Cortisol and GH secretory dynamics, and their interrelationships, in healthy aged women and men

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Numerous studies have shown that physiological amounts of glucocorticoids are required for normal growth hormone (GH) production. Indeed, cortisol drives GH gene expression by pituitary cells in vitro (19). Paradoxically, small increases in glucocorticoids stimulate, whereas excessive concentrations of glucocorticoids inhibit, spontaneous and secretagogue-stimulated GH secretion (19). GH production is reduced in glucocorticoid deficiency states, normalized after glucocorticoid replacement (16, 17), and further augmented (60) by glucocorticoid administration at levels equivalent to two times normal cortisol secretion. In contrast, in states of cortisol excess, such as Cushing’s syndrome (48, 63) or depression (13, 40), there is an inverse relationship between the secretion of cortisol and that of GH. This paradigm of opposition between cortisol and GH is also evident in the metabolic and catabolic/anabolic effects that each of these hormones exerts on peripheral tissues. For example, both glucocorticoid excess and GH deficiency in nonelderly adults are associated with osteopenia (26, 33), relative sarcopenia (8, 19), and increased total and intra-abdominal fat (2, 15).

With advancing age, most recent studies describe spontaneous secretion of cortisol as increased in non-stressed individuals (9, 11, 32, 50), whereas GH release decreases in women and men, particularly at night (8). Cortisol production is generally comparable (39) or somewhat greater (50, 70) in young men vs. women, whereas in postmenopausal women serum concentrations of cortisol are similar to (50), or higher than (21), those in elderly men. GH secretion is greater in young women than in comparably aged men, whereas GH secretion is reduced to approximately similar levels in older postmenopausal women and men (22, 23).

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To date, the interrelationships between cortisol and GH secretion in the healthy elderly, and whether these relationships differ by sex, have not been elucidated. In the present study, we employed deconvolution analysis, and assessments of approximate entropy and cross-approximate entropy, to examine the detailed interrelationships between nocturnal cortisol and GH release in healthy aged women and men.

MATERIALS AND METHODS

Subjects

One hundred and thirty healthy, ambulatory, community-dwelling elderly volunteers (57 women and 73 men), 65–88 yr of age, were recruited by local advertisement. Subjects were selected to be moderately active by self report and performed usual household activities. Persons engaged in regular strenuous or athletic exercise training were excluded. All were healthy by screening history and physical examination, routine blood studies, urinalysis, and graded treadmill electrocardiogram. None of the women had taken any estrogen or progestogen for at least 3 mo before study. All subjects were encouraged to sleep beginning at 11:00 PM, to 8:00 AM, blood samples (2 ml) were collected at 20-min intervals from an intravenous catheter inserted into a forearm vein and kept open with heparinized (1,000 U/l) 0.9% sodium chloride at a rate of 83.3 U/h. From 8:00 PM to 8:00 AM, blood samples (2 ml) were collected at 20-min intervals for subsequent cortisol and GH determinations. All subjects were encouraged to sleep beginning at 11:00 PM, and room lights were turned off from 12:00 AM to 7:00 AM. At 8:00 AM on the morning of day 2, after an overnight fast, blood was collected for measurements of testosterone and estradiol. All sera were saved at −80°C until assayed.

