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An Amplitude-Specific Divergence in the Pulsatile Mode of Growth Hormone (GH) Secretion Underlies the Gender Difference in Mean GH Concentrations in Men and Premenopausal Women*

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ABSTRACT

Although many studies have discerned higher serum GH concentrations in women than in men, based on measurements of single random blood samples or integrated 24-h means, the neuroendocrine mechanisms that underlie such gender differences have not been defined. Such mechanisms might entail an increase in GH-secretory burst frequency, amplitude, or duration, heightened interpulse basal GH release, or a prolonged half-life of GH. These mechanisms can be distinguished by deconvolution analysis of appropriate GH time series. Earlier studies employed RIA or IRMA with sensitivities of 0.1–0.5 μg/L, which result in frequently undetectable serum GH concentrations. To address these limitations, we undertook blood sampling at 10-min intervals for 24 h and applied a high-sensitivity immunofluorometric assay of GH (sensitivity 0.0115 μg/L). Multiparameter deconvolution analysis was used to estimate specific features of GH secretion, while simultaneously calculating the half-life of endogenous GH. Eleven men and 11 premenopausal women from the same community were studied. Discrete peak detection by Cluster was employed as a complementary half-life-independent technique to assign variations in serum GH into pulsatile and basal fractions over 24 h. Cluster revealed significantly higher mean serum GH concentrations over 24 h in women (0.78 ± 0.08 μg/L) compared with in men (0.27 ± 0.03 μg/L, $P < 0.00005$). Women exhibited significantly higher maximal serum GH concentration peak values than men (2.08 ± 0.34 μg/L in women, 0.67 ± 0.11 μg/L in men, $P = 0.0008$), which could be, in turn, attributed to a significantly increased incremental serum GH peak amplitude (1.85 ± 0.33 μg/L in women *vs.* 0.60 ± 0.10 μg/L in men, $P = 0.0021$) and a longer peak duration (114 ± 8 min in women, 86 ± 4 min in men, $P = 0.008$). The mean area under the serum GH concentration peak was significantly (3-fold) higher in women than in men (98 ± 17 μg/L · min in women, 34 ± 8 μg/L · min in men, $P = 0.0046$). Serum GH peak frequency was similar in women (9.7 ± 0.8 peak/24 h) and men (10.7 ± 1.1 peak/24 h, P = NS). The mechanisms underlying the increase in serum GH concentration pulse amplitude, duration, and area were investigated further by deconvolution analysis. Deconvolution analysis disclosed equivalent secretory pulse frequencies in women and men (13 ± 0.9 bursts/day in women, 10.5 ± 1.3 bursts/day in men, P = NS), and statistically indistinguishable mean interburst intervals of 106 ± 8 min in women and 150 ± 26 min in men (P = NS). GH-secretory burst mass was significantly higher in women by approximately 2.4-fold ($P = 0.0013$) compared with in men, which was attributed to a greater burst amplitude. Only low levels of basal GH release were inferred in women (5%) and men (9%), which did not differ significantly between genders. Moreover, the calculated half-life of endogenous GH was no different in women compared with in men: 15.8 ± 0.7 min *vs.* 17.1 ± 0.8 min, respectively (P = NS). The calculated daily secretion rate was 3-fold higher in women (47 ± 4.8 μg/L · 24 h) than in men (15 ± 1.8 μg/L · 24 h) ($P < 0.001$). In summary, discrete peak-detection analysis of serum GH concentration profiles collected at 10-min intervals over 24 h in men and premenopausal women discloses significantly different mean serum GH concentrations that are accounted for by higher maximal and incremental serum GH peak amplitudes. Deconvolution analysis demonstrated that the mechanism supporting the amplitude-specific difference in women was an augmentation of the GH-secretory burst mass caused by a higher GH-secretory burst amplitude. These gender differences were highly specific because the frequency of detectable GH-secretory bursts, the calculated endogenous half-life, and the estimated basal GH release were no different in women than in men. (*J Clin Endocrinol Metab* **81:** 2460–2467, 1996)

SINCE the first measurements by immunologically based assays of serum GH concentrations almost three decades ago, many (but not all) reports have inferred higher serum GH concentrations in women than in men (1–4). Many of these studies were based on single, random, morning samples, whereas others depended on continuous or intermittent blood sampling over 24 h. Higher serum GH concentrations in women have been inferred under basal conditions in some studies, and in others, after provocative tests such as arginine infusion (5), exercise (1), estrogen administration (6–8), insulin-induced hypoglycemia (9), and clonidine stimulation (10), as well as after GHRH injection (11–13). Not all investigations have documented such height-

