Twenty-Four Hour Growth Hormone and Leptin Secretion in Active Postpubertal Adolescent Girls: Impact of Fitness, Fatness, and Age at Menarche


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Context: GH is strongly related to body composition, physical activity, and pubertal progression. Adolescent girls decrease physical activity during puberty, whereas their weight increases. Because leptin is a good index of energy balance in active young women, we hypothesized that leptin is related to GH secretion in this population while taking into account fitness, fatness, and age at menarche.

Methods: We measured body composition and maximal oxygen consumption (VO₂max) in 37 postpubertal adolescent girls aged 16–21 yr. GH was sampled every 10 min and leptin hourly for 24 h. We first analyzed 6-h time blocks by repeated measures for GH and leptin, with body mass index (BMI), percent body fat, and VO₂max as covariates for the entire group and a lean subgroup. The deconvolution method was used to characterize GH pulsatility from individual time points.

**Results:** GH varied through the day (P < 0.0001), with the highest concentrations overnight. BMI, percent body fat, and VO₂max were related to GH concentrations in the entire group, whereas leptin predicted GH in the entire group as well as the lean subgroup of girls. Higher leptin was related to lower GH concentrations (P = 0.011), regardless of time. A log leptin level increase by 1 unit decreased GH by 27%. Pulsatility characteristics showed a 1-yr increase of age at menarche increasing total GH input by 20% (P = 0.0035) independently from BMI.

**Conclusion:** In postpubertal adolescent girls, leptin is related to GH concentration across the lean to overweight BMI spectrum. GH pulsatile secretion was greater in girls with later age at menarche.

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**Subjects and Methods**

The protocol was approved by the Institutional Review Board of the University of Michigan Health System. Thirty-seven healthy adolescents aged 16–21 yr with a BMI between the 10th and 95th percentile for age (based on growth charts issued by the National Institute for Health Statistics) were studied a minimum of 2 yr after the age at menarche as part of a study on exercise and the menstrual cycle. Because the range of menarche in the United States is 11–16 yr (14), onset of menarche at 16 yr was selected as the upper limit for inclusion in the study. Young women were recruited from local university campuses and high schools and were enrolled after signing an institutional review board-approved consent form. An additional signature was required from one parent or legal guardian for all participants under 18 yr of age. All participants were healthy and taking no medications. Individuals who had used any hormonal method of birth control or reported a significant weight loss (more than 10%) within the last year were excluded. A 3-d prospective food diary was collected and analyzed. All girls were asked to estimate, if any, how many hours of exercise they practiced each week (reported

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Abbreviations: BMI, Body mass index; CV, coefficient of variation; VO₂max, maximal oxygen consumption.

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exercise) and describe the type of exercise. Self-described sedentary girls who reported no planned physical activity were not excluded. All participants attended classes on a college or high school campus, and all walked from class to class. In addition, we characterized fitness with the measure of maximal aerobic capacity (VO_{2max}) as determined by a treadmill test following the Bruce protocol (15). Relative body fat was obtained by dual-energy x-ray absorptiometry using a total body scanner (model DPX-L; Lunar Radiation Corp., Madison, WI) (16). As a screen for potential causes of pituitary dysfunction, TSH and prolactin were assessed in all adolescents. Pregnancy was excluded by serum measurement of human chorionic gonadotropin.

All participants with an established menstrual cycle were instructed to contact the principal investigator at the start of the menstrual cycle and were admitted to the General Clinical Research Center of the University of Michigan within the first 10 d of the cycle. The follicular phase and were admitted to the General Clinical Research Center was also timed on the night before the study to allow for acclimatization. By 0700 h, an iv catheter was inserted into the forearm, and blood was sampled 24 h for GH every 10 min as well as leptin every hour.

