Fasting Ghrelin Levels in Physically Active Women: Relationship with Menstrual Disturbances and Metabolic Hormones

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Recent findings support a role for ghrelin in the regulation of energy homeostasis and possibly reproductive function. The primary purpose of this study was to test whether differences in fasting ghrelin levels exist in exercising women with differing menstrual and metabolic status. Menstrual cycle status was defined as sedentary ovulatory (SedOvul; n = 10, cycles = 26), exercising ovulatory (ExOvul; n = 11, cycles = 22), exercising luteal phase defect/anoovulatory (ExLPD/Anov; n = 11, cycle = 27), and exercising amenorrheic (ExAmen; n = 8, cycle = 16). Subjects were 27.7 ± 1.2 yr of age, weighed 60.2 ± 3.3 kg, and had menstrual cycle lengths of 28.4 ± 0.9 d. Blood was collected during the follicular phase (d 2–9) of each menstrual cycle and analyzed for total ghrelin, insulin, total T₄, and leptin. Ghrelin was significantly elevated by approximately 85% in the ExAmen category (725.5 ± 40.8 pmol/liter) when compared with all other categories (P < 0.001; SedOvul = 393.6 ± 32.0 pmol/liter, ExOvul = 418.9 ± 34.8 pmol/liter, and ExLPD/Anov = 381.1 ± 314 pmol/liter). Leptin levels were lower in all groups vs. SedOvul (P < 0.001). Insulin was lower in both the ExLPD/Anov and ExAmen categories vs. SedOvul and ExOvul (P < 0.018), and total T₄ was lower in ExAmen compared with all other groups (P < 0.001), with concentrations in ExLPD/Anov and ExOvul exceeding those in SedOvul (P < 0.05). These data clearly indicate a metabolic hormonal profile consistent with chronic energy deficiency in exercising women across a range in menstrual status and introduces ghrelin as a potential supplementary indicator that uniquely discriminates amenorrheic athletes from athletes with other menstrual disturbances. (J Clin Endocrinol Metab 89: 3536–3542, 2004)

**Ghrelin is a 28-amino acid GH-releasing acylated peptide that is primarily produced in a distinct endocrine cell type of the stomach and gastrointestinal tract (1).** It is the endogenous ligand for the GH secretagogue receptor (GHS-R), which is strongly expressed in the arcuate nucleus and ventromedial hypothalamus, regions recognized as critical for the regulation of food intake (2). In humans, plasma ghrelin levels rise before meals and fall within 1–2 h after meals (3), and ghrelin levels increase with fasting (4), hypoglycemia (4), and diet-induced weight loss in obese patients (5) and decrease during refeeding (3, 6). When ghrelin is administered iv in humans, it stimulates both food intake and appetite (6). Fasting levels are decreased in obese individuals (7–9) and increased in anorexic patients (10–12). Interestingly, McConnell et al. (13) recently showed in a prospective study that basal ghrelin levels were responsive to energy-deficit-induced decreases in body weight, fat mass, and resting metabolic rate in normal-weight young women.

Thus, the current literature suggests that ghrelin is a primary peripheral metabolic signal for hunger, meal initiation (food intake), and energy homeostasis, i.e. ghrelin plays a key role as a metabolic signal indicating an energy deficit, potentially acting to restore energy homeostasis (9, 14, 15).

Recently, it has been suggested that, in addition to its role in regulating food intake and energy balance, ghrelin may also play a physiological role in reproductive function via actions on LH pulsatility. Furuta et al. (16) recently demonstrated that the intracerebroventricular administration of 0.1 nmol ghrelin rapidly suppressed LH pulse frequency in ovariectomized rats treated with a small dose of 17-β estradiol. LH pulsatility is controlled by the hypothalamic release of GnRH, primarily from the arcuate nucleus and the ventromedial hypothalamus (17), a region that also expresses GHS-R (2). Ghrelin levels have been shown to be elevated in patients with amenorrhea associated with anorexia nervosa (10, 11) and decreased in women with anorexia nervosa during weight gain (12). The known metabolic actions of ghrelin, combined with a potential role in the modulation of reproductive hormone secretion, make it reasonable to suspect that ghrelin may play a physiological role in the suppression of reproductive function via hypothalamic suppression of GnRH release in women with exercise-associated menstrual disturbances. In support of the latter idea, existing evidence clearly shows a strong association between energy deficiency and menstrual disturbances in physically active

