Gender and Sexual Maturation-Dependent Contrasts in the Neuroregulation of Growth Hormone Secretion in Prepubertal and Late Adolescent Males and Females—A General Clinical Research Center-Based Study*

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ABSTRACT

Although numerous studies have delineated an impact of gender on the neuroendocrine control of GH secretion in the adult, few investigations have defined the nature and extent of sex differences before puberty. This deficit reflects jointly the sensitivity limitations of earlier GH assays and the paucity of intensive sampling protocols in healthy children. Here we have applied a chemiluminescence-based GH assay (sensitivity, 0.005 μg/L) to study GH release in blood sampled every 10 min for 12 h from 1800–0600 h in 58 healthy children. Males and females were evaluated in prepuberty (n = 17 boys; n = 11 girls) and late adolescence (n = 15 males; n = 17 females). We quantitated the principal regulated facets of GH release by 1) deconvolution analysis to assess basal vs. pulsatile GH secretion, 2) approximate entropy to compute the regularity of GH release patterns, and 3) cosine regression analysis to evaluate the overnight rhythmic release of GH. Gender by maturation analysis of variance revealed a mean 2.3-fold increase in the integrated serum GH concentration between prepuberty and late adolescence (P < 10−6). Deconvolution analysis disclosed that 91–97% of total GH secretion was pulsatile. Pulsatile, but not basal, GH release showed marked sexual maturation dependence (P < 10−5). Pulsatile GH release rose in adolescents due to a 2.25-fold greater GH secretory burst mass (P = 0.00011), which reflected joint 1.5-fold increases in GH secretory pulse amplitude and duration (P < 0.01). Pulse-mass enhancement across puberty was gender independent, but mechanistically specific, as GH pulse frequency, intersecretory burst interval, and half-life were invariant of pubertal status. The approximate entropy statistic identified more disorderly GH secretion patterns in adolescent females compared with prepubertal children and adolescent males (P = 0.00074). Cosinor analysis unmasked elevated overnight rhythms in GH secretory burst mass and interburst intervals in late adolescents of both genders compared with prepubertal boys (for burst mass) or girls (for interburst intervals). Linear regression analysis disclosed strong correlations among 1) the plasma insulin-like growth factor I concentration and GH secretory burst mass (P < 10−3), 2) the GH pulse mass and the serum testosterone concentration (P = 10−3), 3) the irregularity (entropy) of GH secretory patterns and the serum estradiol concentration (P < 10−3), and 4) the basal GH secretion rate and the serum estradiol concentration (P = 10−3).

In summary, healthy prepubertal children and late adolescent boys and girls manifest distinctive mechanisms controlling GH release, as appraised for all three of the pulsatile, entropic, and 12-h rhythmic modes of GH neuroregulation. The major maturational contrast in the pulsatile mode of GH secretion is amplified secretory burst mass in adolescents due to jointly heightened GH pulse amplitude and duration. The dominant gender distinction lies in the reduced regularity of GH release patterns in late adolescent girls. Overnight rhythms in GH secretory burst mass and interburst intervals enlarge in both sexes at adolescence, thus signaling enhanced coupling between the rhythmic and pulsatile control of GH release at this time. At the extrema of pubertal development, sex steroid hormones are associated differentially with specific facets of GH release, e.g., an elevated basal GH secretion rate (estrogen), greater irregularity of GH release patterns (estrogen), and amplified GH secretory burst mass and higher plasma insulin-like growth factor I concentrations (testosterone). Accordingly, we postulate that sex steroids supervise selectively each of the dominant facets of GH neurosecretory control across human puberty. (J Clin Endocrinol Metab 85: 2385–2394, 2000)
niques have overcome this sensitivity constraint (17, 18, 20). Concurrently, more informative analytical methods have emerged to quantify the pulsatile, nonpulsatile (basal), end-
tropic (pattern regularity), and rhythmic-episodic coupling of GH secretion in the human (1).

