to this model was based on half-life determinations following exogenous administration of total ghrelin to young male volunteers (27–31 min) (1). Concentration of a hormone at any time point is a function of the half-life of the hormone, basal secretion, secretory burst mass, and burst frequency. Whereas Cluster analysis provides information about concentration characteristics (60), secretory dynamics are best determined using deconvolution methods (59, 61). Total basal secretion was determined as follows: basal secretion rate × number of minutes of sampling (i.e., 720). Total pulsatile secretion was determined as the product of mean burst mass and frequency of secretory bursts. Total secretion was calculated as the sum of total basal and pulsatile secretion. Data for GH and cortisol, but not ghrelin, have been reported earlier (33). In this study, for GH and cortisol we used values of AUC from Cluster analysis, and basal secretion, secretory burst frequency, and pulsatile and total secretion from deconvolution analysis, for determination of relationships with ghrelin concentration and secretion parameters.

Cross-correlation between ghrelin and GH, and ghrelin and cortisol was calculated by lagging the concentration time series of one hormone relative to the concentration time series of the other. A lag period is the time (min) that separates consecutive samples in the paired-hormone series of interest, and various lag times were used in the analysis. Significant cross-correlation values for the two groups at any particular lag time were tested against the null hypothesis of random associations, using the one-sample Kolmogorov-Smirnov statistic. The International Mathematics and Statistical Library routine DCCF was used for the correlational analysis (6, 29).

Approximate entropy (ApEn), a measure of disorderliness of secretion, was determined using the methods described by Pincus (42, 43). This score increases in magnitude as the secretory burst pattern becomes more disorderly. Cross-ApEn (X-ApEn) is the bivariate
Results

Clinical characteristics. Clinical characteristics of adolescents with AN and healthy controls have been reported earlier and are summarized here. As expected, girls with AN had significantly lower BMIs (16.6 ± 1.2 vs. 21.7 ± 3.8 kg/m², P < 0.0001), total fat mass (8.9 ± 2.7 vs. 17.5 ± 6.0 kg, P < 0.0001), and percent body fat (18.5 ± 3.9 vs. 29.6 ± 5.7%, P < 0.0001) compared with healthy adolescents of comparable maturity.

Fasting IGF-I levels were significantly lower in girls with AN compared with controls (309 ± 142 vs. 519 ± 162 ng/ml, P < 0.0001), as were levels of fasting glucose and insulin (79.1 ± 9.2 vs. 85.7 ± 5.5 mg/dl, P = 0.01; 6.6 ± 2.6 vs. 14.5 ± 4.1 μIU/ml, P < 0.0001). Measures of insulin sensitivity, i.e., ratio of glucose to insulin and QUICKI were significantly higher in AN (13.5 ± 4.9 vs. 6.4 ± 2.3 mg/μIU × 10², P < 0.0001; 0.38 ± 0.03 vs. 0.33 ± 0.02, P < 0.0001), whereas HOMA-IR (measure of insulin resistance) was significantly lower in this underweight group (1.3 ± 0.6 vs. 3.1 ± 0.9, P < 0.0001). Pancreatic B-cell function was lower in girls with AN than in controls (6.4 ± 5.8 vs. 17.2 ± 10.6, P = 0.0008). Fasting leptin levels were also markedly lower in AN (4.3 ± 3.0 vs. 15.5 ± 6.5 ng/ml, P < 0.0001).

Girls with AN had higher total AUC for GH (3,991 ± 1,980 vs. 2,515 ± 1,174 ng·ml⁻¹·12 h, P = 0.008), and higher basal GH secretion (14.0 ± 12.4 vs. 7.6 ± 4.3 ng·ml⁻¹·12 h, P = 0.04), GH secretory burst frequency (11.8 ± 1.4 vs. 10.3 ± 1.7/12 h, P = 0.005), pulsatile (230 ± 133 vs. 149 ± 60 ng·ml⁻¹·12 h, P = 0.02) and total GH secretion (244 ± 135 vs. 156 ± 61 ng·ml⁻¹·12 h, P = 0.02) than healthy adolescents. Girls with AN also had significantly higher cortisol AUC (6,142 ± 1,494 vs. 4,183 ± 684 μg·dl⁻¹·12 h, P < 0.0001), cortisol secretory burst frequency (7.0 ± 1.2 vs. 5.8 ± 1.3/12 h, P = 0.004), pulsatile (70.2 ± 14.4 vs. 55.4 ± 10.3 μg·dl⁻¹·12 h, P = 0.0007), and total cortisol secretion (90.4 ± 18.8 vs. 72.9 ± 17.2 μg·dl⁻¹·12 h, P = 0.004) than healthy adolescents. Basal cortisol secretion did not differ between the groups. Mean GH and cortisol concentrations over the period of nocturnal sampling are shown in Fig. 1.