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ened responsiveness of the hypothalamic-pituitary axis in women compared with in men (14). Indeed, other reports have underscored the confounding effects of obesity or relatively increased body mass index, as well as age, on the assessment of the GH axis in women and men (15, 16). For example, in a comprehensive clinical study of the effects of age and gender on 24 h serum GH concentrations, Zadik *et al.* reported that age, but not sex, markedly influenced the amount of GH in serum, which peaked both in males and females by Tanner stage V of normal puberty (14). This study also recognized an approximate 2-fold increase in serum GH concentrations in the midportion of the human menstrual cycle, confirming earlier inferences by Frantz and Rabkin (1) and Yen *et al.* (2). Subsequent studies utilizing 24-h blood sampling at 10-min intervals have corroborated a doubling of serum GH concentrations in the later stages of follicle maturation in young women (17) and documented higher serum GH concentrations in premenopausal, than in postmenopausal, women (18). Overall, available studies permit the hypothesis that estrogen alone, or testosterone after aromatization to estrogen, can stimulate GH secretion in men and women (19). However, despite the important regulatory effects of sex steroid hormones on mean serum GH concentrations, little information is available concerning the specific mechanisms by which gender differences, the menstrual cycle, and steroid hormones modulate pulsatile GH secretion.

To investigate the mechanisms that subserve the gender differences in mean serum GH concentrations over 24 h in men and women, we applied three new complementary strategies: 1) blood was sampled at 10-min intervals for 24 h in age- and community-matched men and women to obtain intensive and extended serum GH time series for comparison; 2) a novel high-sensitivity immunofluorometric GH assay was employed with a sensitivity of 0.0115 μg/L, to detect the majority of serum GH concentrations over 24 h in both men and women; and 3) we used waveform-independent peak detection on the one hand and multiparameter deconvolution analysis on the other hand, to calculate the number, amplitude, duration, and mass of underlying secretory bursts, to estimate the maximal rate of basal secretion, and to compute the apparent half-life of endogenous GH. Our implementation of this 3-fold strategy has permitted us to demonstrate, by two independent pulse analysis techniques, that women maintain a higher amplitude and area of serum GH concentration pulses, which can be attributed to an increased mass of GH secreted per burst. This striking difference in the mode of episodic GH secretion was highly specific because GH-secretory burst frequency, basal GH release, and the endogenous GH half-life were all statistically indistinguishable in men and women.

Materials and Methods

Subjects

Eleven healthy men and 11 women of normal height and body mass index, who were matched for age and originated from the same community, were recruited after obtaining informed consent. The study was approved by ethics committee of the Leiden University Hospital. No subject was receiving medication or had any underlying acute or chronic illness. The mean age of the men was 39.8 (range 30–55) yr, and women 37.9 (range 32–48) yr (NS). The mean body mass index of the women was 22.2 (range 18.3–30.5) and of the men 24.3 (range 21.6–29.3) kg/m^2 (NS). No hormones, including oral contraception, had been administered for at least an interval of 1 year. The volunteers were admitted to the hospital the day before the study to acclimate them to the unit. Subjects underwent blood sampling at 10-min intervals for 24 h beginning at 0900 h the next morning. Between 0900 h and 2200 h, the volunteers were free to move about, and meals were served between 0800–0900 h, 1200–1300 h, and 1700–1800 h. Lights were turned off between 2200–2400 h. No form of sleep monitoring was carried out. Female subjects were evaluated in the follicular phase of the menstrual cycle, namely days 2–7 after the onset of menses. All women were experiencing monthly menstrual bleeding.

Assays

A new sensitive time-resolved immunofluorometric assay was used (Wallac, Turku, Finland). The assay is specific for the 22-kDa GH protein. Standards were human biosynthetic GH (Pharmacia AB, Uppsala, Sweden) diluted in bovine calf serum and also calibrated against the WHO First International Reference Preparation 80–505 (to convert μg/L to mU/L, multiply by 2.6). The limit of detection of the assay was 0.0115 μg/L (20). The intraassay coefficients of variation ranged from 1.6–8.4% over the GH concentration span of 0.1–18 μg/L. All samples from any subject were run in duplicate in the same assay. The total plasma insulin-like growth factor 1 (IGF-I) concentration was measured by RIA (Incstar, Stillwater, MN), after extraction and purification. The interassay coefficient of variation was less than 11%, and normal levels for subjects aged 30–50 yr were 9.0–34.0 nmol/L (21).