**Abbreviations:** BMI, Body mass index; E1C, estrone conjugates; ExAmen, exercising amenorrheic; ExLPD/Anov, exercising luteal phase defect/anoovulatory; ExOvul, exercising ovulatory; GHS-R, growth hormone secretagogue receptor; LPD, luteal phase defect; PdG, pregnanediol-3-glucuronide; SedOvul, sedentary ovulatory; VO₂, oxygen uptake.

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women (18–22), and evidence exists that low energy availability plays a causal role in the induction of exercise-associated amenorrhea (23, 24). However, the relationship of ghrelin levels to menstrual disturbances, including amenorrhea, in exercising women has not been evaluated to date. The primary purpose of this study was to examine ghrelin levels in physically active women who differ in menstrual status and metabolic status.

**Subjects and Methods**

**Experimental design**

This study used a prospective observational design to explore the relationship between ghrelin and other metabolic hormones in women of differing menstrual status. The metabolic hormones include total T₃, leptin, and insulin. Blood samples previously collected from subjects in whom metabolic hormones and menstrual status were carefully documented over three menstrual cycles (18, 19) were analyzed for ghrelin and compared with levels of metabolic hormones and ghrelin in blood samples from amenorrheic subjects from a new cohort of subjects.

**Subjects**

This study was approved by the New Britain General Hospital Institutional Review Board, the University of Connecticut Health Center Institutional Review Board, and the University of Toronto Human Ethics Committee. Specific admission criteria have been previously published (18, 19). Forty subjects participated in this study on ghrelin; 32 women were from the original cohort study on menstrual and metabolic status previously published (18, 19), and eight amenorrheic subjects were tested in association with a different cohort of subjects from the University of Toronto meeting identical admission criteria, except that they had not menstruated for at least 3 months.

**Training status**

Daily training activities were recorded throughout the study. Distance, duration, and heart rate at the termination of exercise were also recorded. Exercise training volume was defined as minutes of activity per week, as recorded on the training logs. Other physical activity performed for 3 or more sustained minutes was also recorded. Weight, menstrual patterns, nutritional and training habits, and any unusual stress events (self report) that might affect ovulatory function were monitored throughout this study. Subjects were weighed weekly.

**Blood sampling**

Overnight fasting blood samples were drawn between d 2–9 of the menstrual cycle in eumenorrheic subjects and weekly for two 30-d periods in the amenorrheic subjects. All serum analyses were made from these samples. Blood samples were processed according to previously described procedures (18, 19). Samples had not been previously thawed and sample pH was determined to be in the physiological range.

**Menstrual categorization**

Menstrual calendars were used 1 month before the study and for the duration of the study to record the first and last day of menses for each cycle. Women who had menstrual cycles of less than 20 d or greater than 38 d during the 3-month monitoring period were excluded from these data analyses. For this substudy on ghrelin levels, 10 sedentary and 22 exercising subjects from the previously published studies (18, 19) and eight exercising amenorrheic women from a different cohort were included in this study on ghrelin.