Girls with AN had lower total T₃ levels compared with controls (88.3 ± 20.0 vs. 159.0 ± 49.3 ng/dl, P < 0.0001), although TSH levels did not differ. Estradiol and LH levels were lower in AN but did not reach statistical significance (16.8 ± 6.7 vs. 20.1 ± 7.4 pg/ml, P not significant; 4.1 ± 3.9 vs. 5.8 ± 7.1 U/l, P not significant). Of importance, healthy controls were examined in the early follicular phase of their cycles, when estradiol and LH levels are very low.

Cluster and deconvolution analysis of ghrelin concentration. Figure 1 shows mean ghrelin concentrations in girls with AN and controls over the period of frequent sampling. Results of Cluster and deconvolution analysis of ghrelin concentration are reported in Table 1 and show that all Cluster parameters are higher in girls with AN than in healthy adolescents. On deconvolution analysis, girls with AN had significantly higher secretory burst amplitude and burst mass than controls. Secretory burst frequency did not differ in the two groups. Increased pulsatile and total ghrelin secretion in AN were thus a conse-
sequence of increased secretory burst mass. Cluster and deconvolution analysis in a girl with AN and a healthy control are shown in Fig. 2. Fasting ghrelin as well as 30- and 60-min postglucose ghrelin levels were significantly higher in girls with AN than in healthy adolescents. ApEn did not differ between the groups (0.906 ± 0.113 in AN vs. 0.879 ± 0.147 in controls, \( P \) not significant).

Following weight recovery (\( n = 9 \) girls with AN), ghrelin concentration and secretory parameters trended lower than at baseline on matched-pair analysis even with a small number of subjects. Ghrelin AUC decreased from 513,155 to 348,353 pg/ml \( -1 \cdot 12 \) h (\( P = 0.05 \)), mean ghrelin from 750 to 485 pg/ml (\( P = 0.04 \)), and valley mean ghrelin from 707 to 382 pg/ml (\( P = 0.09 \)). Median burst mass decreased from 839 to 598 pg/ml (\( P = 0.09 \)), pulsatile secretion from 11,818 to 8,515 pg/ml \( -1 \cdot 12 \) h (\( P = 0.06 \)). In these nine girls, BMI of the three being HOMA-IR. When multiple regression analysis, in which BMI, fat mass, IGF-I and ghrelin trended shorter in AN (18.6 ± 30.9 vs. 56.5 ± 34.4 min, \( P = 0.06 \) on the Wilcoxon rank sum test), with GH secretion preceding ghrelin secretion in both groups. X-ApEn between GH and ghrelin trended higher in AN (0.90 ± 0.09 vs. 0.82 ± 0.18, \( P = 0.09 \)). Lag time between cortisol and ghrelin secretion did not differ between the groups (\(-191.4 ± 213.9 \) min in AN vs. \(-180.4 ± 204.0 \) min in controls, \( P \) not significant), with ghrelin secretion preceding cortisol secretion. X-ApEn between the two groups also did not differ (0.86 ± 0.20 vs. 0.81 ± 0.16, \( P \) not significant).