Methods

Laboratory assays. All samples were assayed in duplicate. Cortisol was measured by RIA (ICN Pharmaceuticals, Diagnostics Division, Costa Mesa, CA). The sensitivity of the cortisol RIA was 0.25 μg/dl. Intra-assay coefficients of variation (CVs) at mean cortisol concentrations of 4.0, 12.5, and 25.6 μg/dl were 4.3, 6.8, and 10.5%, respectively, and inter-assay CVs at mean cortisol levels of 3.9, 10.3, and 25.7 μg/dl were 4.5, 4.5, and 5.5, respectively. GH was measured by immunoradiometric assay (IRMA; Nichols Institute Diagnostics, San Juan Capistrano, CA). The sensitivity of the GH IRMA was 0.05 μg/l. Intra-assay CVs at mean GH concentrations of 2.5, 6.4, and 10.9 μg/l were 2.0, 1.7, and 1.3%, respectively, and interassay CVs at mean GH levels of 2.4, 6.2, and 11.2 μg/l were 3.6, 2.7, and 4.1%, respectively. Testosterone was measured by RIA (Diagnostic Products, Los Angeles, CA) with a sensitivity of 10 ng/dl. Intra-assay CVs at mean testosterone concentrations of 60, 300, 597, and 998 ng/dl were 11.2, 6.7, 1.5, and 3.1%, respectively, and interassay CVs at mean testosterone levels of 76, 299, 707, and 1,041 ng/dl were 5.9, 3.9, 3.2, and 4.8%, respectively. Sex hormone binding globulin (SHBG) was measured by coated tube IRMA (Diagnostic Systems Laboratories, Webster, TX) with a sensitivity of 5 nmol/l. Intra-assay CVs at mean SHBG concentrations of 27, 108, and 194 nmol/l were 11.1, 7.4, and 9.3%, respectively, and interassay CVs at mean SHBG levels of 26, 103, and 182 nmol/l were 11.5, 6.7, and 8.2% respectively. The free testosterone index was derived from division of total testosterone by the concentration of SHBG (T/SHBG). Estradiol was measured by RIA (Diagnostic Products) with an assay sensitivity of 20 pg/ml. Intra-assay CVs of mean estradiol concentrations at 84, 180, and 504 pg/ml were 9.7, 9.3, and 4.3%, respectively, and interassay CVs at mean estradiol levels of 86, 185, and 496 pg/ml were 7.6, 5.0, and 5.7%, respectively. Total serum IGF-I levels were measured by RIA after acid-ethanol extraction (Endocrine Sciences Laboratories, Calabasas Hills, CA). Sensitivity of the IGF-I assay was 30 μg/l, and the intra- and interassay CVs were, respectively, 5.9 and 7.3% at 289 ng/ml and 4.6 and 6.3% at 591 μg/l. IGF-binding protein (IGFBP)-3 was measured using a polyclonal antibody directed against the binding subunit (Endocrine Science Laboratories). Sensitivity of the IGFBP-3 assay was 0.3 ng/ml, with intra- and interassay CVs of 2.7 and 7.5%, respectively.

Analysis of pulsatile hormone secretion. Cortisol and GH secretory profiles were assessed using deconvolution analysis (56, 58). A preliminary fit of the data by a waveform-independent deconvolution methodology (PULSE2) was followed by a multiparameter deconvolution methodology (DECONV; see Ref. 59). The following secretory parameters were characterized for both hormones: secretory burst frequency (number of secretory peaks over the 12-h sampling period), interburst interval, mean burst amplitude (average of calculated maximal rates of secretion for all secretory episodes), mass/burst (average amount of hormone secreted/episode), half-duration (mean duration of calculated half-maximal amplitude of secretory events), calculated secretory half-life, pulsatile production rate, and mean and integrated 12-h concentrations. Basal secretion rates for cortisol were assumed to approach zero (38). Total basal secretion for GH, however, was calculated by multiplying the basal secretion rate per minute by the sampling duration of 720 min. Total basal secretion was then added to the pulsatile production rate to calculate the “grand total” GH secretion. Basal secretion rates were determined for GH by inclusion of nonzero GH secretion (basal). Ten percent of all GH values fell below
the limit of detection of the GH IRMA, and these were assigned a value of 0.025 with an SD of 0.025 μg/l.

**Approximate entropy (ApEn) values** for cortisol and GH were also assessed (MC-ApEn; see Ref. 36). ApEn refers to the relative regularity or orderliness of hormone release, with a greater entropy (higher ApEn) reflecting a more random or disordered pattern of hormone secretion. The cross-ApEn assessment (MC-XApEn; see Refs. 27, 35, 37) was used to determine the conditional regularity, or synchrony, of cortisol and GH secretion, with a greater entropy describing less coordinate secretion. The primary limitations of ApEn and cross-ApEn are that the data should be relatively frequently sampled (at least 20 samples ordinarily), not be contaminated by multiple major outliers, and be evaluated within a consistent parameter family (61). All of these conditions are satisfied here. The sensitivity of the ApEn score can also be limited by major epochs and/or trending in the data. We explored this consideration by first-differencing the data and redoing the ApEn analysis, with similar results.

