Growth Hormone Secretory Cycle Dynamics Over the Menstrual Cycle

CRAIG A. JAFFE, BLANCA OCAMPO-LIM, WENSHENG GUO*, KATHERINE KRUEGER, IKUKO SUGAHARA, ROBERTA DEMOTT-FRIBERG AND ARIEL L. BARKAN

Division of Endocrinology and Metabolism, Department of Internal Medicine, Department of Veterans Affairs Medical Center and University of Michigan Medical Center, Ann Arbor, Michigan 48109, USA
* Department of Biostatistics, School of Public Health, University of Michigan Ann Arbor, Michigan 48109, USA

Abstract. Whether GH secretion in women varies over the menstrual cycle is uncertain. Previous investigations have led to conflicting conclusions; some studies suggested that there is an estrogen mediated rise in GH during the periovulatory (PO) and luteal (L) phases whereas others indicated no change in GH axis over the cycle. Differences in conclusions could relate to heterogeneity of the study populations, GH sampling paradigms or sensitivity of the GH assays used. In order to investigate whether GH secretion varied over the cycle, 24-h GH profiles using every 10-min sampling were obtained in 6 ovulatory women during the early follicular (EF), PO and L phases of the cycle. The TSH response to TRH, GH response to GRH and fasting plasma IGF-I were measured on each occasion. There was a trend toward higher integrated GH concentration (IGHC) during the PO phase, although this difference was not statistically significant (3284±721 vs 4542±872 vs 4071 ±699 pg/min/L; EF vs PO vs L; p=0.09). Similarly, deconvolution estimated GH secretion did not vary over the cycle (p=0.56). There were no differences in GH pulse amplitude or frequency. There were no correlations between IGHC and sex steroids. Serum IGF-I was constant over the cycle (272±38 vs 277±31 vs 265±38 pg/L; p=0.89). The TSH response to TRH and GH response to GRH did not vary over the cycle. We concluded that the effect of changes in the ovarian steroid milieu on the GH axis during spontaneous menstrual cycles is minimal.

Key words: Pituitary, Growth hormone, Menstrual cycle, Growth hormone-releasing hormone, Estrogen

THE effects of sex steroids and gender on GH secretion have been extensively investigated. Several groups have reported that women have higher GH secretion than do men [1, 2]. In contrast to these conclusions, in a carefully matched group of young men and women in the early follicular phase of the cycle, we were not able to demonstrate differences in either mean daily GH or daily GH secretion [3]. Although the mean daily GH concentration tended to be higher in women, the within-gender variability of GH secretion obscured any potential small difference between genders from being statistically significant. This study did, however demonstrate that insulin-like growth factor I (IGF-I) was less effective in suppressing GH secretion in women, suggesting gender-specific differences in GH regulation. Moreover, the pattern of GH secretion differs across genders, with women secreting GH more continuously throughout the day than do men.

Sex steroids could potentially regulate differences in GH neuroendocrine regulation, but the data on the effect of endogenous sex steroids on GH secretion are contradictory. Faria et al. [4] reported that GH concentration varied over the menstrual cycle, with a two-fold increase of mean GH during the late follicular stage over early follicular levels. GH concentrations during the luteal stage were intermediate
and these data suggested that estradiol (E2) increased GH secretion. However, different subjects were studied in each phase of the cycle and the between-subject variability of GH secretory parameters could have contributed to the observed differences. In contrast, Zadik et al. [5] and Holst et al. [6] found no difference in mean GH over the menstrual cycle. The latter studies were based on a single GH sample derived from either a continuous 24-h collection [5] or from a fasting morning blood sample [6].

In the present study, we have addressed the issue of changes in GH secretion over the menstrual cycle. In contrast to several earlier studies, which used either infrequent blood sampling or a continuous draw technique and relatively insensitive GH RIA, we have utilized a paradigm of intensive blood sampling and a highly sensitive GH assay that allows for accurate detection of interpulse GH concentrations. In addition, we have studied a homogeneous group of lean, young women across the menstrual cycle. This has minimized the well-described impact of obesity on GH secretion [7] as well as between subject variability.