In light of the foregoing issues, the present studies evaluated GH secretion in healthy prepubertal and late adolescent boys and girls using 1) repetitive (10-min) blood sampling; 2) a high sensitivity chemiluminescence GH assay (sensitivity, 0.005 μg/L); 3) deconvolution analysis to estimate both basal and pulsatile GH secretion; 4) the approximate entropy (ApEn) statistic to quantify the regularity of GH release patterns, which is strongly gender dependent at least in the adult (21, 22); and 5) cosine regression to assess overnight rhythmic variations in GH secretory burst mass and frequency (i.e., rhythmic-episodic GH coupling). These neuroendocrine analyses unmask prominent interactive effects of gender and sexual maturation on each primary regulated facet of GH secretion.

Subjects and Methods

Study subjects

The 4 study groups comprised a total of 58 healthy children: i.e., prepubertal boys (n = 17) and girls (n = 11), and late adolescent males (n = 13) and females (n = 17). Corresponding ages are given in Table 1. The height and weight of each subject fell between the 5th and 95th percentiles for age based on standards from the National Center for Health Statistics. In prepubertal children, the bone age was within 2 sp of values predicted for chronological age. Prepuberty was defined in boys as stage I genitalia and pubic hair, and in girls as stage I breasts and pubic hair, as described by Tanner. Late adolescence was defined by pubertal developmental stage IV or V for genitalia in boys and for breasts in girls. A nonoverlapping description of the serum GH concentration peak characteristics in some of these profiles was included in a previous body compositional analysis (23).

The study was reviewed and approved by the human investigation committee of the University of Virginia. The reason for the study and the potential risks were discussed with each parent, child, and adolescent before participation. Each volunteer was informed that the study would not provide direct benefit to him/her, although all data would be made available and explained upon request. All subjects under the age of 18 yr gave individual assent, with written consent provided by a parent. The studies were performed at the General Clinical Research Center at the University of Virginia.

Clinical protocol

Volunteers were unmedicated with a normal medical history, physical examination, and screening blood tests of hematological, renal, metabolic, electrolytic, and hepatic function. No volunteers had acute illness, chronic disease, psychiatric disorder, recent medication use (within five biological half-lives), transdermal travel (within 2 weeks), or significant weight changes (e.g., 1 kg or more within 10 days).

The sampling protocol consisted of repetitive blood removal overnight at 10-min intervals (1.5 mL each) for 12 h beginning at 1800 h. To allow adaptation, volunteers were admitted to the General Clinical Research Center in the morning (0800 h). A constant diet was given, beginning with breakfast. An iv catheter was inserted in a forearm vein at least 1 h before blood sampling began. Sleep monitoring was not performed, but lights were put out at 2000 h.

Screening endocrine measurements were made by RIA or immuno-
radiometric assay (IRMA) of (morning) serum concentrations of testos-
terone, estradiol, FSH, LH, PRL, insulin-like growth factor I (IGF-I), T4, and TSH (6, 17), all of which were appropriate to maturational state and gender.

Assays

GH concentrations were measured in each serum sample in duplicate by an automated ultrasensitive chemiluminescence-based assay (mod-
ified Nichols Luma Tag hGH assay; sensitivity, 0.005 μg/L) using hu-
man recombinant GH (22 kDa) as the assay standard, as described previously (17–19). The median inter- and intraassay coefficients of variation were less than 6.5% and 8.5%, respectively. All 73 serum samples from each admission were assayed together. Unknown sample values were interpolated from GH standard curves along with their joint variances, as described recently using a four- or five-parameter best-fit monotonic sigmoidal function (24).

Serum estradiol was measured by RIA using solid phase RIA (Coat-
A-Count, Diagnostic Products, Los Angeles, CA) with a sensitivity of 10 pg/mL (17, 18). Undetectable sample values were arbitrarily assigned this threshold for statistical purposes.

Deconvolution analysis

Deconvolution analysis was applied to estimate basal and pulsatile GH secretion jointly (17, 25, 26). Total pulsatile GH secretion is calculated as the product of secretory burst frequency and the mean mass of GH released per pulse. Basal secretion represents time-invariant (interpulse) GH release (26). Overall GH production is the sum of these components. Deconvolution analysis was carried out using 95% statistical confidence intervals to define significant (nonzero amplitudes of) calculated GH secretory bursts (17, 27). The technician was blinded to study design.

Overnight (12-h) rhythmicity

Cosine regression with a 720-min periodicity was used to quantify the inherent overnight rhythms in deconvolution-calculated GH secretory burst mass and intersecretory pulse intervals in each of the four study groups (27, 28). Only rhythms with significantly nonzero (P < 0.05) amplitudes were considered further.