Discrete peak detection

Cluster analysis was used for the detection of discrete peaks in the serum GH concentration profiles in relation to dose-dependent intraassay measurement error. This methodology is relatively independent of assumptions about peak duration, amplitude, regularity, the presence or absence of baseline, and hormone half-life (22). The locations and widths (min) of all significant GH concentration peaks were identified, the total number of peaks counted (frequency), the mean interpeak interval calculated (time in minutes separating consecutive peak maxima), and the following pulse parameters determined: maximal peak height (highest value obtained within the serum GH concentration peak), incremental peak amplitude (algebraic difference between the peak height and the prepeak nadir), and the area above baseline under the serum GH concentration peak. Interpulse valley regions were identified as serial GH concentrations flanked by pre- and postpeak nadirs. The total area under the 24-h serum GH concentration curve, mean concentration, and the sum of the incremental pulse areas were also calculated.

Deconvolution analysis

A multiparameter deconvolution technique was used to estimate relevant measures of GH secretion from the 24-h serum GH concentration profiles as described previously (23–26). Initial estimates of basal GH secretion were calculated to achieve serum GH concentrations approximately the mean of the lowest 5% of all GH measurements in that individual. Peak detection entailed application of 95% statistical confidence intervals to two thirds of all GH-secretory peaks considered jointly, and individual 95% statistical confidence intervals to the remaining one third smaller putative pulses. We estimated the following specific measures of GH secretion: secretory burst frequency (number of statistically significant secretory pulses per 24 h), amplitude (maximal rate of calculated GH secretion attained within a release episode), mass (integral of the calculated secretory pulse or the amount of hormone secreted per burst per unit distribution volume), half-duration (time in minutes elapsing during a calculated secretory episode at half its maximal amplitude), basal GH-secretion rate (μg/L · min or the amount of hormone secreted per unit distribution volume per unit time), and the endogenous GH half-life (min). Daily pulsatile, basal, and total GH secretion and day/night differences were evaluated as described (22). Twenty-four-hour variations in GH amplitudes, GH mass, and burst intervals were evaluated by cosinor analysis (27).

TABLE 1. Mean pulse parameters of 24-h serum GH concentration profiles in normal male and female subjects as determined by cluster analysis

Cluster pulse parameter	Men	Women	*P*
Mean serum GH concentration (μg/L)	0.27 ± 0.03	0.78 ± 0.08	<0.00005
Total 24-h area (μg/L · min)	390 ± 53	1130 ± 120	<0.00005
Maximal pulse height (μg/L)	0.67 ± 0.11	2.08 ± 0.34	0.0008
Incremental amplitude (μg/L)	0.60 ± 0.10	1.85 ± 0.33	0.0021
Pulse width (min)	86 ± 4	114 ± 8	0.0008
Pulse area (μg/L · min)	34 ± 8.1	98 ± 17	0.0046
Total pulse area (μg/L · min)	314 ± 41	860 ± 96	<0.00005
Nadir concentration (μg/L)	0.06 ± 0.02	0.25 ± 0.07	0.0136
Interpulse interval (min)	152 ± 24	137 ± 9	NS
Number of pulses/24 h	9.7 ± 0.8	10.7 ± 1.1	NS

Data are expressed as mean ± SEM. Venous blood samples were withdrawn at 10-min intervals for 24 h in eleven male and female healthy subjects. Cluster analysis was used with a cluster test configuration of 2 × 2, and a *t* statistic of 2.0. Significance by unpaired two-tailed Student's *t* test and confirmed by the nonparametric Mann-Whitney test.

TABLE 2. Deconvolution analysis of 24 hour plasma GH secretory profiles

Parameter	Males	Females	P^a
Interburst interval (min)	150 ± 26	106 ± 0.8	0.13
Number of secretory bursts/24 h	11 ± 1	13 ± 1	0.11
Secretory burst duration (min)	10.3 ± 1.1	11.6 ± 0.5	0.31
Secretory burst half-duration (min)[b]	23 ± 2.5	27 ± 1.2	0.31
Basal secretion rate (μg/L · min)[c]	0.0009 ± 0.0002	0.0017 ± 0.0004	0.08
Maximal secretory rate/burst (μg/L · min)	0.06 ± 0.01	0.12 ± 0.02	0.007
Mass of GH secreted/burst (μg/L)	1.5 ± 0.3	3.6 ± 0.5	0.0013
Endogenous GH half-life (min)	17.2 ± 0.8	15.8 ± 0.7	0.24
Basal GH production rate (μg/L · day)	1.3 ± 0.09	2.5 ± 0.53	0.08
Pulsatile GH production rate (μg/L/day)	14 ± 2	45 ± 5	0.0005
Total GH production rate (μg/L · day)	15 ± 2	47 ± 2	0.0005

Values are the mean ± SEM. Blood samples were withdrawn at 10-min intervals for 24 h in 11 male and 11 female control subjects. Multiple parameter deconvolution analysis of the plasma GH concentration-time series was used to simultaneously resolve the underlying GH secretory and clearance rates for each individual.