Material and Methods

Subjects

The research protocol was approved by the Institutional Review Board and the General Clinical Research Center (GCRC) Operating Subcommittee of the University of Michigan Medical Center. Written consent was obtained from subjects prior to their participation. Eight healthy and nonobese young women were recruited. Two of the women were subsequently found to have anovulatory cycles and their data were excluded from the analyses. The mean (±SE) age was 25.2 ± 4.0 y (range 21-34 y) and body mass index was 22.9 ± 2.9 kg/m² (range 20-25 kg/m²). All had unremarkable medical histories and physical examinations. Measurements of renal, hepatic and hematological function in all subjects were normal. None of the subjects were on any medications. Only women with regular spontaneous menstrual cycles as confirmed by menstrual diary for at least two months prior to the study were included. Women were studied during the early follicular (EF) phase of the cycle (days 2-5 after the onset of menstrual bleeding), periovulatory phase (PO, 16 to 14 days prior to next expected menses) and luteal phase (days 24-26). Menstrual status was validated by measurement of serum E2 and progesterone (P) concentrations in a sample made by pooling equal aliquots of plasma obtained every 6 h during the period of frequent blood sampling.

Protocol

The subjects were admitted to the GCRC the evening prior to the actual study and antegrade intravenous canulae were placed in forearm veins of each arm. The subjects were fed three meals each day served at 0700 h, 1200 h and 1800 h. Caloric content was standardized as previously described [8]. Lights were turned on at 0630 h and off at 2300 h. Daytime napping was not allowed.

In brief, every 10-min blood sampling was done for GH from 0800 h day 1 until 1200 h day 2. At 0800 h on day 2, thyrotropin-releasing hormone (TRH, Thyphine; Abbot Laboratories, North Chicago, IL) 50 μg was given as an iv. bolus. This was followed by a human GRH-44 (Bachem California, Torrance, CA) 0.33 μg/kg iv. bolus at 1000 h. This dose of GRH-44 results in a GH pulse that is similar in amplitude to spontaneous, nocturnal pulses [9]. Lights were turned on at 0630 h and off at 2300 h. Daytime napping was not allowed.

In brief, every 10-min blood sampling was done for GH from 0800 h day 1 until 1200 h day 2. At 0800 h on day 2, thyrotropin-releasing hormone (TRH, Thyphine; Abbot Laboratories, North Chicago, IL) 50 μg was given as an iv. bolus. This was followed by a human GRH-44 (Bachem California, Torrance, CA) 0.33 μg/kg iv. bolus at 1000 h. This dose of GRH-44 results in a GH pulse that is similar in amplitude to spontaneous, nocturnal pulses [9]. Plasma E2, P and IGF-I were measured in a sample made by pooling equal aliquots of plasma obtained every 6 h during the time period 0800-0800 h.

Assays

Plasma GH was measured in duplicate by a chemiluminescent assay (Nichols Institute Diagnostics, San Juan Capistrano, CA) as previously described [3]. All samples from each particular subject were analyzed in the same assay and the detection limit of the assay was 0.01 μg/L. Mean intraassay CV was 9% between 0.01 and 0.1 μg/L and 5% between 0.1 and 40 μg/L. Interassay CV was 7% at 9 μg/L. Total plasma IGF-I was measured by IRMA (Diagnostics Systems Laboratories; Webster, TX) in the same plasma pools used for sex steroids. All IGF-I measurements for a particular patient were performed in a single assay and the interassay and mean intraassay coefficients of variations were 6% and 5%, respectively. Similarly, all plasma E2, P and T concentrations were measured in single assays.
using commercial kits (Coat-A-Count, Diagnostics Products Corporation; Los Angeles, CA). Total T4, total T3, T3RU and TSH assays were performed by the Ligand Laboratory of the University of Michigan Medical Center.