TABLE 1. Clinical characteristics of study groups

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Range (yr)</th>
<th>Sex</th>
<th>No.</th>
<th>IGF-I (μg/L)</th>
<th>Testosterone (ng/dL)</th>
<th>Estradiol (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prepubertal</td>
<td>10.6 ± 0.31</td>
<td>8.44–12.03</td>
<td>M</td>
<td>17</td>
<td>169 ± 12c</td>
<td>12 ± 1.3c</td>
</tr>
<tr>
<td>9.9 ± 0.34</td>
<td>8.56–10.74</td>
<td>F</td>
<td>11</td>
<td>231 ± 13c</td>
<td>11 ± 1.6c</td>
<td>13 ± 0.4c</td>
</tr>
<tr>
<td>Adolescents</td>
<td>14.9 ± 0.35</td>
<td>13.1–16.9</td>
<td>M</td>
<td>13</td>
<td>489 ± 41d</td>
<td>543 ± 65d</td>
</tr>
<tr>
<td>14.1 ± 0.27</td>
<td>12.2–16.0</td>
<td>F</td>
<td>17</td>
<td>507 ± 40d</td>
<td>31 ± 3.0c</td>
<td>85 ± 18c</td>
</tr>
</tbody>
</table>

P values<0.001<0.001<0.001

Data are the mean ± SEM (absolute range) for age and serum hormone concentrations. Note, serum estradiol concentrations in both prepubertal groups overlap with the detection limit (10 pg/mL) in this assay (see Materials and Methods).

Multiply by 0.0347 to convert to nanomoles per L.

Multiply by 3.67 to convert to picomoles per L.

different alphabetic superscripts within columns denote significantly different means within that column (P values determined by ANOVA).
ApEn

ApEn was applied as a scale- and model-independent statistic to quantify the orderliness or regularity of GH release patterns. Normalized ApEn parameters of \( m = 1 \) (series length) and \( r = 20\% \) (threshold) of the intraseries so were applied, as previously described (6, 22). This ApEn statistic is thus designated ApEn (1, 20\%). ApEn evaluates the consistency of recurrence of subpatterns in the data. For a neuroendocrine axis, ApEn is an indirect barometer of feedback activity. Higher absolute ApEn values given the same series lengths and ApEn parameter values as those used here denote greater disorderliness of subordinate patterns in the time series.

Statistical analysis

Because of nonnormal distributions, measures derived by deconvolution, entropy, and cosinor analyses were compared by ANOVA after logarithmic transformation (1). Mean and integrated (12-h) serum GH concentrations were compared by ANOVA of untransformed data. Computed data are presented as the mean \( \pm \) sem. Statistical significance was construed for \( P < 0.05 \).

Linear regression analysis was applied to test for correlations between GH secretory pulse mass and serum IGF-I or sex steroid hormone concentrations. To allow for multiple comparisons, we used a protected \( P \) value of \( \leq 0.01 \), which, on the average, would yield only one false positive inference for each 100 regressions carried out.

Results

As shown in Fig. 1, overnight frequently sampled serum GH concentration profiles in prepubertal boys and girls and late adolescents of both sexes were visibly pulsatile. Figure 1A shows the observed and deconvolution-predicted fits of the serial serum GH concentrations. Figure 1B shows the calculated underlying GH secretory bursts, highlighting the episodicity of release in males and females.

Figure 2 summarizes the mean and integrated (12-h) serum GH concentrations in the total of 58 prepubertal and late adolescent girls and boys studied. There were 2- to 2.5-fold increases in the mean and integrated serum GH concentrations in late puberty (\( P < 10^{-5} \)), which were gender independent (Fig. 2).

Figure 3 illustrates that the mass of GH secreted per burst (integral of the calculated GH secretory event) was maturation (but not gender) dependent, whereas GH burst frequency was not (Table 2). There were 2.2- to 2.3-fold elevations in GH secretory burst mass in adolescent males and females compared with values in prepubertal children of either gender. The amplification of GH secretory burst mass in adolescents reflected a 1.5-fold augmentation of GH secretory burst amplitude (calculated maximal rate of GH secretion attained within a release episode) and a concomitant 1.5-fold prolongation of the GH secretory burst half-duration (computed duration of the underlying secretory pulse at half-maximal amplitude; \( P < 0.01 \)). In contrast, GH half-life, secretory burst duration, frequency, and interburst interval were invariant of either gender or maturational status (Table 2).