### Assays

Plasma samples were stored at −70 C until assayed. Plasma GH concentrations were measured using a chemiluminescent enzyme immunometric assay for use with the Immulite automated analyzer (Diagnostic Products Corp., Los Angeles, CA). Assay sensitivity was 0.01 ng/ml. The intra- and interassay coefficients of variation (CVs) were 2.0 and 4.5%, respectively. Leptin was measured using reagents from Linco Research (St. Charles, MO) with intra- and interassay CVs of 4.5 and 6.6%. To further characterize the GH axis (17), we measured IGF-I using a chemiluminescent enzyme immunometric assay for use with the Immulite automated analyzer (Diagnostic Products). The assay sensitivity was 20 ng/ml; intra- and interassay CVs were 2.5 and 10.7%, respectively.

### Analysis

The analysis applied two different approaches to first describe more general patterns throughout the day, focused on large time blocks, and then further detailing pulsatile characteristics of GH derived from frequent individual samples. We took into account fitness as measured by maximal oxygen consumption, fatness as measured by total body percent body fat, and age at menarche.

**Time-block means analysis.** As a first step, we delineated 6-h time blocks to see whether there were relationships that could be related to integrated windows of time reflecting different times of the day (overnight, early morning, midday, and evening). Because of the nighttime prominence of GH in youth, 2300 h was arbitrarily chosen as the beginning of the first time block or time 1. The 145 time points of GH and 25 hourly leptin measurements were analyzed as four 6-h blocks defined as follows: time 1 = 2300–0450 h, time 2 = 0500–1050 h, time 3 = 1100–1650 h; time 4 = 1700–2250 h. The mean concentration of both hormones for each time interval was then calculated and referred to as block mean. Repeated-measures ANOVA was used to determine the effects of time as well as the effect of body composition, VO_{2max}, and age at menarche on GH and leptin. A Tukey adjustment was used for post hoc analysis of different time periods. Each time block mean was log (base e) transformed before analysis.

**Pulsatility analysis.** As a second step, we analyzed the pulsatile characteristics of GH over 24 h. Deconvolution (18) was fitted to each series to detect and characterize the pulsatility in GH. Total input was defined as the sum of area under all the recognized GH pulses. Half-life, mean area under the curve, and total input were log (base e) transformed before analysis, and the number of pulses was square root transformed before analysis.

### Results

Subject characteristics are shown in Table 1. The average age at menarche was concordant with national norms (14). The average BMI corresponds to the 50–75th percentile for late adolescent girls and ranged from a minimum at the fifth to a maximum at the 95th percentile for age (19). IGF-I levels were comparable with age-specific norms (20). Fitness range was also comparable with published norms for adolescent girls (21). The range of reported exercise ranged from sedentary (or no planned exercise) to daily planned physical activity and averaged almost 4 h/wk at the time of enrollment. Types of reported exercise were all recreational and varied; they included use of gymnasium, in-line skating, snowboarding, diving, biking, resistance training, jogging, skiing, exercise videotapes, and figure skating.

**Leptin, BMI, percent body fat, and fitness**

We examined relationships between leptin as an index of energy balance and weight, adiposity, and fitness. Regardless of time of collection, a 1% increase in body fat related to a 7% increase of leptin ($P < 0.0001$), whereas a 1-unit increase in BMI increased leptin by 15% ($P < 0.0001$), and a 1-unit increase in VO_{2max} decreased leptin by 6% ($P < 0.0001$). When both percent body fat and VO_{2max} were studied together, percent body fat was still significantly related to leptin ($P < 0.0001$), but the significance of VO_{2max} disappeared ($P = 0.69$). However, BMI and VO_{2max} both retained significance within the same model for leptin ($P = 0.0032$ and 0.0154, respectively). Thus, the relationship between VO_{2max} and leptin is not mediated by BMI changes but appears dependent on differences in percent body fat.

**GH Time block analysis.**

There was marked diurnal variation for GH during the 6-h time block, with the highest concentrations reached overnight between 2300 and 450 h as outlined in Table 2. The relationships among BMI, percent body fat, VO_{2max}, and GH did not depend on the time of the day. However, when the analysis was restricted to participants with a BMI between 18 and 25 kg/m^2, these relationships with GH lost significance ($P$ value for BMI = 0.74, $P$ value for percent body fat = 0.11, $P$ value for VO_{2max} = 0.12).