**Ghrelin, nutritional status, and insulin resistance.** Measures of nutritional status, such as BMI and percent fat mass, correlated inversely with 30-min ghrelin concentration (\( r = -0.40, P = 0.01 \), and \( r = -0.44, P = 0.01 \)), nadir ghrelin (\( r = -0.42, P = 0.03 \), and \( r = -0.38, P = 0.06 \)), and mean valley ghrelin concentrations (\( r = -0.46, P = 0.02 \), and \( r = -0.43, P = 0.03 \)), but not with fasting ghrelin. Fasting glucose levels were inversely associated with ghrelin concentration and secretion parameters, as were fasting insulin levels (Table 2). Ghrelin concentration and secretion correlated strongly and positively with the glucose-to-insulin ratio and inversely with HOMA-IR, a measure of insulin resistance (Table 2). Correlations with QUICKI were similar to those with the glucose/insulin ratio and are not shown. Pancreatic \( \beta \)-cell function correlated inversely with fasting ghrelin (\( r = -0.43, P = 0.01 \)), 30-min ghrelin (\( r = -0.48, P = 0.004 \)), and 60-min ghrelin (\( r = -0.44, P = 0.008 \)), but not with overnight ghrelin concentration and secretion parameters. An inverse relationship was observed between IGF-I levels and 0-min ghrelin (\( r = -0.42, P = 0.008 \)), 30-min ghrelin (\( r = -0.39, P = 0.02 \)), and 60-min ghrelin (\( r = -0.38, P = 0.03 \)). Inverse correlations were observed between IGF-I and ghrelin AUC (\( r = -0.41, P = 0.01 \)), nadir ghrelin (\( r = -0.47, P = 0.02 \)), valley ghrelin (\( r = -0.44, P = 0.02 \)), median burst mass (\( r = -0.33, P = 0.04 \)), and total ghrelin secretion (\( r = -0.31, P = 0.05 \)).

On multiple regression analysis, in which BMI, fat mass, IGF-I, and HOMA-IR were entered into the model, the only significant predictor of different measurements of ghrelin concentration and secretion was HOMA-IR. For ghrelin AUC and nadir and mean valley ghrelin, HOMA-IR contributed to 30.5, 33.9, and 50.1% of the variability, respectively. Similarly, HOMA-IR contributed to 19.8, 25.2, 19.9, and 22% of the variability of mean burst mass, median burst mass, and pulsatile and total ghrelin secretion, respectively. Similar results were noted when QUICKI or glucose/insulin ratio rather than HOMA-IR were entered into the model, the strongest predictor of the three being HOMA-IR. When multiple regression analysis was repeated with leptin or GH parameters entered into the

### Table 1. Comparison of Cluster and deconvolution analysis of ghrelin concentration and secretory characteristics in adolescent girls with anorexia nervosa and controls

<table>
<thead>
<tr>
<th></th>
<th>Healthy Adolescents (( n = 18 ))</th>
<th>Anorexia Nervosa (( n = 22 ))</th>
<th>( P ) Value (( t )-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cluster analysis of ghrelin concentration</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nadir, pg/ml</td>
<td>288 ± 163</td>
<td>548 ± 171‡</td>
<td>0.0006</td>
</tr>
<tr>
<td>Valley mean, pg/ml</td>
<td>391 ± 147</td>
<td>619 ± 181‡</td>
<td>0.002</td>
</tr>
<tr>
<td>Peak amplitude, pg/ml</td>
<td>534 ± 73</td>
<td>847 ± 190‡</td>
<td>0.009</td>
</tr>
<tr>
<td>Peak area, pg/ml</td>
<td>6,799 ± 8,862</td>
<td>31,995 ± 29,570*</td>
<td>0.02</td>
</tr>
<tr>
<td>Mean, pg/ml</td>
<td>458 ± 149</td>
<td>670 ± 246‡</td>
<td>0.003</td>
</tr>
<tr>
<td>Total AUC, pg/ml (-1\cdot12 ) h</td>
<td>320,563 ± 110,594</td>
<td>464,115 ± 158,329‡</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Deconvolution analysis of ghrelin secretory characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total basal secretion, pg/ml (-1\cdot12 ) h</td>
<td>111 ± 250</td>
<td>365 ± 808</td>
<td>NS</td>
</tr>
<tr>
<td>Half-life, min</td>
<td>30.5 ± 0.85</td>
<td>29.5 ± 6.4</td>
<td>NS</td>
</tr>
<tr>
<td>No. of secretory bursts/12 h</td>
<td>14.5 ± 1.0</td>
<td>14.4 ± 1.2</td>
<td>NS</td>
</tr>
<tr>
<td>Mean burst mass, pg/ml</td>
<td>618 ± 263</td>
<td>812 ± 309‡</td>
<td>0.04</td>
</tr>
<tr>
<td>Median burst mass, pg/ml</td>
<td>548 ± 182</td>
<td>789 ± 309‡</td>
<td>0.006</td>
</tr>
<tr>
<td>Mean burst amplitude, pg/ml</td>
<td>25.1 ± 10.7</td>
<td>33.6 ± 12.7‡</td>
<td>0.03</td>
</tr>
<tr>
<td>Median burst amplitude, pg/ml</td>
<td>22.3 ± 7.6</td>
<td>32.5 ± 12.7‡</td>
<td>0.004</td>
</tr>
<tr>
<td>Total pulsatile secretion, pg/ml (-1\cdot12 ) h</td>
<td>9,028 ± 4,189</td>
<td>11,611 ± 4,065†</td>
<td>0.05</td>
</tr>
<tr>
<td>Total secretion, pg/ml (-1\cdot12 ) h</td>
<td>9,139 ± 4,209</td>
<td>11,976 ± 3,781†</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Following a 100-g oral glucose load</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-min Ghrelin, pg/ml</td>
<td>549 ± 192</td>
<td>784 ± 222‡</td>
<td>0.001</td>
</tr>
<tr>
<td>30-min Ghrelin, pg/ml</td>
<td>448 ± 136</td>
<td>753 ± 272‡</td>
<td>0.0002</td>
</tr>
<tr>
<td>60-min Ghrelin, pg/ml</td>
<td>473 ± 177</td>
<td>657 ± 2.8*</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Values are means ± SD. AUC, area under the curve; NS, not significant. *\( P \) ≤ 0.05, †\( P \) ≤ 0.01, ‡\( P \) ≤ 0.001, Wilcoxon rank sum test.
model, HOMA-IR remained the sole significant predictor of ghrelin concentration and secretion characteristics.