**Determination of menstrual status**

Menstrual status in eumenorrheic subjects was determined using previously published methods (18, 19). Daily urine samples were assayed for creatinine, LH, pregnanediol-3-glucoronide (PdG), and estrone conjugates (EIC). Menstrual status was determined in amenor-
software was not available at that site. For the amenorrheic subjects at
the University of Toronto, body fat was determined using dual-energy
x-ray absorptiometry measurements using a total body scanner (en-
CORE 2002 software, version 6.50.069; General Electric Lunar Corpo-
ration, Madison, WI). This instrument has a precision of less than 1%'
coefficient of variation for the total body composition measurements.
The separation of soft tissue into fat (g) and lean tissue (g) was based on
the attenuation ratio of high-energy and low-energy photons. Fat-free
mass (g) was calculated as the sum of lean tissue and bone mineral
content (g).

Statistical methods
Data for demographics, menstrual cycle characteristics, and repro-
ductive hormones were analyzed using one-way ANOVA, with indi-
viduals grouped according to the predominant menstrual cycle status
they exhibited and whether or not they were sedentary or exercised, i.e.
sedentary ovulatory (SedOvul), exercising ovulatory (ExOvul), exercis-
ing luteal phase defect/anovulatory (ExLPD/Anov), or exercising am-
enorrheic (ExAmen). When comparing whether metabolic hormones
differed between menstrual cycles of different status, a mixed-model
ANOVA, accounting for the fact that cycles from a given individual may
fall into more than one menstrual cycle category, was used. When a
significant main (fixed) effect was observed, Student’s t tests were used
with a Bonferroni correction for multiple comparisons to determine
where the significant differences existed. Analysis of covariance was
used to test whether differences in leptin between groups differing in
individual menstrual status depended on percent body fat. Pearson-product
moment correlation analysis was used to examine relationships among
variables. Data were analyzed using the Statistica (Statsoft, Tulsa, OK)
and SPSS (SPSS Inc., Chicago, IL) software packages. A significance level
of \( P < 0.05 \) was used to detect the differences for all statistical
procedures.

Results
Demographic and anthropometric characteristics
The demographic characteristics of the study participants
are presented in Table 1. Subjects in the ExLPD/Anov and
ExAmen groups were significantly younger than the subjects
in the ExOvul group (\( P < 0.05 \)). Height, weight, BMI, and
percent body fat were not significantly different among the
groups, although there was a trend for the ExLPD / Anov and
ExAmen groups to have lower fat mass (\( P < 0.07 \)). As ex-
pected, \( \text{VO}_2 \) peak was significantly higher in the exercise
groups compared with the SedOvul group. The body weight
of the subjects did not differ (\( P > 0.05 \)) from week to week
across the menstrual cycles monitored (data not shown).

Training characteristics
Within the exercise groups of women, exercise time was
spent in weight-bearing physical activities such as bicycling,
step machine, walking or running, resistance training, hik-
ing, tennis, biking, and racquetball. The volume of exercise
performed by these subjects was typical of moderate or rec-
reational runners (eumenorrheic subjects, 5.0 \( \pm \) 0.7 h/wk;
and amenorrheic subjects, 6.2 \( \pm \) 1.0 h/wk).

Subject classification
The sample population consisted of a cohort of women who
were initially classified as either sedentary eumenorrheic
women (performing no more than 1 h of aerobic ex-
ercise per week for the past 12 months, with a peak \( \text{VO}_2 \) of
less than 35 ml/kg/min) or exercising women (performing
at least 2 h of exercise for the past 12 months, with a \( \text{VO}_2 \) peak
greater than 35 ml/kg/min). All subjects completed a max-
imal exercise test to exhaustion to document peak aerobic
power (\( \text{VO}_2 \) peak).