**Assessment of total body fat by DEXA.** Percent total body fat was estimated by DEXA scanning using a threecompartment model of body composition (model DPX-L; Lunar Radiation, Madison, WI; see Ref. 30). The reproducibility of DEXA measurements was confirmed by performing two total body scans on a separate group of 12 elderly men (>65 yr) at 6-wk intervals. Scans were then analyzed by two observers with both intra- and interobserver CVs of ~4%.

**Statistical Analysis**

All outcome variables were analyzed for frequency distribution and log transformed if the distribution was skewed to the right. In the latter cases, log transformation produced a normal distribution; therefore, parametric analyses were employed. The variables that were log transformed were cortisol half-duration, cortisol secretory bursts, cortisol mass/burst, cortisol amplitude, cortisol pulsatile production rate, GH interburst interval, GH mass/burst, GH pulsatile production rate, GH total production rate, GH mean and integrated concentration, and GH grand total secretion. Sex differences in each of the indexes of cortisol and GH secretion were assessed by the Student’s unpaired t-test (Statview version 5.0; SAS Institute, Cary, NC). Simple linear regression analyses (Statview) were performed to examine the relationships between integrated 12-h cortisol concentration and GH half-life, GH burst interval, GH mass/burst, GH frequency, GH production rate, GH integrated concentration, and GH grand total secretion and of integrated GH concentration against the corresponding parameters of cortisol secretion. Possible sex differences in cortisol-GH interrelationships were examined both by comparisons of slopes (t-test) of the regression equations and by ANOVA. Multiple linear regression analyses (Statview) were performed to determine the interrelationships of age, BMI, waist circumference, percent total body fat, free testosterone index, cortisol, and/or GH secretion with the dependent variable (cortisol or GH) of interest. Estradiol was not used in any statistical analysis because of the relative insensitivity of the assay employed (51/57 = 90% of the values were <20 pg/ml). All data are expressed as means ± SE. The level of significance was set at a P value of <0.05.

**RESULTS**

**Subject Characteristics**

Table 1 summarizes the mean ages of women and men, which did not differ significantly. In contrast, weight (P < 0.0001), BMI (P < 0.01), and waist circumference (P < 0.0001) were all greater in men. The mean testosterone level in men was 369 ± 11 ng/dl (range: 143–501) and the free testosterone index was 71 ± 7 ng/dl (range: 2–250). Neither IGF-I levels nor IGFBP-3 levels differed by gender.

**Characteristics of Cortisol and GH Deconvolution Analyses**

Comparison of pulsatile cortisol deconvolution parameters in women and men (Table 2) revealed that cortisol mass/burst, production rate, and mean and integrated cortisol concentrations, but no other cortisol secretory parameters, were significantly greater in women.

Deconvolution analysis of GH profiles (Table 3) revealed a slightly greater (P < 0.05) value for log total basal GH secretion in women but no significant sex differences in any of the other measures of GH secretion.

There were no significant sex differences in ApEn values for cortisol (Table 2) or GH (Table 3). In contrast, the cortisol-GH cross-ApEn score was significantly higher in women (Fig. 1), which denotes relative loss of GH-cortisol pattern synchrony in women.

**Bivariate Regressions of Cortisol vs. GH Secretion**

Simple regression analyses revealed such strong correlations of integrated cortisol levels with cortisol mass/burst (r = 0.687, P < 0.0001), cortisol production rate (r = 0.678, P < 0.0001), and mean cortisol

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**Table 1. Characteristics of the study population**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Women (n = 57)</th>
<th>Range</th>
<th>Mean ± SE</th>
<th>Range</th>
<th>Mean ± SE</th>
<th>Range</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>70.9 ± 0.5</td>
<td>65.1–80.1</td>
<td>71.3 ± 0.6</td>
<td>65.1–88.2</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>65.1 ± 1.2</td>
<td>49.2–89.0</td>
<td>82.3 ± 1.2</td>
<td>57.7–103.5</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.6 ± 0.4</td>
<td>18.7–32.6</td>
<td>27.0 ± 0.3</td>
<td>19.3–32.4</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>82.7 ± 1.2</td>
<td>65.1–107.6</td>
<td>96.9 ± 0.9</td>
<td>78.5–111.5</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean testosterone, ng/dl</td>
<td>369 ± 11</td>
<td></td>
<td>143–501</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free testosterone index, ng/dl</td>
<td>71 ± 7</td>
<td></td>
<td>2–250</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF-I, μg/l</td>
<td>117.5 ± 6.0</td>
<td>48.0–244.0</td>
<td>132.9 ± 46.0</td>
<td>29.0–254.0</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGFBP-3, ng/dl</td>
<td>2.6 ± 0.1</td>
<td>1.5–4.0</td>
<td>2.4 ± 0.08</td>
<td>0.6–4.3</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BMI, body mass index; IGF-I, insulin-like growth factor I; IGFBP, IGF-binding protein; NS, not significant; n, no. of subjects.
Table 2. Cortisol deconvolution data in healthy elderly women and men