Relation between ghrelin and GH. Ghrelin concentration and secretory characteristics predicted basal GH secretion rates, and a positive correlation was observed between basal GH secretion and AUC ghrelin \( (r = 0.49, P = 0.002) \), ghrelin mean burst mass \( (r = 0.43, P = 0.006) \), median burst mass \( (r = 0.46, P = 0.003) \), pulsatile ghrelin secretion \( (r = 0.42, P = 0.007) \), and total ghrelin secretion \( (r = 0.44, P = 0.004) \). Fasting ghrelin was the sole ghrelin parameter that predicted GH secretory burst frequency \( (r = 0.44, P = 0.005; \text{Fig. 3}) \).

For the group as a whole, on multiple regression analysis in which BMI, fat mass, IGF-I, HOMA-IR, and a measurement of ghrelin concentration or secretion were entered into the model, the most significant predictors of basal GH secretion were that measurement of ghrelin concentration or secretion, and fat mass. When the ghrelin parameter entered into the model was the AUC for ghrelin, the latter contributed to 24.4% of the variability of basal GH secretion. When ghrelin secretory burst mass was entered into the model, the significant predictors of basal GH secretion were ghrelin secretory burst mass and fat mass, contributing 19.1 and 10.6% of the variability, respectively. Similarly, ghrelin pulsatile secretion and fat mass contributed to 17.9 and 11.8% of the variability, and total ghrelin secretion and fat mass to 20.5 and 10.7% of the variability of basal GH secretion, respectively.

GH secretory burst frequency correlated positively with fasting ghrelin, and the latter was the most significant predictor.
of GH secretory burst frequency in a multiple regression model including BMI, fat mass, IGF-I, leptin, HOMA-IR, and fasting ghrelin. Fasting ghrelin contributed to 23.1% of the variability of GH secretory burst frequency, other significant predictors being leptin and BMI (3.3 and 10.5% of the variability).

Relation between ghrelin and cortisol. Positive associations were observed between ghrelin concentration parameters and different measurements of cortisol concentration and secretion (Table 3). In a multiple regression model in which BMI, fat mass, IGF-I, and ghrelin parameters were entered into the model, mean valley ghrelin was an independent predictor of cortisol burst frequency (51.9% of the variability) and cortisol AUC (41.7% of the variability) (Fig. 3).

Relation between ghrelin and other hormones. An inverse association was observed between ghrelin concentration and fasting leptin levels (Table 4); however, on regression modeling using BMI, fat mass, IGF-I, and GH and ghrelin parameters, ghrelin was not an independent predictor of leptin levels. Instead, leptin levels were predicted by fat mass and GH secretory burst frequency, contributing to 77.1 and 7.2% of the variability, respectively.