All eumenorrheic subjects were subsequently classified
after a two- or three-cycle prospective evaluation of training
status (determined by analysis of training diaries) and men-
strual status (determined by hormonal evaluations) during a
2- or 3-month study period. For training status, the following
two general categories were used: sedentary and exercising.
For ovulatory status and determination of luteal phase sta-
tus, previously published criteria were used (26, 27). Based
on these criteria, individual menstrual cycles were classified
as ovulatory, luteal phase deficient (LPD), or anovulatory.
Confirmation of amenorrheic status in those subjects self-
reporting the absence of menses was made from the analysis of
LH, estradiol, and progesterone in weekly blood samples
over the 60-d period of observation. Individuals were as-
signed to groups of menstrual status based on the predom-
inant classification of the menstrual cycles observed for each
individual, as previously described (19). Individuals with
either three out of three or two out of three cycles from one
category were placed in that category. The four combined
categories of exercise and menstrual cycles evaluated were as
follows: sedentary women (\( n = 10 \) subjects) with ovulatory
menstrual cycles (SedOvul, \( n = 26 \) cycles), exercising women
(\( n = 11 \) subjects) with ovulatory menstrual cycles (ExOvul,
\( n = 22 \) cycles), exercising women (\( n = 11 \)) with LPD and/or
anovulatory menstrual cycles (ExLPD/ANOV, \( n = 27 \)), and
exercising women (\( n = 8 \)) with amenorrhea (ExAmen, \( n =
16 \) cycles) (30-d periods of observation). One sedentary
woman with LPD menstrual cycles (\( n = 3 \) cycles) was ex-
cluded from this study because the LPD cycles observed did
not meet the SedOvul group status. Two exercising women
with ovulatory and LPD cycles (\( n = 3 \)) were also excluded from
data analyses for this study when inadequate volumes of
serum were available for the ghrelin assays.

Table 1. Demographic characteristics of the individuals in each group

<table>
<thead>
<tr>
<th></th>
<th>SedOvul (n = 10)</th>
<th>ExOvul (n = 11)</th>
<th>ExLPD/Anov (n = 11)</th>
<th>ExAmen (n = 8)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (yr)</strong></td>
<td>26.6 ± 1.2</td>
<td>29.9 ± 1.6</td>
<td>25.6 ± 1.9</td>
<td>23.0 ± 1.2</td>
<td>0.037</td>
</tr>
<tr>
<td><strong>Height (cm)</strong></td>
<td>163.3 ± 1.5</td>
<td>165.3 ± 2.6</td>
<td>163.1 ± 2.2</td>
<td>165.5 ± 1.4</td>
<td>0.770</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>62.4 ± 5.1</td>
<td>58.7 ± 2.4</td>
<td>59.3 ± 2.3</td>
<td>56.7 ± 1.6</td>
<td>0.695</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>23.2 ± 1.5</td>
<td>21.6 ± 0.8</td>
<td>22.2 ± 0.6</td>
<td>20.8 ± 0.8</td>
<td>0.394</td>
</tr>
<tr>
<td><strong>Body fat (%)</strong></td>
<td>24.7 ± 2.4</td>
<td>21.8 ± 1.7</td>
<td>18.6 ± 1.2</td>
<td>20.5 ± 2.5</td>
<td>0.163</td>
</tr>
<tr>
<td><strong>VO₂ peak (ml/kg/min)</strong></td>
<td>30.2 ± 1.4</td>
<td>40.9 ± 2.2</td>
<td>41.8 ± 1.2</td>
<td>44.2 ± 2.0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

\( a \) ExLPD/Anov, ExAmen vs. ExOvul.

\( b \) SedOvul vs. ExOvul, ExLPD/Anov, ExAmen.
Menstrual cycle categorization and characteristics