Pubertal maturational status significantly influenced the pulsatile, and hence total, GH production rate (\( P < 10^{-5} \), by ANOVA). The latter was 2.25-fold higher in adolescents compared with prepubertal children of either gender (Fig. 4). Prepubertal boys and girls had similar pulsatile GH secretion rates, as did adolescent males and females. Basal GH secretion did not vary significantly (Table 2). The mean percentage of GH secretion that was pulsatile was invariant across the four study groups, as follows: 96 \( \pm \) 11\% (girls) and 91 \( \pm \) 9.3\% (boys), 96 \( \pm \) 8.9\% (female adolescents), and 97 \( \pm \) 13\% (male adolescents; \( P = NS \)).

The ApEn statistic was used to quantify the pattern orderliness of GH release and hence provide indirect information regarding feedback activity within the GH-IGF-I axis (6). As highlighted in Fig. 5, mean GH ApEn was significantly higher in adolescent females than in prepubertal girls, prepubertal boys, or adolescent males (\( P = 0.00074 \)). These differences signify that adolescent girls (only) maintain a more irregular GH release process.

Cosinor analysis was applied to quantify the magnitude and timing of the overnight rhythms in 1) the (deconvolution-calculated) mass of the GH secreted per burst, and 2) GH intersecretory burst intervals (directly estimated, but proportionate to the reciprocal of GH pulse frequency). The characteristics of the overnight rhythms in GH secretory burst mass and interburst intervals are summarized in Table 3. The mass of GH secreted per burst varied significantly overnight by a mean amplitude of approximately 35\% of the mesor in all four study groups. The absolute amplitude of this variation was greater in late adolescents of both genders than in prepubertal boys (but not girls). There was no other sex difference either before puberty or in late adolescence. GH interpulse intervals also maintained significant overnight rhythmicity, albeit at a slightly lower fractional amplitude than pulse mass (i.e. by a mean amplitude of \( \approx 21\% \) of the mesor). The mesor of this rhythm was higher in adolescents of both genders than in prepubertal girls (but not boys).

The results of linear regression analyses using data from all four study groups combined are summarized in Table 4. Strongly positive linear correlations were evident between 1) the serum estradiol concentration and the ApEn (irregularity) of GH secretion (\( P < 10^{-5} \)) as well as basal GH secretion (\( P = 0.013 \)), 2) the serum testosterone concentration and GH secretory burst mass (\( P = 0.001 \)) and GH half-life (\( P = 0.0025 \)), and 3) the plasma IGF-I concentration and GH secretory burst mass (\( P < 10^{-5} \)).

Discussion

To our knowledge, no prior clinical studies have investigated the single and joint (interactive) impact of gender and pubertal maturation on GH neuroregulation in children by combining the contemporary methodologies of intensive blood sampling, an ultrasensitive GH assay, deconvolution analysis, ApEn, and measures of rhythmic-episodic pulse coupling. To this end, we studied 28 healthy prepubertal children and 30 late adolescents of both genders. Using the foregoing collective strategies, we could identify 1) a consistent 2.3-fold increase in GH secretory pulse mass in healthy, late adolescent girls and boys compared with prepubertal children of either gender; 2) a strong dependence of GH secretory burst mass on sexual maturational status, but not on gender; 3) invariance of basal GH release, GH pulse frequency, half-life, and percentage of pulsatile GH secretion to gender and pubertal maturation; 4) greater irregularity of GH secretory patterns in adolescent girls; and 5) specific
FIG. 1. Illustrative (10-min sampled) profiles of pulsatile serum GH concentrations measured in an ultrahigh sensitivity chemiluminescence-based assay in a prepubertal and late adolescent male and female volunteer. A, Overnight (1800–0600 h) serum GH concentrations (±SD) and deconvolution-predicted fits of the data (continuous lines). B, Corresponding deconvolution-calculated basal and pulsatile GH secretion rates. Note that 720 min (start of sampling) denotes 1800 h, and 1440 min (end of sampling) designates 0600 h the next morning.
correlations of regulated facets of GH secretion with estrogen or androgen (see below). The present investigation in healthy children reveals that GH secretory burst mass is sex independent before puberty. In contrast, in young and midadulthood, GH secretory pulse mass is strongly (1.5- to 2.0 fold) gender dependent (27). However, male and female adolescents exhibited a prominent amplification of GH pulse mass compared with prepubertal children. The difference was mechanistically specific, as the basal GH secretion rate, burst frequency, interpulse interval and GH half-life were not affected by pubertal status. Deconvolution analysis attributed the elevation in GH pulse mass to a combined (1.5-fold) greater secretory burst duration and amplitude (maximal rate of GH secretion attained within a secretory pulse). We are unaware of any prior recognition of the latter dual mechanism of physiological regulation of pulsatile GH secretion in puberty. Indeed, previous application of a 20-min blood sampling regimen and a lower sensitivity GH IRMA identified only an increase in GH secretory burst amplitude in pubertal boys (4), most likely reflecting the dual technical constraints of limited sampling frequency and imperfect assay sensitivity (see the introduction).