Ghrelin concentration and secretion correlated inversely with total T3 levels, and by use of a regression model including BMI, fat mass, IGF-I, leptin, and ghrelin and GH parameters, nadir ghrelin was determined to be an independent predictor of total T3 levels, contributing to 39.9% of the variability, with other significant predictors being leptin and BMI (11.6 and 11.4%, respectively). Similar results were observed with valley ghrelin.

A strong inverse association was also observed between fasting ghrelin and estradiol levels. Fasting ghrelin was the sole predictor of LH levels ($r = -0.39$, $P = 0.01$), and markers of nutritional status and other hormones did not predict LH levels.

Relation between fasting ghrelin and ghrelin concentration and secretion parameters. Strong correlations were observed between fasting ghrelin and overnight ghrelin parameters (Table 5).

DISCUSSION

We demonstrate that increased ghrelin concentrations in adolescent girls with AN are a consequence of increased ghrelin secretory burst mass resulting in increased ghrelin pulsatile and total secretion. These measurements improve with weight recovery. Ghrelin levels are predicted by markers of nutritional status, particularly by insulin resistance. Ghrelin concentrations predict basal GH secretion and GH secretory burst frequency, cortisol burst frequency, and total T3 and LH levels independently of markers of nutritional status, and on the basis of these data we hypothesize an intriguing scenario wherein elevations in ghrelin may drive, or contribute to, many of the neuroendocrine changes observed in conditions of undernutrition. A limiting factor in our study is that the relation between ghrelin and other hormones examined is correlational and thus does not imply causation. Intervventional experimental strategies, for example, the possible use of ghrelin antagonists in AN, would help differentiate whether elevations in GH and cortisol and lowering of total T3 and LH levels in AN are direct

Table 2. Relation between ghrelin and measurements of insulin sensitivity and resistance

<table>
<thead>
<tr>
<th>Glucose (0-min)</th>
<th>Insulin (0-min)</th>
<th>Glucose/Insulin</th>
<th>HOMA-IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r$</td>
<td>$P$</td>
<td>$r$</td>
<td>$P$</td>
</tr>
<tr>
<td>0-min Ghrelin</td>
<td>NS</td>
<td>0.35</td>
<td>0.02</td>
</tr>
<tr>
<td>30-min Ghrelin</td>
<td>0.42</td>
<td>0.01</td>
<td>0.44</td>
</tr>
<tr>
<td>60-min Ghrelin</td>
<td>0.44</td>
<td>0.03</td>
<td>0.47</td>
</tr>
<tr>
<td>AUC ghrelin</td>
<td>0.47</td>
<td>0.004</td>
<td>0.44</td>
</tr>
<tr>
<td>Nadir ghrelin</td>
<td>0.44</td>
<td>0.003</td>
<td>0.50</td>
</tr>
<tr>
<td>Mean valley ghrelin</td>
<td>0.47</td>
<td>0.02</td>
<td>0.44</td>
</tr>
<tr>
<td>Mean burst mass</td>
<td>0.47</td>
<td>0.02</td>
<td>0.44</td>
</tr>
<tr>
<td>Median burst mass</td>
<td>0.47</td>
<td>0.004</td>
<td>0.44</td>
</tr>
<tr>
<td>Pulsatile secretion</td>
<td>0.47</td>
<td>0.01</td>
<td>0.44</td>
</tr>
<tr>
<td>Total secretion</td>
<td>0.47</td>
<td>0.003</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Glucose/insulin, glucose-to-insulin ratio; HOMA-IR, homeostasis model assessment of insulin resistance.
effects of undernutrition, a consequence of hyperghrelinemia, or both.

To our knowledge, this is the first study that has examined ghrelin secretory characteristics by deconvolution analysis in adolescents. Koutkia et al. (28) reported Cluster analysis of ghrelin levels measured overnight in healthy young men. Cluster analysis is an established, effective method to measure hormone concentration characteristics; however, deconvolution analysis provides additional information about individual secretory events. In our study, we demonstrate that ghrelin secretory bursts in girls with AN have increased burst amplitude and burst mass compared with healthy controls and that this contributes to increased pulsatile secretion of ghrelin in AN. Ghrelin is orexigenic (40, 53, 56), and high ghrelin levels in AN, a state of negative energy balance, are likely a compensatory mechanism to increase caloric intake and induce a state of positive energy balance. The decreased food intake in AN despite elevated ghrelin levels suggests a resistance to the orexigenic effects of ghrelin in AN or complex interactions at the level of the arcuate and periventricular nuclei that modify the orexigenic effects of ghrelin. As expected, ghrelin parameters improved with weight recovery. Ghrelin secretory parameters obtained in our study in healthy adolescents differ from those reported by Ghizzoni et al. (15) in prepubertal children with idiopathic short stature. These differences may be related to the difference in age of the populations studied, the program used (the older program, Pulsar, was used in the study with the younger program, P). The fact that idiopathic short stature may be associated with the differences reported by Ghizzoni et al. (15) in prepubertal children and the fact that idiopathic short stature may be associated with subclinical changes in hypothalamic-pituitary function.