<table>
<thead>
<tr>
<th>Variable</th>
<th>Women (n = 57)</th>
<th>Men (n = 73)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log cortisol half-duration, min</td>
<td>0.99 ± 0.07</td>
<td>0.94 ± 0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Cortisol half-life, min</td>
<td>89.60 ± 2.66</td>
<td>95.22 ± 2.18</td>
<td>NS</td>
</tr>
<tr>
<td>Cortisol burst frequency, no. of peaks/12 h</td>
<td>8.30 ± 0.19</td>
<td>8.54 ± 0.17</td>
<td>NS</td>
</tr>
<tr>
<td>Cortisol burst interval, min</td>
<td>83.52 ± 1.33</td>
<td>81.77 ± 1.42</td>
<td>NS</td>
</tr>
<tr>
<td>Log cortisol mass/burst, µg/ml</td>
<td>0.80 ± 0.02</td>
<td>0.71 ± 0.01</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Log cortisol amplitude, µg·ml⁻¹·min⁻¹</td>
<td>−0.22 ± 0.06</td>
<td>−0.26 ± 0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Log cortisol pulsatile production rate, µg·ml⁻¹·12 h⁻¹</td>
<td>1.71 ± 0.02</td>
<td>1.62 ± 0.02</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mean cortisol, µg/dl</td>
<td>7.96 ± 0.20</td>
<td>6.96 ± 0.20</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Integrated cortisol, µg·dl⁻¹·min⁻¹</td>
<td>5717 ± 142</td>
<td>4948 ± 126</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cortisol approximate entropy</td>
<td>0.69 ± 0.02</td>
<td>0.66 ± 0.02</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. *Women vs. men.

Table 3. GH deconvolution data in healthy elderly women and men

<table>
<thead>
<tr>
<th>Variable</th>
<th>Women (n = 57)</th>
<th>Men (n = 73)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH half-duration, min</td>
<td>31.34 ± 1.52</td>
<td>33.27 ± 1.60</td>
<td>NS</td>
</tr>
<tr>
<td>GH half-life, min</td>
<td>16.99 ± 0.75</td>
<td>16.41 ± 0.71</td>
<td>NS</td>
</tr>
<tr>
<td>GH burst frequency, no. of peaks/12 h</td>
<td>6.67 ± 0.20</td>
<td>6.27 ± 0.20</td>
<td>NS</td>
</tr>
<tr>
<td>Log GH burst interval, min</td>
<td>2.02 ± 0.01</td>
<td>2.03 ± 0.02</td>
<td>NS</td>
</tr>
<tr>
<td>Log GH mass/burst, µg/l</td>
<td>0.48 ± 0.06</td>
<td>0.48 ± 0.04</td>
<td>NS</td>
</tr>
<tr>
<td>GH amplitude, µg·l⁻¹·min⁻¹</td>
<td>0.14 ± 0.01</td>
<td>0.19 ± 0.07</td>
<td>NS</td>
</tr>
<tr>
<td>Log GH pulsatile production rate, µg·l⁻¹·12 h⁻¹</td>
<td>1.32 ± 0.05</td>
<td>1.26 ± 0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Log GH total production rate, µg·l⁻¹·12 h⁻¹</td>
<td>1.40 ± 0.05</td>
<td>1.30 ± 0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Log mean GH, µg/l</td>
<td>−0.13 ± 0.05</td>
<td>−0.18 ± 0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Log integrated GH, µg·l⁻¹·min⁻¹</td>
<td>2.72 ± 0.05</td>
<td>2.68 ± 0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Log GH total basal secretion, µg·l⁻¹·12 h⁻¹</td>
<td>0.30 ± 0.09</td>
<td>0.06 ± 0.07</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Log GH grand total secretion, µg·l⁻¹·12 h⁻¹</td>
<td>1.40 ± 0.05</td>
<td>1.30 ± 0.04</td>
<td>NS</td>
</tr>
<tr>
<td>GH approximate entropy</td>
<td>0.61 ± 0.03</td>
<td>0.64 ± 0.02</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. GH, growth hormone. *Women vs. men.