According to current neuroregulatory concepts, GH secretory pulses are evoked when an adequate hypothalamic (GHRH) stimulus is delivered via the hypothalamo-pituitary portal microvasculature to responsive somatotrope in the absence of excessive restraint by somatostatin (1). Single hypothalamo-pituitary portal venous blood samples in urethane-anesthetized adult male rats disclosed simultaneous somatostatin withdrawal and GHRH release during a pulse of GH secretion (29). In other mammals, such as the sheep and pig, high frequency repeated portal blood sampling in conscious animals has unveiled a wider variety of soma-
tostatin-GHRH interactions (30, 31). This disparity may reflect technical differences, species distinctions, and/or modulatory signaling by other putative coregulators of GH secretion (1). Considering the simplest model of reciprocal release of GHRH and somatostatin, our finding of an elevated mass of GH secreted per burst in late adolescence would probably reflect enhanced competing pituitary actions of GHRH over somatostatin. Inferred GHRH predominance could be mediated, in turn, via an absolute increase in hypothalamic GHRH release or action and/or a reduction in somatostatin restraint. In the present analysis the unchanging GH secretory burst frequency would speak against a mechanism that involves solely a reduction in hypothalamic somatostatin secretion in late puberty. This reasoning follows from analyses in other clinical states marked by presumptive somatostatin withdrawal, e.g. fasting, type I diabetes mellitus, and sleep, wherein the same sampling and analytical techniques readily detect accelerated GH secretory burst frequency (17, 27, 32). Conversely, GH peak frequency falls in response to short-term iv infusion of somatostatin in young men (20). The present new data thus favor (but do not prove) an interpretation of amplified endogenous GHRH release and/or actions in late adolescence, rather than exclusively or predominantly somatostatin withdrawal during the nighttime phase of GH release. To our knowledge, analogous data are not available for the daytime fed state.

Although the identities of postulated endogenous (non-GHRH) cosecretogogues of GH remain unknown in the hu-

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**Fig. 3.** Pulsatile (top), basal (middle), and total (basal plus pulsatile; bottom) GH secretion rates (micrograms per L/12 h) as quantitated by deconvolution analysis in a total of 88 prepubertal children and late adolescents of both genders. Children underwent blood sampling at 10-min intervals from 1800–0600 h. Data were obtained by GH chemiluminescence assay and deconvolution analysis (see Subjects and Methods and Fig. 2).
man, constant iv infusion of GH-releasing peptide-2 (GHRP-2), a novel (non-GHRH) synthetic hexapeptide stimulus of GH release selectively amplifies GH secretory burst mass and concurrently elevates the plasma IGF-I concentration without altering the half-life of GH, the interpulse interval, or the pulse frequency (33). These GHRP-2-driven dynamics in the adult closely emulate the foregoing physiology of the maturing GH axis in adolescents of both sexes. Thus, albeit unproven, the present clinical data do not exclude a possible role for (putative) endogenous GHRP-like cosecretagogues in augmenting pulsatile GH secretion in late puberty.