We measured total ghrelin levels in this study, and future studies measuring acylated vs. desacylated ghrelin by frequent sampling may reveal further differences between the groups not determined in this study. Administration of desacylated ghrelin is not associated with increases in GH or ACTH, unlike administration of acylated ghrelin (7, 8). Data reported thus far regarding acylated and desacylated ghrelin levels in AN are contradictory. Hotta et al. (21) reported higher desacylated ghrelin levels in adult women with AN, whereas Nakai et al. (39) have reported higher acylated ghrelin levels in this condition.

Nutritional and hormonal predictors of ghrelin secretion are not well understood. Exogenous ghrelin administration increases GH and cortisol secretion in healthy adults (4, 50, 52) and animals (51, 66) and decreases TSH (66) and LH secretion (63) in animal models. Ghrelin was administered as a single intravenous dose in humans in these studies and as either a single injection or continuously over several hours in the animal models. The longest studies in animals with ghrelin involve ghrelin administration either continuously or intermittently for a week (51, 55). However, in one patient with a malignant ghrelinoma and high ghrelin levels, levels of GH, IGF-I, cortisol, TSH, total T<sub>3</sub>, FSH, and LH were well within the normal range (57), raising the question whether effects of short-term exogenous ghrelin administration on the endocrine system differ from those of endogenous chronic ghrelin concentrations. We thus examined the associations between endogenous ghrelin and various markers of nutritional status and hormones in this study.

Ghrelin levels are high in AN (21, 36) and low in obesity (13, 32), suggesting that ghrelin secretion is nutritionally regulated. This is corroborated by the trend toward a decrease in ghrelin parameters observed in the nine subjects with AN who recovered weight over the follow-up period. Inverse correlations have been reported between ghrelin and BMI, fat mass, glucose, insulin, and IGF-I levels (21, 28, 36, 41). However, BMI and fat mass predicted only 30-min ghrelin and nadir and valley mean ghrelin levels in this study and did not predict ghrelin secretory characteristics. Thus BMI and fat mass may predict the lows in ghrelin concentrations but do not predict basal or pulsatile secretion or total AUC for ghrelin. The strongest predictor of ghrelin concentration and secretion characteristics was the HOMA-IR, a measurement of insulin resistance incorporating values of both glucose and insulin. Wasko et al. (65) similarly reported inverse correlations between insulin and ghrelin in women with polycystic ovary syndrome (PCOS). Conversely, Moran et al. (37) reported lower ghrelin levels in obese women with PCOS compared with obese

---

**Table 3. Relation between ghrelin and cortisol**

<table>
<thead>
<tr>
<th>AUC cortisol</th>
<th>Nadir cortisol</th>
<th>Mean valley cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.48 0.002</td>
<td>0.63 0.0005</td>
<td>0.66 0.0002</td>
</tr>
</tbody>
</table>

**Table 4. Relation between ghrelin and other hormones**

<table>
<thead>
<tr>
<th>Leptin</th>
<th>Total T&lt;sub&gt;3&lt;/sub&gt;</th>
<th>Estradiol</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-min Ghrelin</td>
<td>0.36 0.02</td>
<td>0.63 0.0001</td>
</tr>
<tr>
<td>30-min Ghrelin</td>
<td>0.50 0.002</td>
<td>0.44 0.008</td>
</tr>
<tr>
<td>60-min Ghrelin</td>
<td>0.37 0.03</td>
<td>0.42 0.02</td>
</tr>
<tr>
<td>AUC ghrelin</td>
<td>0.34 0.03</td>
<td>0.44 0.004</td>
</tr>
<tr>
<td>Nadir ghrelin</td>
<td>0.41 0.04</td>
<td>0.57 0.002</td>
</tr>
<tr>
<td>Valley ghrelin</td>
<td>0.43 0.03</td>
<td>0.59 0.002</td>
</tr>
<tr>
<td>Mean burst mass</td>
<td>0.59 0.0001</td>
<td>0.52 0.001</td>
</tr>
<tr>
<td>Median burst mass</td>
<td>0.27 0.09</td>
<td>0.43 0.006</td>
</tr>
<tr>
<td>Pulsatile secretion</td>
<td>0.41 0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Total secretion</td>
<td>NS</td>
<td>0.42 0.007</td>
</tr>
</tbody>
</table>