Data analysis

Integrated GH concentration (IGHC) was calculated as the area under the GH versus time curve using the trapezoidal rule for the time period 0800-0800 h. The GH response to GRH was defined as the maximal increase in the hormone concentrations over the baseline or the integrated hormone concentration over one hour following GRH administration. Similar calculations were used for the TSH response to the TRH bolus.

Parameters of GH pulsatility were analyzed with regard to both concentration and secretion as previously described [3]. The baseline GH concentration was set at the lowest 5% of the daily GH concentration values. Waveform dependent deconvolution analysis of the 24-h GH profiles was used to calculate GH half-life, GH pulse frequency, and GH secretion profile [3]. Parameters of spontaneous and GRH stimulated GH secretion and of thyroid hormones and TSH were compared between the different menstrual phases by repeated measures analysis of variance (ANOVA). Repeated measures ANOVA comparing IGHC between menstrual phases was also performed for daytime (0800-2000 h) and nighttime (2000-0800 h) GH concentrations. Pairwise comparisons were made by the Tukey-Kramer test when a significant difference was found by ANOVA. In all comparisons, data that were not normally distributed were logarithmically or square root transformed as appropriate prior to analyses in order to maintain homogeneity of the variance. P ≤ 0.05 was considered statistically significant. Data are presented as M ± SE.

Results

Table 1 lists the plasma concentrations of sex steroids, IGF-I and thyroid hormones in the 6 women who were studied over the menstrual cycle. There was a four-fold increase in plasma E2 between the EF and PO phases, whereas E2 concentrations in the PO and L phases were indistinguishable. EF progesterone was low as expected. PO plasma progesterone was slightly higher than during EF and this difference was statistically significant. Both EF and PO progesterone were lower than L progesterone. EF T was significantly lower than T measured during the other two phases. There were no differences in total IGF-I, thyroid hormones, T3RU or baseline TSH across the menstrual cycle.

Fig. 1 shows the mean (± SE) 24-h GH profiles for the 6 women. Discrete parameters of GH concentration and secretion are given in Table 2. There was no overall effect of menstrual phase on 24-h IGHC (p = 0.09 by repeated measures ANOVA), although there was a trend toward higher IGHC in the PO over the EF phase (3284 ± 721 vs 4542 ± 872 pg x min/L). By paired t-test, this latter difference did not quite reach statistical significance (p = 0.06). Similarly, there was no effect of menstrual phase on either

<table>
<thead>
<tr>
<th>Parameter</th>
<th>EF</th>
<th>PO</th>
<th>L</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol (ng/L)</td>
<td>29±7a</td>
<td>105±18b</td>
<td>97±17b</td>
<td>0.0005</td>
</tr>
<tr>
<td>Progesterone (µg/L)</td>
<td>0.49±0.06a</td>
<td>1.51±0.40b</td>
<td>8.03±2.00b</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Testosterone (µg/L)</td>
<td>0.36±0.06a</td>
<td>0.53±0.06b</td>
<td>0.52±0.04b</td>
<td>0.006</td>
</tr>
<tr>
<td>IGF-I (µg/L)</td>
<td>272±38</td>
<td>277±31</td>
<td>265±38</td>
<td>0.89</td>
</tr>
<tr>
<td>T4 (µg/dL)</td>
<td>6.60±0.49</td>
<td>5.60±0.37</td>
<td>5.68±0.49</td>
<td>0.11</td>
</tr>
<tr>
<td>T3RU (%)</td>
<td>96.5±4.4</td>
<td>96.6±5.1</td>
<td>99.8±2.9</td>
<td>0.47</td>
</tr>
<tr>
<td>Total T3 (ng/dL)</td>
<td>106±8</td>
<td>104±8</td>
<td>106±2</td>
<td>0.88</td>
</tr>
<tr>
<td>Baseline TSH (mU/L)</td>
<td>1.51±0.3</td>
<td>1.58±0.41</td>
<td>1.83±0.48</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Different subscripts denote significant differences as determined by Tukey-Kramer.
daytime (P=0.45) or nighttime (P=0.54) IGHC. There were no differences across the cycle in mean pulse amplitude, median pulse amplitude, maximum pulse amplitude, nadir or pulse frequency. GH secretion and half-life as determined by deconvolution analysis were identical. There were no significant relationships between GH concentration or GH secretion and sex steroids in univariate linear regression analyses in which estrogen, progesterone or testosterone were used as the dependent variables or in a stepwise multivariate regression using these same independent variables. The coefficients of variation for Cluster identified GH pulse amplitudes, as previously defined [3], were identical across the