[a] Significance determined by unpaired two-tailed Student's *t* test.

[b] Half-duration is the duration (min) of the calculated GH secretory burst at half-maximal amplitude.

[c] GH mass and rates are expressed per L distribution volume.

Statistical analysis

Data are presented as mean ± SEM, unless stated otherwise. Statistical comparisons were made with the Student's *t* test or with the Wilcoxon test in case of nonnormal distribution of the data before and after appropriate statistical transformations. *P* values <0.05 were considered significant.

Results

Table 1 presents the results of Cluster analysis (discrete peak detection) of the serum GH concentration profiles in men and women. Mean and integrated 24-h serum GH concentrations were 2.9-fold higher in women than in men ($P < 0.0001$). Women had nearly 3-fold higher serum GH-peak height ($P = 0.0008$), which resulted primarily from a significantly higher incremental serum peak amplitude ($P = 0.0021$). The serum GH concentration pulse width was significantly prolonged by 30% in women ($P = 0.008$). Because serum GH-pulse area above baseline is proportionate to the incremental amplitude and width of a peak, this value also was significantly (almost 3-fold) higher in women than in men ($P = 0.0046$). The sum of the GH pulse areas over 24 h was 2.7-fold larger in women than in men ($P < 0.0001$). Differences in interpulse nadir and valley serum GH concentrations between sexes, although statistically significant, were minor in absolute amounts. In addition, GH-interpulse interval and GH pulse frequency were not distinguishable in men and women.

Deconvolution analysis was used to define and quantitate the underlying GH-secretory measures and GH half-life simultaneously. As summarized in Table 2, the mean maximal rate of GH secretion attained within each secretory burst (secretory burst amplitude) was 0.06 ± 0.01 μg/L distribution volume/min in normal men and 0.12 ± 0.02 μg/L distribution volume/min in normal women ($P = 0.007$). The mean mass of GH secreted per burst was 1.5 ± 0.3 μg/L in normal males and 3.6 ± 0.5 μg/L in normal females ($P = 0.0013$). Assuming a GH distribution volume of 7.9% BW (36), the GH-secretory burst amplitude or maximal secretory rate would be 0.42 ± 0.09 μg/min in men and 0.60 ± 0.07 μg/min in women ($P = 0.1145$), with a corresponding average mass of 9.6 ± 1.6 μg in males and 17.2 ± 1.9 μg in females ($P = 0.0078$).

The numbers of statistically significant GH-secretory pulses in healthy men ranged from 5–18 per day and in women, from 7–19 per 24 h, with mean values of 11 and 13 bursts per 24 h, respectively (P = NS). Intersecretory burst intervals were 150 ± 26 min in men and 106 ± 8 min in females (P = NS).

Deconvolution-estimated, subject-specific half-lives of endogenous GH were 17.2 ± 0.8 min in men and 15.9 ± 0.7 min