The menstrual cycle parameters are presented in Table 2. Thirty-five women participated in the prospective evaluation of menstrual status, which was previously published (19). For this substudy examining ghrelin, 10 sedentary and 30 exercising subjects, in whom ghrelin measurements were obtained, were included. For menstrual category comparisons, women were classified according to the predominant menstrual cycle type (ovulatory, LPD, or anovulatory) that they displayed in at least two of the three or two of the two menstrual cycles that were evaluated. The sedentary group included 10 women; six had three of three and four had two of two ovulatory menstrual cycles. These 26 menstrual cycles comprised the SedOvul categorization of menstrual cycles. The exercising women included 11 women with 22 menstrual cycles that comprised the ExOvul category of menstrual cycles; three women had three of three, two women had two of two, five women had two of three, and one woman had one of two ovulatory menstrual cycles. The exercising women also included nine women with 20 menstrual cycles that comprised the ExLPD category of menstrual cycles; four women had three of three, one woman had two of two, three women had two of three, and one woman had one of two LPD menstrual cycles. The subject groups were similar with respect to age of menarche (Table 2), but gynecological age was significantly lower in the ExLPD/Anov and Ex/Amen vs. the SedOvul groups (P < 0.05) due to their lower chronological age (P < 0.05). Although menstrual cycle length was similar among eumenorrheic subjects, the ExOvul subjects had a significantly longer follicular phase than both the SedOvul and ExLPD/Anov groups; similarly, the ExOvul category had significantly lower PdG concentrations in these same two categories of PdG excretion (P < 0.05) than the SedOvul group. These characteristics are consistent with suppressed luteal phase production of progesterone in exercising women (19).

Estrogen and progesterone production in menstruating subjects

Concentrations of E1C excretion are presented in Table 3. E1C was significantly lower in the follicular and luteal phases of the ExLPD/Anov group compared with the SedOvul and ExOvul groups (P < 0.05). Concentrations of PdG excretion are presented in Table 3. The ExLPD/Anov category of cycles had significantly lower luteal phase PdG levels and a lower 3-d sum of PdG when compared with concentrations in the SedOvul and ExOvul groups; similarly, the ExOvul category had significantly lower PdG concentrations in these same two categories of PdG excretion (P < 0.05) than the SedOvul group. These characteristics are consistent with suppressed luteal phase production of progesterone in exercising women (19).

Serum reproductive hormone levels in the amenorrheic subjects

In the ExAmen subjects, serum concentrations of estradiol remained constantly low and less than 30 pg/ml (110 pmol/liter), which is consistent with suppression of follicular development. Serum LH levels remained less than 10 mIU/liter (10 mIU/ml), confirming the absence of ovulation. The amenorrheic subjects failed to demonstrate any evidence of luteinization because levels of serum progesterone were less than 1 ng/ml (3.18 nmol/liter) (26). These reproductive hormone concentrations confirm the amenorrheic status of these subjects.

Ghrelin

Ghrelin concentrations were approximately 100% higher in the ExAmen group, whereas no differences in concentrations existed across all other menstruating groups, as shown in Fig. 1. Regression analyses to examine the contribution of

**TABLE 2.** Menstrual cycle characteristics of the eumenorrheic and amenorrheic subjects

<table>
<thead>
<tr>
<th></th>
<th>SedOvul (n = 10)</th>
<th>ExOvul (n = 11)</th>
<th>ExLPD/Anov (n = 11)</th>
<th>ExAmen (n = 8)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of menarche (yr)</td>
<td>13.3 ± 0.4</td>
<td>12.4 ± 0.3</td>
<td>12.9 ± 0.3</td>
<td>13.5 ± 0.5</td>
<td>0.417</td>
</tr>
<tr>
<td>Eumenorrheic subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Menstrual cycle length (d)</td>
<td>28.8 ± 0.8</td>
<td>28.9 ± 1.7</td>
<td>26.0 ± 0.7</td>
<td>NA</td>
<td>0.161</td>
</tr>
<tr>
<td>Folllicular phase (d)</td>
<td>16.1 ± 0.7</td>
<td>14.0 ± 0.7^a</td>
<td>16.5 ± 0.8</td>
<td>NA</td>
<td>0.038</td>
</tr>
<tr>
<td>Luteal phase (d)</td>
<td>12.5 ± 0.5</td>
<td>13.1 ± 0.5</td>
<td>9.8 ± 0.7^b</td>
<td>NA</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Amenorrheic subjects</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>8.1 ± 0.9</td>
<td>NA</td>
</tr>
<tr>
<td>Duration of amenorrhea (months)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NA, Not applicable. Values are mean ± SEM.