Deconvolution analysis and an ultrasensitive GH assay here predicted equivalent basal (interpulse) GH secretion in prepubertal and late adolescent girls and boys. Whereas analysis of basal hormone release is technically difficult, especially if there are few interpulse measurements of serum hormone concentrations (34), the present 12-h GH profiles typically displayed intervals of secretory quiescence at the onset (1800 h) and/or offset (0600 h) of the blood-sampling session. Basal GH secretion in puberty is of interest, because virtually nothing is known about its physiological regulation in the human. Indeed, until recently, interpulse serum GH concentrations often remained undetectable in both the experimental animal (e.g. adult male rat as studied by RIA) and human (e.g. adults and children evaluated in the daytime fed state using earlier IRMAs). The present analysis unexpectedly demonstrated apparently unchanging basal GH secretion rates in pre- and late puberty. This finding is probably not artifactual, as the same deconvolution technique disclosed severalfold elevated basal GH release in premenopausal women compared with similarly aged men (27, 35).

Recent clinical application of ultrasensitive GH assays has revealed that (basal) interpulse GH release persists in hypopituitary patients (36), somatostatin-infused men (20), estrogen-withdrawn postmenopausal women (37), and hypothyroid, obese, and older volunteers (17, 18). The origin and regulation of nonpulsatile GH secretion are poorly understood. However, in one recent study, repeated pulsatile iv infusions of GHRH (0.33 \( \mu g/kg \) bolus every 90 min for 3 days) in healthy men significantly increased basal GH secretion, as estimated by deconvolution analysis (38).

### TABLE 2. Selected deconvolution measures of GH release in male and female prepubertal children and adolescents

<table>
<thead>
<tr>
<th>GH measure</th>
<th>Prepubertal children</th>
<th>Adolescents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Boys (n = 17)</td>
<td>Girls (n = 11)</td>
</tr>
<tr>
<td>GH half-life (min)</td>
<td>17 ± 0.95</td>
<td>18 ± 0.65</td>
</tr>
<tr>
<td>GH burst frequency/12 h</td>
<td>11 ± 0.74</td>
<td>9.2 ± 0.48</td>
</tr>
<tr>
<td>Basal secretory rate (( \mu g/L \times 10^{-3} ))</td>
<td>8.7 ± 4.7</td>
<td>8.5 ± 1.1</td>
</tr>
<tr>
<td>Total GH secretory rate (( \mu g/L \times 12 \ h ))</td>
<td>65 ± 11b</td>
<td>74 ± 9b</td>
</tr>
<tr>
<td>Interburst interval (min)</td>
<td>71 ± 5</td>
<td>76 ± 6</td>
</tr>
<tr>
<td>Burst half-duration (min)</td>
<td>22 ± 2b</td>
<td>23 ± 1b</td>
</tr>
</tbody>
</table>

Data are the mean ± SEM. *n*, The number of volunteers studied.

* P > 0.05 for comparisons among the four study groups (as assessed by ANOVA).

b,c Different alphabetic superscripts denote significantly different mean values.

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**FIG. 4.** GH secretory burst mass in a total of 58 prepubertal and late adolescent males and females. A chemiluminescence-based GH assay and deconvolution analysis were applied to quantitate overnight pulsatile GH secretion (see Subjects and Methods). Data are presented otherwise as described in Fig. 2. Different superscripts define different means. NS, P > 0.05.
Continuous infusion of GHRP-2 (1 mg/kg·z h, iv, for 24 h) in postmenopausal women also elevated basal GH secretion (39). In contrast, in a similar population, oral estrogen administration raised interpulse serum GH concentrations solely by augmenting GH secretory pulse mass (37). In the latter context, we emphasize that increases in interpeak serum GH concentrations can arise from one of several distinguishable secretory mechanisms, e.g. more frequent GH secretory bursts, a higher burst mass, a prolonged hormone half-life, and/or greater basal secretion (25, 26, 40). Thus, appropriate analytical tools must be applied to dissect the individual contributions of the foregoing mechanisms.