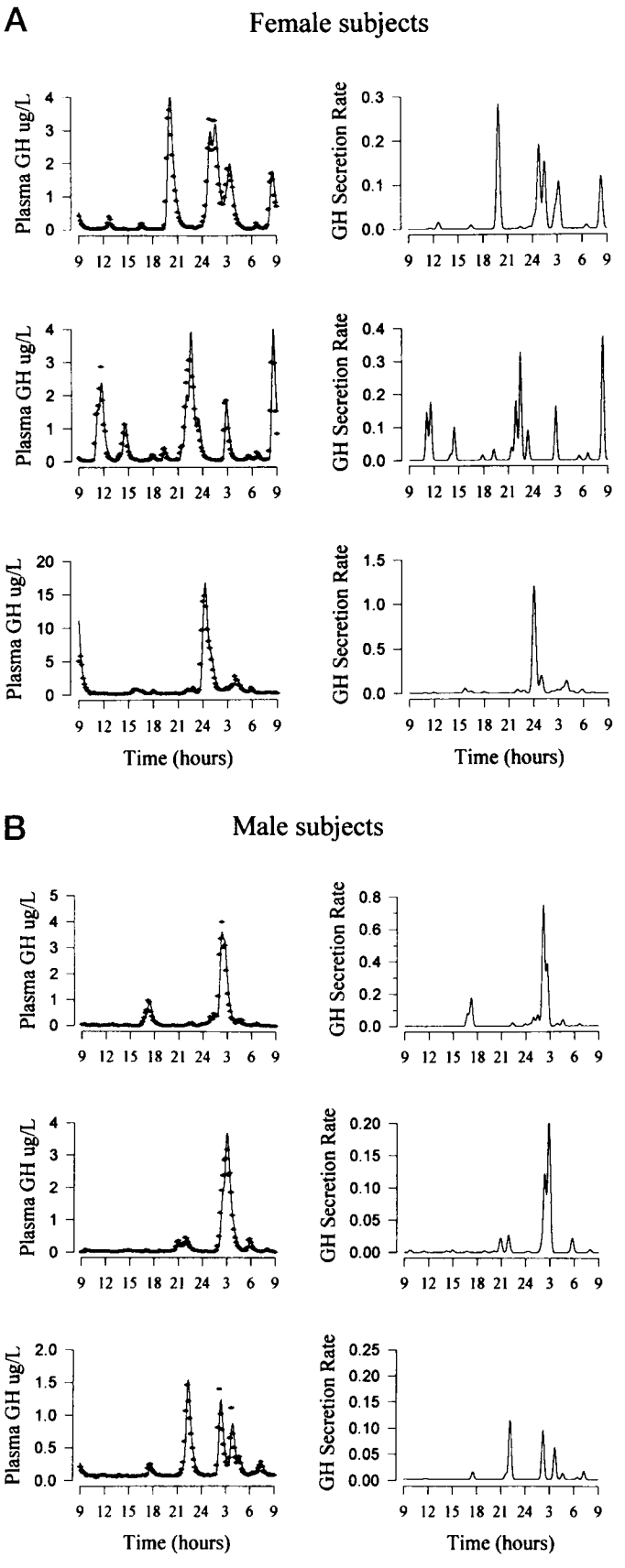

FIG. 1, A and B, Illustrative profiles of 24-h serum GH concentrations measured by time-resolved immunofluorometric assay in 3 women (A) and 3 men (B). Blood was sampled at 10-min intervals for 24 h in 11 women and 11 men, and results are illustrated for 3 volunteers of each gender. The *left* panels depict the measured serum GH concentrations over time with their intrasample dose-dependent standard deviations and the fitted curves predicted by deconvolution analysis. The *right* panels depict the deconvolution-calculated GH-secretory profiles consisting of almost exclusively burst-like GH-secretory episodes widely distributed over 24 h. Very low rates of basal secretion are also shown but constitute less than 10% of total daily GH production in these healthy middle-aged individuals.

in women (P = NS). In both sexes, more than 90% of GH production was secreted in a pulsatile mode. The total daily GH production rate (pulsatile plus nonpulsatile) was 15 ± 1.8 μg/L distribution volume in men and 47 ± 4.8 μg/L distribution volume in women ($P < 0.001$). The corresponding values for total GH secretion (assuming a GH distribution volume of 7.9% BW) were 95 ± 11 μg in men and 227 ± 18 μg in women ($P < 0.0001$). IGF-I plasma levels were 16.9 ± 1.3 nmol/L in males and 21.2 ± 1.9 nmol/L in females (P = 0.06). The ratio of serum IGF-I concentration and GH-secretion rate per liter distribution volume per day was 1.23 ± 0.13 nmol/L · μg in men and 0.48 ± 0.05 nmol/L · μg in women ($P < 0.0005$)

Typical 24-h profiles of serum GH concentrations submitted to deconvolution analysis in three men and three women are shown in Fig. 1A and 1B. In Fig. 2, the 24-h variations in GH-secretory burst interval (min), secretory-burst amplitude (μg/min · L distribution volume), and secretory burst mass (μg/L distribution volume) are shown for genders (Table 3).

Mesors (mean levels) for secretory-burst amplitudes and mass secreted per burst were higher in females than in males. The amplitudes and acrophases for these rhythms did not differ significantly between genders. Pulse frequency was slowest during the afternoon, for both sexes, with no differences in timing, amplitude, or mean level.

The nyctohemeral variations in GH secretion inferred by the cosinor analysis were supported by independent comparisons of daytime (0800–2200 h) *vs.* night (2200–0800 h) interburst intervals, secretory burst mass, and amplitude. Nighttime pulse intervals averaged 90 ± 11 min *vs.* daytime values of 218 ± 36 min in males (P = 0.01). The corresponding values in women were 85 ± 9 min and 145 ± 18 min (P = 0.008). The secretory burst amplitude in men was 0.009 ± 0.002 μg/L · min during daytime and increased significantly to 0.050 ± 0.012 μg/L · min during nighttime (P = 0.0044). The corresponding values for GH mass secreted in men were 0.35 ± 0.17 μg/L and 1.05 ± 0.48 μg/L (P = 0.0619). In women, the values were larger than in men; the secretory burst amplitude during daytime was 0.042 ± 0.014 μg/L · min and, during night, 0.11 ± 0.03 μg/L · min (P = 0.0044), and the corresponding GH mass secreted during these time sections was 1.68 ± 0.76 μg/L and 3.47 ± 0.90 μg/L (P = 0.0069).