^a ExOvul vs. SedOvul, ExLPD/Anov.

^b ExLPD/Anov vs. SedOvul, ExOvul.

**TABLE 3.** Ovarian steroid data of the menstrual categories of eumenorrheic cycles

<table>
<thead>
<tr>
<th>Eumenorrheic subjects</th>
<th>SedOvul (n = 26 cycles)</th>
<th>ExOvul (n = 22 cycles)</th>
<th>ExLPD/Anov (n = 27 cycles)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean E1C, follicular phase^a</td>
<td>42.7 ± 2.8</td>
<td>43.3 ± 3.2</td>
<td>34.7 ± 2.8^c</td>
<td>0.045</td>
</tr>
<tr>
<td>Mean E1C, luteal phase^a</td>
<td>49.8 ± 4.0</td>
<td>53.2 ± 4.4</td>
<td>41.4 ± 4.0^c</td>
<td>0.048</td>
</tr>
<tr>
<td>Mean PdG, luteal phase^b</td>
<td>4.9 ± 0.3</td>
<td>3.5 ± 0.3^d</td>
<td>2.3 ± 0.3^c</td>
<td>0.029</td>
</tr>
<tr>
<td>3-d sum of PdG peak^c (≥1 d of PdG peak)</td>
<td>21.4 ± 1.6</td>
<td>16.9 ± 1.7^d</td>
<td>9.0 ± 1.6^c</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

^a All E1C concentrations are expressed as ng/mg creatinine.

^b All PdG concentrations are expressed as µg/mg creatinine.

^c ExLPD/Anov vs. Sed Ovul, ExOvul.

^d Sed Ovul vs. ExOvul.
Table 4.

SedOvul, ExOvul, ExLPD/Anov) as seen in the SedOvul group (Table 4). However, concentrations in ExAmen were significantly lower than all other groups. Leptin was significantly lower in all exercising groups when compared with the SedOvul group. An analysis of covariance, with percent body fat selected as a covariate, revealed that leptin concentrations remained significantly different between the SedOvul group and all other groups (*P < 0.05). Insulin was significantly lower in ExLPD/Anov and ExAmen groups when compared with the ExOvul and SedOvul groups.

Discussion

This represents the first attempt to explore the association between fasting ghrelin levels and a spectrum of exercise-associated menstrual disturbances, including amenorrhea, in healthy young exercising women. Fasting ghrelin concentrations were at least 85% greater in the subjects with exercise-associated amenorrhea. More subtle menstrual disturbances, including LPD and anovulation that are associated with normal menstrual cycle intervals of approximately 28 days, failed to significantly impact fasting ghrelin levels.

The observed elevation of fasting ghrelin in our amenorrheic athletes is similar to numerous observations of elevated ghrelin with amenorrhea that accompanies anorexia nervosa (11, 12, 32) and, thus, may be explained by the mutual association of the two conditions with chronic energy deficiency. In contrast to healthy women, anorexic patients do not exhibit the typical fall in plasma ghrelin levels after a standardized or fiber meal (even 2 h after the meal), representing an impaired ghrelin response to an acute meal (32). Presumably, the single meal is inadequate to restore energy homeostasis, and thus, ghrelin levels remain elevated to force an ongoing peripheral signal in the direction of energy intake. Nedvidkova et al. (32) suggest that these findings in anorexic patients represent an adaptive metabolic strategy secondary to prolonged food restriction and inadequate energy stores that attempts to restore normal feeding patterns by maintaining the drive to eat. When anorexic patients regain body weight and replenish energy stores, decreased ghrelin levels are observed (12), presumably indicating more adequate energy stores and weight regain that may possibly

Metabolic hormones

Total T₃ was significantly lower in both the ExOvul and ExLPD/Anov groups when compared with concentrations in the SedOvul group (Table 4). However, concentrations in ExAmen were significantly lower than all other groups. Leptin was significantly lower in all exercising groups when compared with the SedOvul group. An analysis of covariance, with percent body fat selected as a covariate, revealed that leptin concentrations remained significantly different between the SedOvul group and all other groups (*P < 0.05). Insulin was significantly lower in ExLPD/Anov and ExAmen groups when compared with the ExOvul and SedOvul groups.

