Sex Differences in Nocturnal Growth Hormone and Prolactin Secretion in Healthy Older Adults: Relationships With Sleep EEG Variables

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Study Objectives: To examine sex differences in nocturnal growth hormone and prolactin release in older adults.

Design: Sleep was polygraphically recorded for 2 consecutive nights, and blood was sampled at frequent intervals during the last 24 hours.

Setting: The University of Chicago Clinical Research Center.

Participants: Two groups of healthy nonobese older subjects: 10 men (59 ± 2 years, mean ± SEM), and 10 postmenopausal women (63 ± 2 years).

Interventions: N/A.

Measurements and Results: A spectral analysis of the electroencephalogram was performed in the delta and alpha bands. When delta activity was normalized for the activity in rapid eye movement sleep, women had lower delta activity than men. Growth hormone secretion was estimated by deconvolution. The prolactin profile was quantified by a best-fit curve. In both sexes, growth hormone was released both before and after sleep onset. In men, there was no relationship between presleep growth hormone release and subsequent sleep quality and postsleep growth hormone release correlated with delta activity. In women, presleep growth hormone release appeared to inhibit both postsleep growth hormone release and sleep consolidation. Prolactin release was related to rapid eye movement sleep and was lower in men than in women. Women with poor sleep maintenance had a lower prolactin acrophase.

Conclusions: Major sex differences in the nocturnal profiles of growth hormone and prolactin and their relationship to sleep electroencephalogram variables are present in healthy older adults. Our analyses suggest that lower sleep-onset release of growth hormone in women as compared with men could be related to lower levels of delta activity. Improvements in the homeostatic control of sleep could have hormonal benefits in older adults.

Keywords: Aging, sex differences, delta activity, growth hormone, prolactin.

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tecture, including decreased SWS and delta activity, and increased sleep fragmentation. While most studies have concluded that sleep quality is better preserved in women than in men, we report, in a companion paper, a novel analysis of delta and alpha activities in older adults that suggests that the sex difference could in fact be in the opposite direction. Indeed, when delta activity in non-rapid eye movement (NREM) sleep (which decreased, as expected, across successive sleep cycles) was normalized for delta activity in rapid eye movement (REM) sleep (which remained constant across successive sleep cycles), women had lower levels of delta activity than men, consistent with the higher prevalence of sleep complaints in women than in men. Furthermore, in women, but not in men, delta activity in NREM sleep was associated with alpha activity. Whether these sex differences in delta activity in the sleep electroencephalogram of older adults are associated with differences in hormones that are preferentially released during SWS is not known.

The present study was, therefore, designed to analyze sex differences in sleep-related GH and prolactin releases in healthy older adults and to characterize the relationship between sleep and hormone variables.

METHODS

Subjects

Twenty healthy fully self-sufficient older adults participated in the study: 10 men (59 ± 2 years, body mass index of 25 ± 0.7 kg/m², mean ± SEM), and 10 women (63 ± 2 years, 24 ± 0.7 kg/m²). The study protocol was approved by the Institutional Review Board of the University of Chicago, and all volunteers gave informed written consent.

Only healthy subjects with regular life habits who did not take any drugs were included. Only women who were at least 1 year past menopause and did not suffer from hot flashes were included. None of the women was on hormone replacement therapy. Screening procedures included a clinical interview, routine laboratory blood tests, evaluation of mood and cognition, a physical examination, and an oral glucose tolerance test. All participants had normal findings on clinical examination, normal results in laboratory tests (including thyroid function tests, biochemistry, complete blood count, and lipid panel), and no history of psychiatric or endocrine illness, sleep disorders, or neurologic disorders. Other details of the inclusion criteria are given in the companion paper.

Experimental Protocol

For 1 week before the study, the subjects were required to maintain regular rest-activity cycles and to adhere to a fixed mutually agreed upon bedtimes and wake-up times (± 30 minutes). The bedtime schedule was individually designed for each subject, taking into account the usual life habits. After 1 night of habituation, 2 consecutive nights were recorded, 1 without and 1 with blood sampling, as described in the companion manuscript. At noon of the second day of the study, a catheter for blood sampling was placed in the nondominant arm, and blood was collected at 20-minute intervals for 24 hours for the measurement of hormone profiles. The intravenous line was kept patent by a slow drip of saline. A 2-hour delay between insertion of the sampling catheter and beginning of sample collection ensured that stress effects related to venipuncture had subsided. At night, the indwelling catheter was connected to a plastic tube extending to an adjacent room to collect blood samples without disturbing the subject. The total amount of blood withdrawn was in conformity with the regulations of the Institutional Review Board. Each blood sample was immediately centrifuged at 4°C. Plasma samples were frozen at -20°C until assay.