T<sub>3</sub>, triiodothyronine.
women without PCOS but did not find any association between the degree of insulin resistance and fasting or postprandial ghrelin levels.

In our subjects, endogenous GH concentration and secretory characteristics did not predict ghrelin values, suggesting that feedback by endogenous GH may not be an important regulator of ghrelin. This is in contrast to exogenous administration of recombinant human (rh)GH to GH-deficient adults, which results in a decrease in ghrelin levels, and suggests that GH may regulate ghrelin (12). However, because GH-deficient individuals were not compared with healthy individuals, it is uncertain whether ghrelin levels were elevated in GH-deficient adults prior to beginning rhGH therapy.

In this study, AN serves as a unique model of undernutrition. However, it would be interesting to compare adolescent girls suffering from AN with age-, BMI-, and fat mass-matched, naturally thin adolescent girls and adolescent athletes without eating disorders to determine any contribution of the psychological alterations in AN to ghrelin levels and to better delineate the effects of nutrition on ghrelin concentrations.

Ghrelin concentration was the most important predictor of basal GH secretion and GH secretory burst frequency in this study, indicating that high ghrelin levels in AN may drive, or contribute to, the increased pituitary secretion of GH. Traditionally, low IGF-I levels have been assumed to drive pituitary GH secretion in AN, and our data suggest that ghrelin may, in fact, be a more important regulator of GH secretion than IGF-I, given that ghrelin, but not IGF-I, predicted GH levels. However, such conclusions are limited by our study design in that correlational analyses do not determine causality. Our results are in contrast to the marked reduction in GH secretion observed in adults being infused with rhIGF-I (10, 19). However, a sexual dimorphism in the response of GH to rhIGF-I has been demonstrated (22), and it is uncertain whether in addition to sex there are modifying effects of age on the relation between GH and IGF-I. GH secretion induced by continuous exogenous ghrelin is modified by somatostatin and requires intact growth hormone-releasing hormone (GHRH) secretion, as demonstrated by rat models (51). In healthy adults, somatostatin infusion decreases endogenous ghrelin levels (5). In addition, growth hormone-related peptide-2 induced GH secretion is decreased markedly in people with inactivating mutations of the GHRH receptor (16). High ghrelin levels in AN may thus increase basal GH secretion and increase the number of secretory bursts by overriding background somatostatin tone, and by a synergistic effect on underlying GHRH secretion.

Even though stepwise regression analysis in this study suggests that ghrelin rather than undernutrition is the strongest predictor of GH basal secretion and burst frequency, a study design in which a ghrelin antagonist or placebo is administered to girls with AN would help confirm whether elevations in GH basal secretion and burst frequency in AN are a consequence of hyperghrelinemia, undernutrition per se, or both. Of note, studies with severe caloric deprivation and fasting do not demonstrate a change in ghrelin levels (9, 11). Conversely, the physiology of short-term fasting may significantly differ from that of the long-term caloric deprivation of AN, and chronic undernutrition may result in high levels of ghrelin, which may, in turn, potentiate the effects of undernutrition on GH. In addition, in both studies with short-term fasting, there was no change in fat mass at the end of the study period, and given the association between fat mass and insulin sensitivity, this may explain why ghrelin levels did not increase in these studies.

In this study of adolescent girls, we observed strong positive associations between ghrelin concentration parameters and cortisol concentration and secretion characteristics. Our observations are consistent with data from animal and adult studies, which demonstrate increased ACTH and cortisol concentration following exogenous ghrelin administration (4, 38, 52, 63). However, studies examining endogenous ghrelin levels in relation to cortisol concentrations report contradictory results. A lack of any relation between endogenous ghrelin and cortisol concentrations has been reported (45), as well as an inverse relationship in adults (13). We determined that ghrelin is an independent predictor of cortisol burst frequency, suggesting that elevated ghrelin levels in AN may in part be responsible for high cortisol levels in this condition. This is supported by data from our cross-correlation analysis, which showed that cortisol secretion followed ghrelin secretion, both in healthy adolescents and in girls with AN. Of note, the limitations of correlational analysis described earlier with respect to the relationship between ghrelin and GH also apply to the relationship we report between ghrelin and cortisol.