Values are mean ± SEM.
a SI units.
b ExAmen vs. SedOvul, ExOvul, ExLPD/Anov.
c ExOvul, ExLPD/Anov vs. SedOvul.
d ExOvul, ExLPD/Anov, ExAmen vs. SedOvul.
e ExLPD/Anov, ExAmen vs. SedOvul.

<table>
<thead>
<tr>
<th></th>
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<th>ExLPD/Anov (n = 27 cycles)</th>
<th>ExAmen (n = 16 cycles)</th>
<th>P</th>
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<tbody>
<tr>
<td>Ghrelin</td>
<td></td>
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<td></td>
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<tr>
<td>pg/ml</td>
<td>1326.8 ± 107.9</td>
<td>1412.1 ± 117.3</td>
<td>1284.7 ± 105.85</td>
<td>2445.7 ± 137.5ᵇ</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pmol/literᵃ</td>
<td>393.6 ± 32.0</td>
<td>418.9 ± 34.8</td>
<td>381.1 ± 31.4</td>
<td>725.5 ± 40.8ᵇ</td>
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<tr>
<td>Total T₃</td>
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<tr>
<td>ng/ml</td>
<td>140.0 ± 6.2</td>
<td>108.7 ± 6.5ᵃ</td>
<td>115.5 ± 6.5ᵇ</td>
<td>52.1 ± 6.4ᵇ</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pmol/literᵃ</td>
<td>2.15 ± 0.1</td>
<td>1.67 ± 0.1ᵇ</td>
<td>1.775 ± 0.1ᵇ</td>
<td>0.8 ± 0.1ᵇ</td>
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<tr>
<td>Leptin</td>
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</tr>
<tr>
<td>ng/ml</td>
<td>13.5 ± 1.0</td>
<td>5.0 ± 1.0ᵈ</td>
<td>6.2 ± 0.9ᵈ</td>
<td>4.1 ± 1.3ᵈ</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>µg/literᵃ</td>
<td>13.5 ± 1.0</td>
<td>5.0 ± 1.0ᵈ</td>
<td>6.2 ± 0.9ᵈ</td>
<td>4.1 ± 1.3ᵈ</td>
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<tr>
<td>Insulin</td>
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<tr>
<td>µIU/ml</td>
<td>8.2 ± 0.9</td>
<td>8.3 ± 1.1</td>
<td>4.1 ± 1.1ᵇ</td>
<td>4.3 ± 1.2ᵇ</td>
<td>0.018</td>
</tr>
<tr>
<td>pmol/literᵃ</td>
<td>59.0 ± 6.9</td>
<td>59.7 ± 8.3</td>
<td>30.0 ± 8.2ᵇ</td>
<td>31.2 ± 9.0ᵇ</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Serum concentrations of ghrelin (pmol/liter) for the groups of cycles for the SedOvul (n = 26), ExOvul (n = 22), ExLPD/Anov (n = 27), and ExAmen groups (n = 16). Data are expressed as the mean ± SEM (to convert to picograms per milliliter, multiply by 3.371). *, Significantly (*P < 0.001) different from the other groups (ExAmen vs. SedOvul, ExOvul, ExLPD/Anov).

TABLE 4. Ghrelin and metabolic hormone concentrations of the menstrual categories of eumenorrheic and amenorrheic cycles
restore the normal acute ghrelin response to a meal. Whether amenorrheic athletes exhibit a similarly impaired ghrelin meal response remains to be determined.