Hormonal Assays and Analysis

Determination of plasma GH and prolactin levels were performed using a chemiluminescent enzyme immunoassay (Immulite, Diagnostic Products, Los Angeles, CA). For GH, the lower limit of sensitivity of the assay was 0.05 µg/L, and the intraassay coefficient of variation averaged 6%. For prolactin, the limit of sensitivity was 0.5 µg/L, and the intraassay coefficient of variation averaged 5.5%. All samples collected in the same individual were analyzed in the same assay run in order to avoid inter assay variations.

Analysis of Hormone Profiles

The wave shape of the plasma prolactin profiles was quantified by a best-fit curve obtained using a robust locally weighted regression procedure with a regression window of 4 hours. The acrophases and the nadirs were defined as the respective maxima and minima of the best-fit curves. The nocturnal acrophase was defined as the highest acrophase occurring during the sleep period. In analyses of the relationships between hormone and sleep variables, the nocturnal acrophase was used as a summary measure for prolactin.

Because the 24-hour profile of plasma GH levels normally consists of a series of secretory pulses interrupting low GH concentrations, these profiles were quantified by pulse analysis rather than by variables derived from a best-fit curve. GH secretory rates were first derived from the corresponding plasma GH concentrations by mathematical deconvolution using a 1-compartment model for GH clearance and subject-adjusted half-lives (in the range 13 to 21 minutes) as previously described. The distribution volume was assumed to be 7% of the body weight. Significant GH secretory pulses were identified using a modification of the pulse-detection program ULTRA, as previously described. The total amount of GH secreted over a given time interval was determined by summing the amounts secreted in each of the significant pulses occurring during that time interval. If a pulse overlapped 2 time intervals, the amount of GH secreted was divided proportionally. In analyses of the relationships between hormone and sleep variables, the amount of GH secreted in the 3-hour interval preceding sleep onset i.e., GH secretion before sleep onset) and the amount of GH released during the first 3 hours after sleep onset i.e., GH secretion after sleep onset) were used as summary measures for GH.

Sleep Recording and Analysis

Polygraphic sleep recordings were visually scored using standardized criteria. A spectral analysis on the central electroencephalogram lead was performed (PRANA, PhiTools, Strasbourg, France), and power spectra were obtained in the delta and alpha bands. All the details are given in the companion paper.
Statistical Analysis

All group values are expressed as mean ± SEM. An unpaired t test was used to examine sex differences in hormone variables. Associations between hormone variables, sex, and sleep variables were explored using analyses of covariance (ANCOVA). When significant interactions between sex and sleep variables were detected, sex-specific correlations were calculated. For GH, we first examined associations between GH secretion before sleep onset and sleep variables and then associations between GH secretion after sleep onset and sleep variables. For prolactin, we examined associations between the nocturnal acrophase and sleep variables. All statistical calculations were performed using the StatViewSE+ and SuperAnova software packages for Macintosh (Abacus Concepts Inc., Berkeley, CA).

RESULTS

Sleep

A detailed analysis of sleep stages and power spectra in both men and women has been reported in the companion manuscript.26 In the present report, we focus on delta activity during NREM sleep because of its known temporal association with the release of both GH and prolactin.

Figure 1 shows the mean profiles of absolute (upper panels) delta activity in men (left panel) and women (right panel) for the first 4 non-rapid eye movement (NREM)/rapid eye movement (REM) sleep cycles in the night of blood sampling. Lower panels: Mean profiles of delta activity in men (left panel) and women (right panel) for the first 4 NREM/REM sleep cycles in the night of blood sampling when, in each epoch and for each subject, delta activity is expressed as a percentage of the mean delta activity in REM sleep. Dashed vertical lines delimit REM sleep episodes.

Table 1—Impact of Sex on Growth Hormone Secretion and Prolactin Levels.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Sex</th>
<th>P level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth hormone secretion</td>
<td>Men (n=10)</td>
<td>Women (n=10)</td>
</tr>
<tr>
<td>Level, µg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-h</td>
<td>355 ± 67</td>
<td>248 ± 32</td>
</tr>
<tr>
<td>During sleep</td>
<td>118 ± 17</td>
<td>51 ± 13</td>
</tr>
<tr>
<td>During wake</td>
<td>238 ± 55</td>
<td>197 ± 33</td>
</tr>
<tr>
<td>Before sleep onset</td>
<td>97 ± 19</td>
<td>59 ± 18</td>
</tr>
<tr>
<td>After sleep onset</td>
<td>98 ± 15</td>
<td>35 ± 14</td>
</tr>
<tr>
<td>Percentage of 24-h total, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>During sleep</td>
<td>39.3 ± 5.2</td>
<td>23.7 ± 5.6</td>
</tr>
<tr>
<td>After sleep onset</td>
<td>31.5 ± 4.0</td>
<td>16.0 ± 5.8</td>
</tr>
<tr>
<td>Before sleep onset</td>
<td>25.1 ± 3.7</td>
<td>21.1 ± 5.1</td>
</tr>
<tr>
<td>Prolactin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Level, µg/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-h</td>
<td>7.8 ± 0.6</td>
<td>10 ± 0.5</td>
</tr>
<tr>
<td>During daytime</td>
<td>194 ± 13</td>
<td>225 ± 15</td>
</tr>
<tr>
<td>During nighttime</td>
<td>220 ± 17</td>
<td>337 ± 18</td>
</tr>
<tr>
<td>Acrophase</td>
<td>11.7 ± 1.0</td>
<td>16.0 ± 1.2</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM.