As a complementary, but statistically independent, measure of neuroregulation within the GH axis, we used the ApEn statistic. This new regularity metric quantifies the reproducibility or orderliness of biological time series, such as hormone release patterns, and can serve as a barometer of feedback activity within an axis (41–43). The ApEn measure showed that neither gender nor pubertal maturation singly controls the orderliness of GH release, as only late adolescent girls exhibited more disorderly GH secretory patterns (P = 0.00074). Elevated ApEn values in older girls are consistent with the emergence of new or stronger feedback interactions within the adolescent female GH-IGF-I axis (6). More intense interactions could reflect changing patterns of hypothalamic somatostatin and GHRH release, altered intrapituitary (paracrine or autocrine) regulation of somatotrope function, and/or reduced negative feedback effects of GH or IGF-I on the hypothalamo-pituitary unit. The available clinical data cannot yet distinguish definitively among these primary hypotheses. However, experiments in the rodent have unmasked consistently attenuated GH autonegative feedback in the female compared with the male animal (44). In thematic analogy, a recent clinical study showed that young women are relatively resistant (compared with young men) to the GH-suppressing effect of peripherally infused IGF-I (45). Thus, we can suggest that the elevated ApEn of GH profiles in late adolescent girls may mirror reduced GH and/or IGF-I autonegative feedback at this time (6, 17, 21). This change could be due to relative estrogen predominance, as gonadectomy or GnRH agonist-induced down-regulation of the reproductive axis in the immature rat blunts the vivid gender contrast in GH release patterns otherwise evident in the adult (21). In corollary, in clinical studies, the administration of estrogen (or aromatizable androgen) elicits more irregular patterns of GH release (6, 37, 46), and thereby mim-

### FIG. 5. ApEn (1, 20%) of frequently sampled serum GH concentration profiles in 58 prepubertal children and adolescents of both genders. Higher ApEn values denote greater irregularity or disorderliness of GH release patterns. Data are presented as defined in Fig. 2.

### TABLE 3. Overnight rhythms in deconvolution-calculated GH secretory burst mass and interpulse intervals in male and female prepubertal children and adolescents

<table>
<thead>
<tr>
<th>Measure</th>
<th>Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prepubertal boys</td>
</tr>
<tr>
<td>A. GH secretory burst mass (μg/L)</td>
<td></td>
</tr>
<tr>
<td>Mesor (mean)</td>
<td>2.3 (1.8–2.8)</td>
</tr>
<tr>
<td>Amplitude</td>
<td>1.2 (0.55–1.9)</td>
</tr>
<tr>
<td>Acrophase</td>
<td>1159 (±68)</td>
</tr>
<tr>
<td>B. Interburst interval (min)</td>
<td></td>
</tr>
<tr>
<td>Mesor (mean)</td>
<td>69 (60–78)</td>
</tr>
<tr>
<td>Amplitude</td>
<td>18 (5–31)</td>
</tr>
<tr>
<td>Acrophase</td>
<td>1912 (±79)</td>
</tr>
</tbody>
</table>

Data are the mean best-fit values by cosine regression (95% statistical confidence intervals or ±SEM). The acrophase is the clocktime (±minutes). The interburst interval is proportionate to the reciprocal of pulse frequency.

a, b, c Means with unshared alphabetic superscripts within rows are significantly different at P < 0.05.

c No significant group differences.
The following greater disorderliness of GH evident in late pubertal girls. Thus, we speculate that heightened irregularity of GH release patterns in women (22) and late adolescent girls (present data) originates from estrogen-dependent regulation of GH and/or IGF-I feedback control mechanisms. Further interventional experiments will be needed to confirm or refute this clinical hypothesis.

Diurnal rhythms in serum concentrations in the adult can be ascribed to corresponding 24-h variations in (ultradian) secretory burst amplitude (mass) and/or frequency (28). With respect to both sleep and the smaller circadian contribution (17), nyctohemeral rhythmicity of GH release is probably under the supervision of one or more central nervous system (CNS) regulatory centers (1). Here, we applied cosine regression analysis to evaluate overnight rhythmic variations in GH secretory pulse mass and interpulse interval. Thereby, we could assess the degree of coupling of the rhythmic and episodic modes of GH secretion in children of both sexes before and during late puberty. GH secretory burst mass and interpulse interval both varied significantly (respectively, by 35% and 21% of their mean values) overnight in prepubertal and adolescent males and females. The mass of GH secreted per burst was maximal (acrophase) at approximately midnight. Although sleep monitoring was not performed in these children, the acrophase mirrors the expected rise in GH secretion during first onset deep (slow wave) sleep (47). The amplitude as well as the cosine mean of the nighttime variation in the mass of GH secreted per burst increased by 2-fold in adolescents of both sexes compared with prepubertal boys (but not girls, who showed intermediate values). GH interpulse interval values likewise exhibited a significant nighttime rhythmicity in all four study groups, with an estimated nadir (corresponding to the time of the maximum in GH secretory burst frequency) at approximately 0645 h. This acrophase was independent of gender and maturation. On the other hand, the cosine mean of the rhythm in interpulse intervals was higher in adolescents of both sexes than in prepubertal girls (but not prepubertal boys). The foregoing sex-distinctive variations in GH secretory burst mass and frequency in puberty may reflect developmental modulation of GH pulsatility via a combination of relevant CNS inputs, e.g. neural signals originating in the suprachiasmatic nucleus, and other inputs associated with the sleep-wake activity cycle (1). Additional clinical experiments will be required to identify the nature of CNS pathways that confer these gender and maturation-dependent changes in rhythmic-pulsatile GH coupling.