daytime (P=0.45) or nighttime (P=0.54) IGHC. There were no differences across the cycle in mean pulse amplitude, median pulse amplitude, maximum pulse amplitude, nadir or pulse frequency. GH secretion and half-life as determined by deconvolution analysis were identical. There were no significant relationships between GH concentration or GH secretion and sex steroids in univariate linear regression analyses in which estrogen, progesterone or testosterone were used as the dependent variables or in a stepwise multivariate regression using these same independent variables. The coefficients of variation for Cluster identified GH pulse amplitudes, as previously defined [3], were identical across the

**Table 2.** Comparison of 24-Hour GH Secretion Parameters During Different Menstrual Phases

<table>
<thead>
<tr>
<th>Parameter</th>
<th>EF</th>
<th>PO</th>
<th>L</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH Concentration Analysis (Cluster)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGHC (µg × min/L)</td>
<td>3284 ± 721</td>
<td>4542 ± 872</td>
<td>4071 ± 699</td>
<td>0.09</td>
</tr>
<tr>
<td>Mean pulse amplitude (µg/L)</td>
<td>4.07 ± 1.00</td>
<td>5.72 ± 0.90</td>
<td>5.44 ± 1.22</td>
<td>0.35</td>
</tr>
<tr>
<td>Median pulse amplitude (µg/L)</td>
<td>1.63 ± 0.30</td>
<td>1.97 ± 0.46</td>
<td>1.96 ± 1.49</td>
<td>0.68</td>
</tr>
<tr>
<td>Maximum pulse amplitude (µg/L)</td>
<td>13.00 ± 3.38</td>
<td>25.94 ± 5.94</td>
<td>18.73 ± 4.05</td>
<td>0.06</td>
</tr>
<tr>
<td>Coefficient of variation of pulse amplitudes (%)</td>
<td>113 ± 6</td>
<td>138 ± 12</td>
<td>132 ± 16</td>
<td>0.21</td>
</tr>
<tr>
<td>Pulse frequency (per 24 h)</td>
<td>10.8 ± 0.6</td>
<td>11 ± 0.4</td>
<td>11.5 ± 1.2</td>
<td>0.67</td>
</tr>
<tr>
<td>Valley (µg/L)</td>
<td>0.12 ± 0.03</td>
<td>0.18 ± 0.03</td>
<td>0.14 ± 0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>GH secretory analysis (Deconvolution)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily GH secretion rate (µg/L × 24 h)</td>
<td>97.2 ± 18.58</td>
<td>123.9 ± 24.87</td>
<td>113.2 ± 18.05</td>
<td>0.56</td>
</tr>
<tr>
<td>Pulse frequency (per 24 h)</td>
<td>20.3 ± 2.81</td>
<td>18.2 ± 2.5</td>
<td>17.0 ± 2.41</td>
<td>0.5</td>
</tr>
<tr>
<td>Mean amplitude (µg/L)</td>
<td>3.8 ± 1.38</td>
<td>5.1 ± 1.25</td>
<td>5.2 ± 1.20</td>
<td>0.16</td>
</tr>
<tr>
<td>GH half-life (min)</td>
<td>17.9 ± 1.2</td>
<td>21.9 ± 2.5</td>
<td>20.0 ± 2.4</td>
<td>0.46</td>
</tr>
</tbody>
</table>
menstrual cycle. The intrasubject variability across the cycle, as measured by CV of the IGHC, was half the intersubject variability within a particular menstrual phase (24±6 vs 48±3%; p=0.03).