Growth Hormone

Figure 2 shows mean profiles of plasma GH levels. The top panels show the mean profiles aligned to clock time (the dark bars representing the sleep periods), while the bottom panels show the mean profiles aligned to sleep onset. The Table summarizes the quantitative analysis of the GH profiles.

Confirming previous reports,26,27 there was no significant sex difference in the 24-hour GH secretion between older men and age- and weight-matched postmenopausal women. During the sleep period, however, GH secretion was lower in women than in men, whether expressed in absolute value (µg) or as a percentage of the 24-hour secretion (Table). This sex difference in sleep-related GH secretion was already apparent during the first 3 hours of sleep.

Both men and women showed elevations of GH levels in the late evening, prior to sleep onset. In the present group of healthy older adults, a negative correlation between the percentage of 24-hour GH secreted before sleep onset and the percentage of 24-hour GH secreted after sleep onset was present in women (r=-0.6313, P = .0503). This finding is consistent with the well-documented negative feedback regulation of GH on its own release.28,29 In contrast, in men, GH secretion before sleep onset did not seem to influence GH release after sleep onset (r=0.1786, P = .6215). We conducted exploratory analyses to detect a putative impact of GH secretion before sleep onset on subsequent sleep quality (characterized by sleep stages as well as electroencephalogram delta and alpha activities). In women, there was a robust positive association between GH secretion before sleep onset and subsequent sleep fragmentation, as quantified by the amount of time spent in wake (r=0.8362, P = .0026, right upper panel of Figure 3) whereas, in men, no such association was present (r=0.0070, P = .9848, upper left panel of Figure 3). In women, similar significant correlations between GH secretion before sleep onset and subsequent sleep quality were found for sleep maintenance (r=-0.8345 P = .0027), and time spent in REM sleep (r=-0.7863, P = .007). While such associations cannot determine the direction of causality, it is noteworthy that no correlations were present in either sex.
between measures of sleep fragmentation in the previous night (without blood sampling) and GH release before sleep onset on the following day.

Analysis of covariance with GH secretion during sleep as dependent variable, sex as factor and min of SWS and REM sleep as regressors revealed that the amount of GH released during sleep was predicted by sex ($P = .0024$), the amount of REM sleep ($P = .0398$) but was not significantly associated with the time spent in stages III and IV ($P = .1506$).

When the relationship of GH released during sleep and absolute delta activity during NREM sleep was examined, sex differences were clearly apparent. As illustrated in the lower panels of Figure 3, in women, there were no significant associations between absolute delta activity and GH release ($r=0.0587$, $P = .8808$) whereas a robust correlation was found in men ($r=0.7431$, $P = .0218$). Similar findings were obtained when delta activity in NREM sleep was normalized for delta activity in REM (men: $r=0.6943$, $P = .038$; women: $r=0.0849$, $P = .8280$).

There were no associations between absolute or normalized alpha activity and nocturnal GH release in either sex group.

**Prolactin**

Mean profiles of plasma prolactin are shown in Figure 4, and the variables quantifying the prolactin profiles are summarized in the Table. Men had significantly lower 24-hour mean prolactin levels than women. While daytime levels were essentially similar in both groups, the nighttime levels were markedly blunted in men.

A clear rise in prolactin release following bedtime could be identified in both men and women. The timing of the nocturnal prolactin acrophase was similar in men and women and occurred 3 to 4 hours after sleep onset. The value of the acrophase was significantly lower in men than in women.

Associations between nocturnal prolactin release, sex, and amounts of SWS and REM sleep were explored by ANCOVA, with the nocturnal prolactin acrophase as the dependent variable. Significant effects of sex ($P = .0025$) and of the amount of REM sleep ($P = .0084$), but not of the amount of SWS ($P = .6395$), were found for the prolactin acrophase. There were no significant interactions, suggesting that the impact of REM sleep on the nocturnal prolactin acrophase was similar in both men and women. Figure 5 illustrates the positive relationship between the nocturnal prolactin acrophase and the amount of REM sleep in all subjects.

In women but not in men, the prolactin acrophase was negatively correlated with intrasleep awakenings ($r=-0.6639$, $P = .0363$) and positively correlated with sleep maintenance ($r=0.6487$, $P = .0424$).