In summary, the present multifold analyses of GH secretory dynamics in 58 prepubertal and late adolescent healthy boys and girls indicate that 1) basal GH secretion is evident before puberty in both sexes; 2) GH half-life, pulse frequency, interburst interval, pulse duration, burst amplitude and mass, and the ApEn of GH release patterns are gender-independent before puberty; 3) the ApEn of GH secretory patterns increases significantly in the late adolescent female compared with the late pubertal male and prepubertal children of both genders; 4) basal GH secretion, GH half-life, and GH pulse frequency do not differ before and after puberty; 5) GH secretion is predominantly pulsatile (global mean, 95% of total secretion) in both prepuberty and late puberty in both sexes; and 6) the overnight rhythms in GH secretory burst mass and frequency are amplified in late adolescents compared with prepubertal boys and girls, respectively. Correlation analysis reveals that both basal GH secretion and the irregularity of GH release patterns relate positively to the serum estradiol concentration, whereas plasma IGF-I and GH secretory burst mass vary directly with the serum testosterone concentration. These different statistical associations point to differential roles of estrogen and androgen in mediating developmental changes in the pubertal GH-IGF-I axis. Although we did not correlate the dynamic measure of GH release with linear growth velocities in this cross-sectional study, an earlier longitudinal assessment across male pubertal development revealed a marked rise in 24-h GH ApEn approximately 4 months before attainment of maximal height velocity (48). Other cross-sectional analyses of normal growing children also relate GH pulse mass to plasma IGF-I levels and height increments (1, 2, 4, 5, 8–11). We conclude that gender and sexual maturation jointly modulate specific facets of neuroregulatory control of the human GH-IGF-I axis via preferential sex hormone drive of the basal, pulsatile, entropic, and rhythmic modalities of GH secretion.

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**References**

3. Mauras N, Rogol AD, Veldhuis JD. 1989 Specific, time-dependent actions of

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**TABLE 4.** Linear regression analysis of GH dynamic properties in prepubertal and adolescent females and males

<table>
<thead>
<tr>
<th>Independent</th>
<th>Dependent</th>
<th>r value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum estradiol conc.</td>
<td>GH burst mass</td>
<td>0.247</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>GH burst frequency</td>
<td>-0.022</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>GH half-life</td>
<td>-0.105</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Basal GH secretion</td>
<td>0.326</td>
<td>&lt;10^-2</td>
</tr>
<tr>
<td></td>
<td>ApEn</td>
<td>0.492</td>
<td>&lt;10^-4</td>
</tr>
<tr>
<td>Serum testosterone conc.</td>
<td>GH burst mass</td>
<td>0.426</td>
<td>&lt;10^-3</td>
</tr>
<tr>
<td></td>
<td>GH burst frequency</td>
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<td>NS</td>
</tr>
<tr>
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<td>GH half-life</td>
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<tr>
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<td>Basal GH secretion</td>
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<tr>
<td></td>
<td>ApEn</td>
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<tr>
<td>Plasma IGF-I conc.</td>
<td>GH burst mass</td>
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<td>GH burst frequency</td>
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<td>ApEn</td>
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<td>NS</td>
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</tbody>
</table>

Combined data from 58 volunteers (28 prepubertal children and 30 adolescents). NS, Protected P > 0.01. ApEn, Approximate entropy statistic (see Subjects and Methods).