Fig. 2 shows the mean TSH responses to the iv. TRH bolus. There were no differences in either the rise in TSH over the baseline (P = 0.39) or the area under the TSH versus time curve (P = 0.17) over the cycle. Similarly, Fig. 3 demonstrates that the GH responses to the iv. GRH bolus were indistinguishable across the menstrual cycle (P=0.58 and 0.53 for GH rise and IGHC respectively). The mean GH increases were 22.58±8.03, 40.45±17.20 and 37.86±12.83 μg/L for the EF, PO and L phases respectively.

Discussion

In the present study, which was performed in a homogenous group of 6 young, healthy ovulatory women, we were not able to demonstrate any significant differences in discrete parameters of GH concentration or secretion over the menstrual cycle. Although there was a trend toward higher IGHC and maximum pulse amplitude during the PO versus the EF phase, this difference did not reach statistical significance. The CV of GH pulse amplitudes, which is a measure of uniformity of pulse amplitude and is higher in men than in women [3], was the same in each phase. There were no differences in GH responses to GRH, in TSH responses to TRH or in mean daily IGF-I across the menstrual cycle.

Whether GH secretion varies or not over the normal menstrual cycle has been uncertain. Early studies on single morning blood samples reported that mean GH was higher during the late follicular phase [10, 11]. Once daily or infrequent sampling, however, is not representative of GH secretion since the hormone is secreted in a pulsatile pattern. More intensive sampling protocols were needed to reach a more definitive conclusion about GH secretion.

This same question has been asked more recently in studies where daily GH secretion was assessed by either a continuous draw technique [5] or frequent blood sampling [4, 12, 13]. Faria et al. [4] reported that integrated GH concentration was higher in the late versus the early follicular phase of the cycle. GH pulses, as determined by Cluster, were not different in the two phases and the increased integrated GH secretion during the late follicular phase was attributed to an increase in peak amplitude. Ovesen et al. [12] recently reported that mean GH was higher during the PO phase than during the EF phase in a group of 10 women studied during both time periods. Similar to the study from Faria et al., they found no difference in Cluster defined GH concentration peaks although they did report an increase in deconvolution identified secretory events during the PO phase.

In contrast to these studies, the present and several other studies found that menstrual phase had little impact on GH secretion. Zadik et al., using a continuous withdrawal technique found no difference in mean daily GH over the menstrual cycle [5]. Holst et al. reached identical conclusions with data based on a single morning fasting serum GH measurement [6]. Klein et al. [13] sampled GH every 20 minutes for 24 hours in both young women and in cycling older women during the EF and mid luteal phases. They found no differences in either integrated GH concentration or parameters of pulsatile GH concentration between the two sampling periods. In the present study, we were not able to demonstrate a statistically significant difference in mean daily GH over the menstrual cycle. There was, however, a strong trend toward higher GH during the PO phase than during EF phase. A power analysis based on the measured differences and variances suggests at least 15 subjects would be needed to observe a significant difference if only the EF and PO phases were studied. Since the
changes in GH secretion as estimated by deconvolution were smaller, at least 22 subjects would be needed to observe a significant difference in daily secretory rate.

Despite our failure to demonstrate statistically significant changes, our data are largely in agreement with those of Faria et al. [4]; there appeared to be a trend toward a rise in mean GH secretion between EF and PO phases of the cycle, with the LF data being intermediate between the two. Although these data indicate that estrogen might have some effect on GH secretion, the effect appears to be quite small. The reproducibility of mean daily GH, expressed as the CV, has been estimated by multiple investigators to be approximately 30% [14, 15]. In our hands, the mean intrasubject CV for IGHC over the menstrual cycle was 24% (range 7-39%). This suggests that the expected daily fluctuations in GH secretion are at least as great as the potential effects of hormone milieu.