(r = 1.000, P < 0.0001) and, similarly, of integrated GH levels with GH mass/burst (r = 0.881, P < 0.0001), GH total production rate (r = 0.804, P < 0.0001), and mean GH (r = 0.999, P < 0.0001) in both women and men that they were essentially interchangeable. Therefore, only values for integrated cortisol and GH concentrations were used in subsequent correlational analyses.

Bivariate analyses identified significant positive correlations (Table 4) between integrated cortisol concentrations and GH mass/burst, amplitude, pulsatile production rate, total production rate, and mean, integrated, and grand total secretion in women, and of integrated cortisol concentration with mean and integrated GH concentrations in men. These apparent sex differences in the relationships of integrated cortisol secretion with measures of GH secretion were not significant (Fig. 2, Table 4) for any of the GH secretory measures except for GH amplitude, which was greater in women (Table 4). Moreover, there were no significant correlations between integrated cortisol concentration and GH half-life, GH burst frequency, GH burst interval, and GH ApEn in women or men (Table 4).

Integrated GH concentrations were significantly and directly related to mean and integrated cortisol concentrations in both women (r = 0.40, P < 0.005) and men (r = 0.25, P < 0.05), but these relationships did not differ significantly by sex. There were no significant relationships of integrated GH concentrations with any of the other cortisol secretory parameters, or ApEn, in either sex (data not shown).

There were no significant associations of age or total testosterone (in men) with any of the cortisol or GH secretory parameters, including ApEn (data not shown). In contrast, free testosterone index (in men) was inversely related to cortisol interburst interval (r = −0.26, P < 0.05) and directly related to GH total basal secretion (r = 0.38, P < 0.005). There were no significant relationships of free testosterone index with ApEn or cross-ApEn scores.

**Bivariate Relations of Cortisol and GH Secretion With Body Composition**

BMI was directly related to waist circumference and percent total body fat in women (r = 0.79, P < 0.0001; r = 0.38, P < 0.005; respectively) and men.
Waist circumference and percent total body fat were also directly related in women ($r = 0.28, P = 0.05$) and men ($r = 0.71, P < 0.0001$). BMI was inversely related to cortisol mass/burst and cortisol pulsatile production rate in women and to integrated cortisol levels in women and men (Table 5). Slope comparisons revealed no significant sex differences in these relationships (data not shown).

In women, BMI was inversely related to GH mass/burst, GH amplitude, GH pulsatile production rate, GH total production rate, integrated GH secretion, and grand total secretion and was directly related to GH burst frequency. In men, BMI was inversely related to integrated GH concentrations (Table 5).

Waist circumference was inversely related to integrated cortisol secretion only in women and to integrated GH secretion in women and men. Percent total body fat was inversely related to integrated cortisol secretion and to GH half-life, GH mass/burst, GH pulsatile production rate, GH total production rate, integrated GH secretion, and GH grand total secretion in men only (Table 5).

Waist circumference was directly related to cross-ApEn ($r = 0.38, P = 0.005$) in women, but there were no other significant relationships of BMI, waist circumference, or percent total body fat with ApEn and cross-ApEn in either sex.

### Multivariate Regressions of Cortisol, GH, Age, and Body Composition

Multivariate regression analyses were performed to further assess the relative influences of age and body composition on the cortisol and GH secretory interrelationships (Table 6). BMI, but not age or integrated cortisol secretion, was the strongest predictor of integrated GH secretion in women and men. Additionally, GH secretion was influenced by waist circumference more than age, but not cortisol secretion, in women, and to waist circumference, but not age or cortisol secretion, in men (Table 6). When we substituted all other measures of body composition with percent total body fat, integrated GH secretion, but not age or percent total body fat, was the strongest predictor of cortisol secretion in women. Similarly, integrated cortisol secretion, but not age or percent total body fat, had the greatest influence on integrated GH secretion in women, whereas percent total body fat, and not integrated