Discussion

We have used a 3-fold strategy to clarify the mechanistic basis for increased-mean-serum GH concentrations in women compared with in men of similar age. In particular, the combination of an intensive and extended blood sampling paradigm, a new ultrasensitive immunofluorometric assay, and multiparameter deconvolution analysis un-

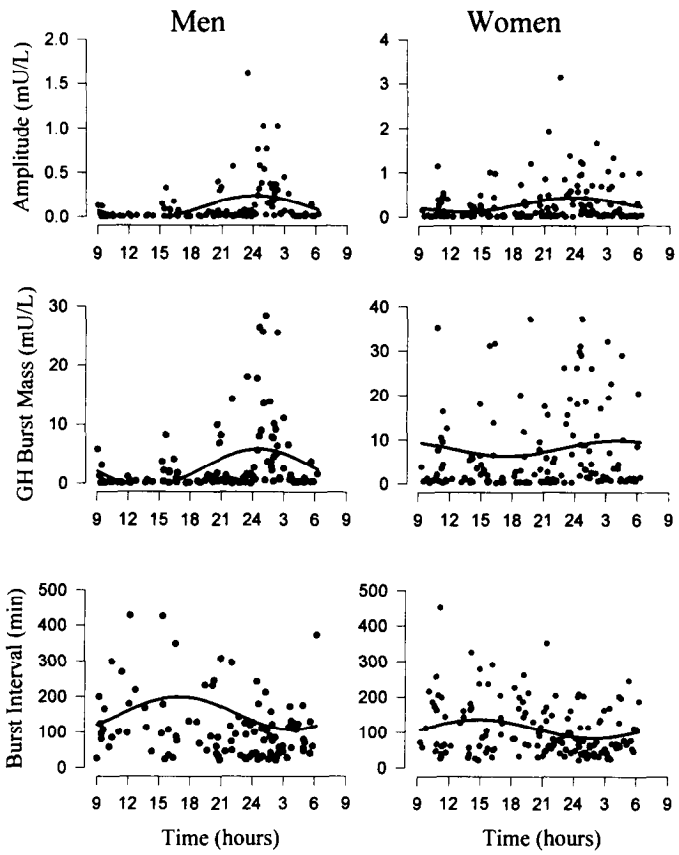

FIG. 2. Cosinor analysis of the deconvolution-derived maximal burst amplitude, burst mass, and interburst interval. The *drawn lines* represent the cosinor fitting.

masked a highly specific mechanism subserving increased serum GH concentrations in the adult female compared with male. Whereas men and women exhibited statistically similar calculated endogenous half-lives of GH disappearance from plasma, an indistinguishable mean GH-secretory burst frequency, and equivalent low-basal rates of GH secretion, women produced approximately 2-fold more GH within each secretory burst. This increased mass of GH secreted per release episode was, in turn, attributable to a higher amplitude (maximal rate of GH secretion attained within the calculated burst) of each inferred release episode. Based on computer modeling studies, we can infer that the higher mass of GH secreted per burst in women is sufficient to explain quantitatively the commensurately higher mean-serum-GH concentration and higher nadir levels in adult females compared with in males (28).

Our principal inference that women secrete more GH per burst, without generating more GH pulses per 24 h, increasing low basal rates of GH secretion or prolonging the half-life of endogenous GH, is also consistent with available data regarding the mechanisms by which estrogen and aromatizable androgen increase circulating GH concentrations. Specifically, estrogen administration to prepubertal girls with gonadal dysgenesis results primarily in an increase in the amplitude of detectable GH-secretory bursts akin to the effect of testosterone administration to boys with constitutionally delayed puberty (29–31). Moreover, the spontaneous increase (approximate doubling) of serum GH concentrations in mid- to late puberty in healthy boys is also accounted for by a selective increase in the mass of GH secreted per pulse, with no alteration in the half-life of GH or the GH-secretory burst frequency (32). Although some of these studies employed deconvolution analysis, low basal rates of GH secretion could not be estimated because blood sampling was carried out only every 20 min and assay sensitivity was 0.25–0.5 μg/L in these studies, which rendered GH levels in many samples collected during daytime undetectable (33). On the other hand, our use of an ultrasensitive GH assay here disclosed low (<10% of total) rates of basal GH secretion, which did not differ in men and women. Although the regulation and origin of such putative basal GH secretion are not known, the present analysis shows that time-invariant GH release is not gender specific.