There were no associations between measures of prolactin release during sleep and measures of delta or alpha activities (whether absolute or normalized) in either sex group.

**DISCUSSION**

The present study demonstrates important sex differences in the nocturnal release of GH and prolactin in healthy nonobese older men and postmenopausal women who did not take any medication. The findings also suggest that sleep quality may be an important determinant of the secretion of these 2 hormones in normal aging.

Aging appears to be associated not only with an overall decrease in the secretion of GH, but also with a different temporal organization of the release of this “antiaging” hormone. Indeed, in both sex groups, a significant amount of GH secretion occurred in the late evening, before habitual bedtime, at a time when GH secretion is usually quiescent in young men. Such GH pulses before sleep onset may, however, appear in young subjects when studied in a state of sleep debt resulting from experimental bedtime restriction. Since total sleep time is consistently lower in older adults, it is possible that this secretion before sleep onset might reflect an accumulation of sleep loss resulting from an inability to achieve sufficient sleep in older adults. Sleep need or sleep capacity in older adults remains, however, to be defined.

In older men, there was no relationship between GH release...
before sleep onset and subsequent sleep fragmentation. Furthermore, the robust quantitative relationship between SWS/delta activity and GH release after sleep onset, which is well documented in young men, was still present. In contrast, in older women, GH release before sleep onset appeared to negatively impact both the amount of GH secreted during sleep and sleep consolidation. Indeed, robust negative correlations were found between GH secretion before and after sleep onset, as well as between GH secretion before sleep onset and sleep maintenance. In contrast to men, no significant correlation between GH release after sleep onset and measures of delta activity could be detected in women. The persistence of an association between GH release during sleep and delta activity in older women is, however, supported by preliminary findings from our laboratory that indicate that pharmacologic enhancement of delta activity in older women results in increased GH release. One possible explanation for the lack of correlation between GH release and delta activity in the women included in the present study is that the variable influence of GH release before sleep onset prevented the detection of this association in a relatively small number of subjects. Another possible reason is suggested by our analysis of delta and alpha activities described in detail in the companion paper. This analysis reveals that, when normalized for delta activity in REM sleep, delta activity in NREM sleep is actually lower in women than in men and is associated with concomitant alpha activity. The combination of lower normalized delta activity with concomitant alpha activity during NREM sleep could be involved in limiting GH release after sleep onset in postmenopausal women.

The present study demonstrates for the first time the existence of a robust sex difference in prolactin levels in older adults, with clearly lower nighttime release in men than in postmenopausal women. Daytime levels were comparable in both sex groups, contrasting with findings in young adults, in which women have higher prolactin levels than men throughout the 24-hour cycle. While prolactin release is concomitant with delta activity in young adults, a quantitative relationship between amounts of delta activity and amount of prolactin levels was not reported and was also not detected in our older volunteers. Instead, a robust relationship between the nocturnal prolactin elevation and the amount of REM sleep emerged from our analysis. There is increasing evidence, demonstrated in animal studies, for a link between prolactin and REM sleep. Rodents exposed to stressors that elicit prolactin release show significant increases in REM sleep for several hours. Short-term administration of prolactin promotes REM sleep in rats, cats, and rabbits, and long-term hyperprolactinemia and hypoprolactinemia increase and decrease, respectively REM sleep in rodents. While these animal studies support a role for prolactin in sleep regulation, there is also evidence for the reverse direction of causality, i.e., that nocturnal prolactin release is dependent on sleep quality. In particular, awakenings interrupting sleep inhibit human prolactin release in young subjects, and our finding of a negative correlation between the prolactin acrophase and measures of sleep fragmentation in older women suggests the persistence of this effect in older adults.

The positive relationships between nocturnal GH and prolactin release and amounts of REM sleep detected in the present study of older adults have not been found in studies of younger adults and may reflect a relationship between the preservation of overall sleep quality and of sleep-related endocrine release, although the direction of causality cannot be inferred from our data. The amount of REM sleep is inversely related to the presence of a sleep debt, as both experimental sleep restriction and sleep loss due to pathologic conditions such as sleep-disordered breathing are associated with reduced amounts of REM sleep. While the clinical implications of decreased SWS and REM sleep have not been well investigated, multiple studies have shown that the relative GH deficiency of the elderly is associated with increased fat tissue and abdominal obesity, reduced muscle mass and strength, and reduced exercise capacity. Reduced prolactin levels may be involved in compromised immune function in older adulthood. It remains to be determined whether the correction of age-related sleep disturbances could be associated with multiple health benefits.

In conclusion, the present findings in a small sample of healthy nonobese drug-free older men and women suggest the presence of important sex differences in the associations between sleep variables and the nocturnal profiles of GH and prolactin.
REFERENCES