**TABLE 1. Subjects’ characteristics (n = 37)**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SE</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>18.9 ± 1.5</td>
<td>16.0–21.0</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>23.1 ± 0.5</td>
<td>18.2–31.8</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>30.0 ± 1.4</td>
<td>17.0–49.7</td>
</tr>
<tr>
<td>Age at menarche (yr)</td>
<td>12.7 ± 0.2</td>
<td>10.0–16.0</td>
</tr>
<tr>
<td>Years from menarche</td>
<td>6.1 ± 0.3</td>
<td>2.0–11.0</td>
</tr>
<tr>
<td>Average caloric intake (kcal/d)</td>
<td>1896.2 ± 72.4</td>
<td>1027.7–3027.0</td>
</tr>
<tr>
<td>Average dietary fat intake (%)</td>
<td>30.0 ± 1.3</td>
<td>13.0–51.8</td>
</tr>
<tr>
<td>VO_{2max} (ml/kg/min)</td>
<td>41.1 ± 1.1</td>
<td>25.7–52.2</td>
</tr>
<tr>
<td>Reported exercise (h/wk)</td>
<td>3.8 ± 0.4</td>
<td>0.0–7.0</td>
</tr>
<tr>
<td>IGF-I (ng/ml)</td>
<td>316.0 ± 16.0</td>
<td>110.0–573.0</td>
</tr>
</tbody>
</table>
TABLE 2. Summary for mean GH (ng/ml) for four time-block periods

<table>
<thead>
<tr>
<th>Time</th>
<th>Mean ± SE</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.9 ± 0.4</td>
<td>0.5</td>
<td>11.5</td>
</tr>
<tr>
<td>2</td>
<td>1.5 ± 0.2</td>
<td>0.1</td>
<td>6.6</td>
</tr>
<tr>
<td>3</td>
<td>1.9 ± 0.2</td>
<td>0.2</td>
<td>5.7</td>
</tr>
<tr>
<td>4</td>
<td>2.0 ± 0.2</td>
<td>0.2</td>
<td>7.2</td>
</tr>
</tbody>
</table>

Time blocks in hours: time 1 = 2300–0450 h; time 2 = 0500–1050 h; time 3 = 1100–1650 h; time 4 = 1700–2250 h.

Univariate relationships between GH and BMI and percent body fat and VO2max for the entire group are shown in Fig. 1. VO2max, BMI, and percent body fat were all highly related. The Pearson coefficients of correlation were 0.87 for BMI and percent body fat, −0.58 for BMI and VO2max, and −0.75 for percent body fat and VO2max. As a result, none of the three variables emerged as a predominant predictor of GH when analyzed in a multiple regression model.

Leptin and GH

Leptin showed a marked diurnal variation with a peak between 2300 and 0450 h and a nadir in the afternoon followed by an evening rise (Table 3). Overall, higher leptin concentrations were related to lower GH concentrations ($P = 0.011$) and a log leptin level increase by 1 unit related to a mean GH decrease of 27%. This relationship was maintained regardless of the time block of observation. This relationship was also maintained when the analysis was restricted to the leanest girls with a BMI between 18 and 25 kg/m² ($P$ value for leptin = 0.015). Within the lean subgroup, a log leptin level increase by 1 unit related to a mean GH decrease of 26%. The correlation of leptin with weight and fitness indices was strong, and there was no independent effect of either variable on mean GH concentration.

GH pulsatility analysis

We studied five pulsatility parameters of GH: half-life, mean area under the curve, total input, input, and mean peak amplitude. Representative profiles are shown in Fig. 2. Half-life, number of pulses, and mean amplitude of GH were not significantly related to VO2max, BMI, or percent body fat (results not shown). The total input or pulse-related GH was linked to age at menarche ($P = 0.0035$). For every additional year at menarche GH total input increased by 20%. The total input of GH was not related to VO2max ($P = 0.16$) or BMI ($P = 0.11$); however, there was a negative trend with percent body fat that did not reach statistical significance ($P = 0.066$).