Although simulation studies show that sampling intensity, assay sensitivity, experimental uncertainty in the data, hormone half-life, analytical methodology, etc., all can influence the absolute estimate of hormone pulse frequency (34), in the present analysis, the serum GH concentration profiles in men and women were established in the same highly sensitive assay, sampled at the same interval, analyzed by the same deconvolution technique, and found to have statistically indistinguishable half-lives. Accordingly, the 2-fold differences in GH-secretory burst mass between men and women is likely to reflect a true gender effect, rather than a procedural difference. Nonetheless, the present estimates of GH-secretory burst frequency in men and women represent relative values rather than absolute measures because the implications of high-sampling intensity, combined with an ultrasensitive assay technology to the deconvolution-based detection of GH-secretory pulses during the awake and fed state in men and women, are not known (35, 36). Even so, because 99% of serum samples contained detectable GH in the women, our estimates of GH-secretory pulse frequency in the female should approach a true value. On the other hand, if anything, we may have underestimated slightly the GH burst frequency in men, whose 50% lower mean serum GH concentrations were somewhat more often undetectable (94% detectable). Indeed, occasional false-negative errors in GH peak detection in men would be expected to lead to an underestimate of pulse frequency in the male. Thus, our inference that women do not secrete GH at a higher pulse frequency than men is likely to be correct.

The calculated half-life of endogenous GH as estimated by the present study by deconvolution analysis conforms well to the range of GH half-lives quantitated earlier by direct infusion studies at steady-state or after bolus injections of GH either purified from human pituitary extracts or prepared as 22-kDa protein (37–39). The present estimates of 16–18 min are very similar to those reported by Faria *et al.* for endogenous GH disappearance determined directly after sequential GHRH and somatostatin administration in young men, or Hartman *et al.* for spontaneous pulses (17, 36). Although there are considerable interindividual differences in GH half-life estimates, even within apparently homogenous study populations (22), we are unaware of systemic differences observed in GH half-lives under nonequilibrium conditions in men compared with in women. On the other hand, one

TABLE 3. Cosinor analysis of the 24-h serum GH concentration profiles

		Mean	Amplitude	Acrophase
Maximal rate of GH secretion/burst (μg/L · min)	Men	0.04 ± 0.01	0.05 ± 0.01	0200 (0009–0352)
	Women	0.10 ± 0.01[a]	0.06 ± 0.01	0136 (2308–0403)
Mass of GH secreted/burst (μg/L)	Men	1.02 ± 0.22	1.24 ± 0.30	0221 (0030–0410)
	Women	3.12 ± 0.40[b]	1.83 ± 0.55	0134 (2207–0400)
Interburst interval (min)	Men	154 ± 17	46 ± 23	1754 (1403–2145)
	Women	110 ± 6	27 ± 8	1536 (1300–1810)

Data are expressed as the mean ± SD. The first two digits of the acrophase indicate the clock hour; the last two digits show the number of minutes. Mesor and amplitude are given as microgram per L. The values between parentheses refer to the 95% confidence interval. Statistical comparisons between men and women were made with the Student's t test for unpaired data.

[a] $P < 0.00001$.

[b] $P < 0.001$.

study, employing steady-state infusions of GH, inferred a decrease in metabolic clearance in women compared with in men (37). Because the metabolic clearance rate of a hormone is controlled linearly by the plasma distribution volume and inversely by the hormone half-life, a decrease in the metabolic clearance rate of GH in women could be caused by either an increase in GH half-life or a decrease in distribution volume or both (39). Although the distribution volume was not measured in the study by Rosenbaum (37), other investigators have reported similar GH distribution volumes in men and women (40), as well as similar GH-binding protein concentrations (41). The latter similarity in men and women is of interest because the plasma GH-binding protein concentration may influence both the half-life and the apparent distribution volume for GH (42). Furthermore, the equilibrium estimate of the half-life of GH also is influenced by body mass or adiposity (23, 43), as well as the absolute concentration of GH, with an increase of apparent half-life from 10–20 min observed as serum GH concentration rises from 0–20 μg/L (34). These two additional factors would tend to have opposite effects on the GH half-life estimate in women, who have higher relative adiposity (conducive to a shorter half-life) and higher mean serum GH concentrations, potentially increasing GH plasma half-life. Here, we found statistically indistinguishable calculated half-lives of endogenous GH in women and men. The present clear gender distinctions in the temporal mode of GH secretion apply to a relatively small group of healthy, middle-aged volunteers. As such, they do not necessarily predict equivalent female-male contrasts in pre- or peripubertal children and young adults or in postmenopausal individuals. In addition to age-dependent differences in absolute GH secretion rates and the relative disorderliness of GH secretion, adiposity (as inferred by body mass index and hydrostatic weighing) also determines pulsatile GH secretion (44). Consequently, inferences derived from small heterogeneous study groups must be extrapolated with caution to other populations.