We observed inverse correlations between ghrelin and leptin in this study, in agreement with a reciprocal relationship postulated for ghrelin and leptin in energy balance (40, 54). Leptin levels are low in AN and high in obesity (18, 20, 27, 31, 34, 54, 58), whereas ghrelin levels are high in AN and low in obesity (13, 21, 32, 36). This is appropriate given that leptin suppresses appetite by its inhibitory effects on neuropeptide Y (NPY) and agouti-related peptide (AgRP) neurons and stimulatory effects on the proopiomelanocortin neurons, and ghrelin increases appetite by activating NPY/AgRP neurons. It is uncertain, however, whether one regulates levels of the other. Chan et al. (9) found no change in ghrelin levels after short-term administration of recombinant human leptin to healthy adults. In our study, measurements of ghrelin concentration and secretion were not independent predictors of fasting leptin levels, consistent with interventional studies.

We observed inverse correlations between endogenous ghrelin concentration and secretion parameters and total T3 levels, consistent with studies showing a decrease in TSH secretion in rodent models after exogenous ghrelin administration (66). In fact, nadir and valley mean ghrelin were independent predictors of total T3 levels. It is uncertain whether this is a direct effect of ghrelin or is indirectly mediated by increased GH secretion associated with high ghrelin levels. Indeed, GH secretory burst frequency was also an important predictor of total T3 values. Conversely, ghrelin did not predict TSH levels.

In this study, we observed inverse relationships between fasting ghrelin concentration and estradiol and LH levels. These data are consistent with results from a recent study in Rhesus monkeys in which continuous exogenous ghrelin infusion over a 3-h period resulted in a decrease in LH pulse frequency (63). Similarly, a study in rodents reported that decreased gonadotropin-releasing hormone (GnRH) stimulated LH secretion after ghrelin administration (14). Although girls with AN have a decrease in LH pulse amplitude rather than a decrease in LH pulse frequency, it is tempting to consider an effect of elevated ghrelin levels on gonadotropin secretion that may be mediated via effects of NPY on GnRH neurons. Our study was limited in that only a single LH level was obtained.

AJP-Endocrinol Metab • VOL 289 • AUGUST 2005 • www.ajpendo.org
Frequent sampling for LH may be necessary to definitely uncover associations between ghrelin and LH.

We thus demonstrate elevated ghrelin concentration parameters in girls with AN compared with controls, a consequence of increased burst mass and thus increased pulsatile and total secretion. The most significant predictor of ghrelin levels is insulin resistance, and endogenous ghrelin independently predicts basal GH secretion and secretory burst frequency. Ghrelin is also an independent predictor of cortisol, total T3, and LH values. Endogenous ghrelin parameters in adolescents thus demonstrate associations with GH, cortisol, total T3, and LH that are consistent with intervention studies using exogenous ghrelin in adults and in animal models. Determining the dynamics of ghrelin secretion and effects on hypothalamic neuropeptides may be key to better understanding the regulators of secretion of other pituitary hormones, including GH, ACTH, TSH, and gonadotropins. More studies are also necessary to separate out the effects of hyperghrelinemia from those of undernutrition on pituitary hormones and to determine whether in fact hyperghrelinemia resulting from chronic undernutrition drives or contributes to alterations in nutritionally regulated hormones.

ACKNOWLEDGMENTS

We thank Ellen Anderson and the Bionutrition team, as well as the skilled nursing staff, of the General Clinical Research Center, Massachusetts General Hospital, for help in competing this study. In addition, we thank Jeffrey Breu of the Core Laboratory of the Massachusetts Institute of Technology for help in analyzing our ghrelin samples, and Rita Tsay and team for performing and analyzing the DEXA scans. Most of all, we thank deeply our subjects, without whose participation this study would not have been possible.

GRANTS

This work was supported in part by National Institutes of Health Grants M01-RR-01066, DK-062249, and K23-RR-018851.

REFERENCES

Ghrelin secretion in anorexia nervosa