Failure to see a significant effect of menstrual phase on spontaneous GH secretion is consistent with our observations that pituitary responsiveness to TRH or to GRH stimulation and daily mean plasma IGF-I did not vary over the menstrual cycle. Reports of increased GH responses to GRH in women over men [16, 17] and during the late follicular as opposed to the early follicular phase of the cycle [17] suggest estradiol-mediated lower hypothalamic somatostatin tone in women. In contrast, we were not able to demonstrate a difference in the GH response to GRH in lean and healthy young men and women [3]. Moreover, greater GH responses to GRH in men than in women [18] and no change in pituitary responsiveness to GRH over the cycle have also been reported [19]. There are similar conflicting data with regard to the TSH response to TRH. The TSH response to TRH has been reported by some [20, 21] but not all [22] investigators to be greater in women than in men. Our failure to see a significant change in TSH response across the menstrual cycle is consistent with data from other studies [20, 23]. The effects of estrogen on hypothalamic somatostatin message and secretion are poorly understood but appear to be gender- and species-specific [24]. Our data suggest that any potential effect of fluctuations of endogenous E2 upon GH secretion in normally cycling women is of minimal physiologic importance.

In contrast to our observation of no or minimal effects of estrogen on the GH axis in these healthy, regularly cycling women, exposure to higher concentrations of estrogen clearly affects GH secretion. Superovulation with resultant grossly elevated serum estrogen levels results in a two- to four-fold increase in mean daily GH concentration [25, 26]. Serum IGF-I also falls during superovulation. The increase in serum GH during this treatment is, at least in part, due to estrogen-mediated down-regulation of hepatic GH receptors [24] and a decrement in negative feedback subsequent to a fall in hepatic IGF-I production [3]. In addition, an estrogen mediated fall in pituitary IGF-I likely contributes to the increased GH [27]. There is controversy regarding the effects of more modest increases in estrogen [24, 28]. The bulk of the data suggest that oral estrogens increase GH secretion in postmenopausal women similarly through a fall in IGF-I negative feedback [24]. In contrast, transdermal estrogen, which does not subject the liver to the first pass effects of oral estrogen, does not lead to changes in GH or IGF-I secretion [28].

The remarkable constancy of plasma IGF-I between groups is consistent with the observation that GH secretion did not vary over the cycle. Although several studies [12, 29, 30] reported increases in IGF-I in association with periods of high estrogen, this is clearly not a consistent finding. There are multiple reports that serum IGF-I is stable over the menstrual cycle [13, 31-33]. Any potential effect of endogenous estrogen on circulating IGF-I appears to be small so that even when IGF-I was found to vary over the cycle, the normal daily variance in IGF-I was equivalent to the magnitude of the reported estrogen effect on IGF-I.

In summary, in a group of healthy young and lean women, studied across the menstrual cycle, the differences in the daily GH output were small and discrete parameters of GH pulsatility were stable despite physiological changes in gonadal steroid milieu. We concluded that the effects of gonadal E2 and P fluctuations within the hormonal ovulatory menstrual cycle are only marginally important. Thus, the previously described powerful effect of E2 upon GH secretion is likely to be manifest only at pharmacological estrogen concentrations.
GH SECRETION OVER MENSTRUAL CYCLE

Acknowledgments

This work was supported by: RO1-DK38449 (A.L.B.), MO1-RR0043-34S3 (Clinical Associate Physician Award to C.A.J.), U54-HD29184 (C.A.J.), MO1-RR0042 (General Clinical Research Center) and the Research Service of the Department of Veterans Affairs. We thank the General Clinical Research Center staff for their excellent nursing support.

References