TABLE 3. Summary for mean leptin (ng/ml) for four time-block periods

<table>
<thead>
<tr>
<th>Time</th>
<th>Mean ± SE</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.9 ± 2.0</td>
<td>3.1</td>
<td>46.1</td>
</tr>
<tr>
<td>2</td>
<td>12.3 ± 1.7</td>
<td>2.7</td>
<td>41.9</td>
</tr>
<tr>
<td>3</td>
<td>11.7 ± 1.6</td>
<td>2.3</td>
<td>41.6</td>
</tr>
<tr>
<td>4</td>
<td>13.6 ± 1.6</td>
<td>2.7</td>
<td>37.3</td>
</tr>
</tbody>
</table>

Time 1 = 2300–0450 h; time 2 = 0500–1050 h; time 3 = 1100–1650 h; time 4 = 1700–2250 h.

We did not find a significant effect of leptin on the five pulsatility parameters.

Discussion

GH concentrations were higher in those girls who had higher VO2max and girls who were leaner. There was a high correlation between fitness and body composition as well. During and immediately after puberty, adolescent girls can experience substantial changes in levels of physical activity and body composition. By using VO2max as a surrogate measure of fitness (22), we observed that its effect on GH secretion pattern was not distinguishable from that of percent body fat. To characterize adiposity further, we measured leptin concentrations (4, 23), and we found, as expected, that percent body fat was the most critical determinant of leptin. Our focus was a population spanning a wide range of fitness from sedentary to very active and an equally wide range of BMI, from lean to obese. Our findings are in agreement with previous work in which athleticism in women represents a hypo leptinemic state, even when relative adiposity is accounted for (10, 24). The wide range of leptin at all time blocks was likely a reflection of the wide range of fat mass as well as the range of athletic activities in our participants.

One additional possibility, which was not within the scope of our study, is that there may be a range of set points for leptin.

Although leptin was closely related to the other surrogate measures of energy balance, namely BMI, VO2max, and percent body fat, it was associated to GH mean concentration in the subgroup of lean girls with a BMI between 18 and 25 kg/m². Our study further illustrates the limitations of BMI as a weight measure and/or a surrogate measure of adiposity in young subjects who can represent a wide range of physical fitness and body composition. The importance of fitness is likely to be more evident in youth because baseline activity tends to be greater than in older adults. Analyzed together, both BMI and VO2max remained significantly related to leptin. We propose that in obesity-related studies in adolescent and young women, the assessment of BMI should be complemented by a measure of adiposity as well as a measure of fitness or endurance to exercise.

Although all girls were studied a minimum of 2 yr after menarche, there was higher GH secretion with menarche at an older age. Because GH decreases after puberty (25) and declines further with aging (26), one possible explanation is that the girls with a later age at menarche were still immature with stronger GH pulses, as can be seen in younger individuals. Our study highlights that the postpubertal maturation of neuroendocrine function continues for at least longer than 2–3 yr. Because exercise habits are acquired over a long period of time (27), one can speculate that girls with a later age at menarche were likely to be more active as they entered puberty, thus delaying their menarche. This would in part explain why higher VO2max was related to significantly higher GH secretion. Because adolescents with delayed puberty may also have a more prolonged course until they achieve maturation, it is also possible that a slower tempo of puberty would influence the results in girls with a later age at menarche.

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In recent years, there have been reports suggesting that GH is involved in pubertal maturation (28), facilitates folliculogenesis (29), and enhances the effectiveness of assisted reproductive techniques in some women (30). Attempts to discriminate between women in which GH was effective at facilitating ovulation from those who were not GH responsive have relied on the response to GH-provocative tests as predictors (31, 32). Salat-Baroux et al. (33) reported that basal GH levels were higher in women who were poor responders to assisted ovulation techniques than those who had a good response. Due to our participants’ young age and the cross-sectional nature of this study, fertility potential could not be assessed here. It is possible, however, that the concurrent finding of higher GH secretion and decreased fertility merely represents the impact of acquired fitness during puberty by delaying puberty and fertility and enhancing GH secretion.
Acknowledgments

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The authors have nothing to declare.

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Fig. 2. Selected individual GH profiles of girls with menarche before (left) or after (right) 13 yr.

FIG. 2. Selected individual GH profiles of girls with menarche before (left) or after (right) 13 yr.
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