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**Table 4. Bivariate regressions of integrated cortisol secretion with GH deconvolution parameters**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Women ($n = 57$)</th>
<th>Men ($n = 73$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH half-duration, min</td>
<td>0.25 NS</td>
<td>0.19 NS</td>
</tr>
<tr>
<td>GH half-life, min</td>
<td>0.02 NS</td>
<td>0.06 NS</td>
</tr>
<tr>
<td>GH burst frequency, no. of peaks/12 h</td>
<td>-0.12 NS</td>
<td>0.01 NS</td>
</tr>
<tr>
<td>Log GH burst interval, min</td>
<td>0.07 NS</td>
<td>0.10 NS</td>
</tr>
<tr>
<td>Log GH mass/burst, $\mu$g/l</td>
<td>0.38 &lt;0.005</td>
<td>0.21 NS</td>
</tr>
<tr>
<td>GH amplitude, $\mu$g$^{-1}$ min$^{-1}$</td>
<td>0.31 &lt;0.05</td>
<td>-0.01 NS</td>
</tr>
<tr>
<td>Log GH pulsatile production rate, $\mu$g$^{-1}$·12 h$^{-1}$</td>
<td>0.38 &lt;0.005</td>
<td>0.20 NS</td>
</tr>
<tr>
<td>Log GH total production rate, $\mu$g$^{-1}$·12 h$^{-1}$</td>
<td>0.35 &lt;0.01</td>
<td>0.20 NS</td>
</tr>
<tr>
<td>Log mean GH, $\mu$g/l</td>
<td>0.41 &lt;0.005</td>
<td>0.26 &lt;0.05</td>
</tr>
<tr>
<td>Log integrated GH, $\mu$g$^{-1}$·min$^{-1}$</td>
<td>0.40 &lt;0.005</td>
<td>0.25 &lt;0.05</td>
</tr>
<tr>
<td>Log total basal secretion, $\mu$g$^{-1}$·12 h$^{-1}$</td>
<td>0.03 NS</td>
<td>0.09 NS</td>
</tr>
<tr>
<td>Log grand total secretion, $\mu$g$^{-1}$·12 h$^{-1}$</td>
<td>0.35 &lt;0.01</td>
<td>0.20 NS</td>
</tr>
<tr>
<td>GH approximate entropy</td>
<td>-0.20 NS</td>
<td>0.15 NS</td>
</tr>
</tbody>
</table>

* $T$ value indicates slope comparison. $P$ values determined by ANOVA.
cortisol or age, influenced integrated GH secretion in men (Table 6).

Because of the influence of free testosterone index on cortisol and GH deconvolution parameters, we investigated whether free testosterone index was a stronger predictor of cortisol or GH secretion than was body composition. In men, percent total body fat, and not free testosterone index, was the strongest predictor of GH ($P < 0.0005$).

We also assessed the relative influence of cortisol and GH secretion, and waist circumference, on the cross-ApEn score. Substitution of percent total body fat for waist circumference in the above paradigm revealed identical relationships, yet the relationship in women was somewhat stronger ($P < 0.0006$).

**DISCUSSION**

Although increased (11), unaltered (64), and decreased (44) spontaneous cortisol secretion has been reported with aging, more recent studies reveal an increase in cortisol production in healthy, nonstressed elderly individuals (9, 50), perhaps in association with a decreased resiliency of cortisol secretion (41). Cortisol secretion has been reported to be similar in men and women <55 yr old by some investigators (5, 39), whereas others have described somewhat lower cortisol secretion in younger women, as estimated by urinary free cortisol excretion (14) or 24-h plasma sampling (50). In the elderly, several studies have shown no sex differences in cortisol secretion (5, 32, 50), whereas others have demonstrated significantly higher unstimulated cortisol production in elderly women (9, 20, 21).