GH secretion is subject to exquisite metabolic regulation, including rapid negative feedback regulation by plasma IGF-I. Indeed, many physiological situations are characterized by a reciprocal relationship between the mean serum GH concentration and the plasma IGF-I concentration (45). In puberty and acromegaly, this relationship is modified as plasma IGF-I and GH concentrations increase concomitantly. In the present comparison between middle-aged men and women, we observed higher mean serum GH concentrations in women, but similar plasma IGF-I concentrations in men and women, leading to a 2.5-fold lower IGF-I/GH ratio in females compared with in men. Assuming comparable free IGF-I levels, this observation is consistent with possible interpretations that the increase of GH in women is less biologically active, that IGF-I clearance is higher in women, and/or that the feedback sensitivity of the hypothalamo-pituitary axis to suppressive effects of IGF-I is relatively attenuated in women compared with in men. Of note, the physiological premenopausal state represented by relatively increased blood GH, but not IGF-I concentrations compared with in men, is mimicked qualitatively in studies of oral, but not necessarily transdermal estrogen replacement in ovariprival states (6, 7, 8, 46). On the other hand, treatment with testosterone stimulates an increase in both serum GH and plasma IGF-I concentrations in GH-sufficient boys (31), which emulates the later stages of normal puberty in boys and girls.

Direct actions of estrogen on anterior pituitary GH secretion *in vitro* have been reported in some, but not most, studies (47). On the other hand, steroid hormones do influence somatostatin peptide and messenger RNA content in the hypothalamus, as well as GHRH concentrations (48). Moreover, steroid hormones influence multiple neurotransmitters in the brain, some of which impinge on somatostatin and GHRH-secreting neuronal systems (49). Although the changes in hypothalamic somatostatin and GHRH release that occur in the primate in response to steroid hormone treatment or physiological steroidal changes are not known, the ability of estrogen or aromatizable androgens to increase the mass of GH secreted per burst without altering the GH pulse frequency (36) is consistent with enhanced GHRH secretion per pulse, relative withdrawal of somatostatin inhibition, and/or increased pituitary sensitivity to GHRH or other secretagogue. In this regard, it is of interest that aging female rats can still respond to the hexapeptide GHRP combined with GHRH with remarkably increased rates of GH secretion (50). However, the identity and role of any putative endogenous ligand that might activate the GHRP receptor are not known.

Sampling of the hypothalamo-pituitary portal blood system in the rat and sheep indicate that a GH pulse is closely associated with a concurrent or prior burst of GHRH secretion with (in the rat) or without (in the ovariectomized sheep) concomitant suppression of somatostatin release (51, 52). Consequently, the true frequency of detectable GH-release episodes is controlled in its upper limit by the frequency of hypothalamic GHRH burst generation and, on its lower

bound, by ambient somatostatin inhibition. Indeed, continuous delivery of GHRH in the human results in pulsatile GH secretion, putatively because of intermittent somatostatin withdrawal (53). Whether somatostatin inhibitory tone is similar in men and women is not known, although one study describes and another report denies a gender difference in GH secretion stimulated by pyridostigmine, an indirect cholinergic agonist that reduces hypothalamic somatostatin release, at least in the rat (54). Our cosinor analysis revealed increased nocturnal GH-secretory release that was larger in men than women. Whether these differences are caused by different secretory patterns of GHRH and somatostatin in men and women is not known. This hypothesis is suggested further by our recent demonstration of remarkable differences in the serial regularity of serum GH profiles in male and female animals and in men and women. In this study, an approximate entropy statistic was used to demonstrate that the GH-release process is significantly less orderly in female than male (55). These observations imply both quantitative and qualitative differences in the regulatory interactions between GHRH and somatostatin in women and men. Further investigations using strategies to modify selectively hypothalamic somatostatin and GHRH secretion in the human will be necessary ultimately to establish the relative and absolute roles of these regulatory peptides in mediating the clear gender distinction in pulsatile GH secretion examined here.

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