Fasting ghrelin levels in obese and anorexic individuals are inversely correlated with body energy stores (7, 9, 11, 12). One possible explanation for this is that ghrelin acts as a compensatory mechanism to return individuals to a body weight set point. In this study, subjects in groups of differing menstrual status were similar in body weight, BMI, and body composition, perhaps limiting the opportunity to observe a correlation with ghrelin in such a homogenous subject sample. When compared with the results for insulin, leptin, and T

β

the disproportionately high ghrelin levels in amenorrheic athletes suggest that ghrelin may be unique in reflecting a prolonged chronic state of chronic energy deficiency that is no longer effecting changes in body weight and composition but is associated with a strong signal to increase food intake and return body weight and body composition to its original state of energy homeostasis. Although detailed information about energy balance, weight, and dieting history was not gathered in the current study, it would be interesting to know whether the subjects in the amenorrheic group differed in their current state of energy balance, weight, level of body fat stores, or resting energy expenditure, representing deviations from earlier, higher levels. If the latter were true, it might indicate that ghrelin is acting to return an individual to some weight, metabolic, or body composition set point. In support of this, studies have reported that amenorrheic athletes report higher scores for cognitive restraint (33), a subscale of the Three Factor Eating Questionnaire (34) that indicates a conscious restriction of calories that presumably overrides normal physiological signals of hunger. Although data pertaining to estimates of energy balance are not available in our amenorrheic subjects, it is interesting to note that, in comparison to similar studies in amenorrheic athletes (20, 22), our subjects exhibit a lower maximum VO

2

and a lower weekly training volume. In light of this, it is possible that chronic energy deficiency in our subjects may be more due to a lower than expected dietary intake that may be secondary to cognitive restraint (34) than a high exercise energy expenditure. In fact, data collected only in the amenorrheic subjects in this study revealed that the average score for this subscale (34) was in the clinical range, i.e. greater than 14. Whether a difference in how an energy deficit is created, i.e. increased exercise energy expenditure vs. decreased food intake, corresponds to differences in ghrelin concentrations is unknown.

The association of elevated fasting ghrelin levels with a state of inadequate energy availability in the amenorrheic athletes is supported by associated changes in insulin, T

β

and leptin, and strong corroboration for disruptions in energy homeostasis with exercise-associated disturbances comes from numerous previous studies (18–21, 23, 35–37). These studies support the existence of metabolic and endocrine adaptations to conserve energy in the face of inadequate energy intake. These adaptations include lower resting metabolic rates (35, 36), low plasma glucose levels (22), low T

β

(20), low insulin (22), low leptin (38), low IGF-1 (22) and low IGF-1/IGF binding protein-1 (22) and increased levels of human GH (22, 37) and cortisol (39, 40). These hormonal and metabolic shifts are also observed in other nonathletic women with functional hypothalamic amenorrhea and are also secondary to self-imposed severe nutritional restrictions, particularly of dietary fat (41, 42).

Although many of the endocrine and metabolic alterations in exercise-associated amenorrhea represent changes in key metabolic signals that have been shown to modulate GnRH neuronal activity in human and animal studies examining the link between metabolism and reproduction (43), a direct causal relationship between one or several of these factors and exercise-associated amenorrhea has not been established. Interestingly, intracerebroventricular administration of ghrelin has been shown to rapidly suppress LH pulse frequency in ovariectomized rats (16). Ghrelin has also been found in the hypothalamus (44), and in the rat, GHS-R receptors have been found in the arcuate nucleus (45, 46). If evidence for ghrelin as a modulator of the reproductive axis continues to mount, the finding in this study that elevated ghrelin levels uniquely discriminated the amenorrheic athletes from those with less severe menstrual disturbances may represent an important contribution to the potential role for ghrelin in the complete suppression of reproductive function in extreme states of chronic energy deficiency. Future studies examining whether changes in both fasting- and meal-related responses in ghrelin correlate with the development of exercise-associated menstrual disturbances will undoubtedly unravel the relationship between this important regulator of energy homeostasis and reproductive function.

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