Using deconvolution analyses and 20-min overnight blood sampling, we found a slightly shorter cortisol half-duration, longer half-life, greater interburst interval, and lesser mass/burst in elderly women and men than previously estimated in premenopausal women and young men (39). Mean cortisol concentrations were also lower in our older men than reported earlier in middle-aged men (56). Our data also demonstrated significantly greater mean and integrated cortisol concentrations, and cortisol production rate, in elderly women than men, which was primarily due to an increase in cortisol mass/burst. There were no sex differences in other indexes of cortisol secretion in our study. The higher circulating levels of total cortisol previously reported in aged women compared with men (9, 21) may have been due to the greater concentrations of corticosteroid-binding globulin (CBG) in women, as it has been reported that there are no significant sex differences in free cortisol levels (10). CBG concentra-
tions have been reported to be unchanged with age in men (34, 47), whereas they decrease in elderly women but remain higher than those of elderly men (10, 47). It was not possible to determine whether there were sex differences in free vs. bound cortisol in our subjects, as serum CBG concentrations and/or cortisol binding was not assessed.

Our GH deconvolution data obtained using a GH IRMA were comparable to those previously reported and confirmed the reported decrease in GH secretion with age described in healthy elderly men and women using more sensitive chemiluminescent and immuno-fluorimetric assays and more frequent sampling intervals in smaller groups of volunteers (22, 24, 25). We found no significant sex differences in estimated GH secretory parameters, except for a slightly, but statistically greater total basal GH secretion in women. Similarly, there were no apparent gender differences in IGF-I or IGFBP-3 levels in our subjects. Our finding that mean and integrated GH secretion, as well as GH production rate, did not differ between elderly women and men confirms the observations of Ho et al. (23). In contrast, premenopausal women exhibit higher GH pulse amplitude (53, 68), GH mass/burst and GH secretion rates (53), integrated serum GH concentrations (IGHC) (23), baseline serum GH levels (6, 46), and increased numbers of GH pulses when measured during the early follicular phase (1) compared with similarly aged men. In one recent analysis using an ultra-sensitive chemiluminescence-based GH assay and a 20-min blood sampling for 24 h, Hindmarsh et al. (22) reported a 50% higher daily GH secretion rate in men than in women. Our assessments differ by way of both assay and sampling paradigm.

Our observations of direct relationships between cortisol and GH are consistent with the results of numerous studies showing that near-physiological amounts of glucocorticoid can stimulate GH synthesis and secretion (19) and extend the prior findings to healthy, nonstressed aged individuals. In humans, minimal amounts of glucocorticoids are necessary for normal in vivo GH production, as evidenced by the clinical recognition that GH secretion is reduced in glucocorticoid deficiency states and normalized after glucocorticoid replacement (16, 17). Short-term administration of moderate doses of dexamethasone to patients with isolated ACTH deficiency restored the subnormal GH secretory response to the insulin tolerance test by increasing daily GH burst frequency, GH mass/burst, and GH secretion rate and decreasing GH burst interval (18). Daily GH production in young men can be further augmented (60) by glucocorticoid administration at levels equivalent to two times normal cortisol secretion. In healthy young women, physiological cortisol and GH secretion are coordinately increased during fasting (3). In healthy young men, ACTH or corticotropin-releasing hormone administration stimulates GH pulses and enhances daytime GH release, whereas at nighttime, ACTH or corticotropin-releasing hormone blunts the surges of GH, thereby causing no alteration in the integrated serum GH concentration (67).

In vitro experiments using human and rat pituitary cells, and rat pituitary tumor cell lines, have consistently demonstrated that glucocorticoids directly enhance somatotrope gene expression, production of GH, and the GH response to GH-releasing hormone (31, 49, 65). One possible mechanism for the stimulatory effect of cortisol on GH is suggested by the presence of a corticosteroid response element (CRE) in the first intron of the GH gene (12, 45). Binding of the activated glucocorticoid receptor to the CRE in the GH gene enhances GH gene transcription and increases intracellular levels of GH mRNA. Another possible mechanism might entail the known effect of glucocorticoids to increase GH-releasing hormone receptors on pituitary somatotropes (42).

Our results in healthy aged individuals contrast with the usual observation of an inverse correlation between the secretion of cortisol and that of GH in states of pathological hypercortisolism, such as Cushing’s syndrome (63, 69) or depression (13, 40). Consistent with such findings are the observations that in vivo administration of glucocorticoid to rats inhibits GH secretion by decreasing GH-releasing hormone and by increasing hypothalamic immunoreactive somatostatin (31) and that administration of anti-somatostatin antibodies reverses glucocorticoid suppression of GH (66).

The interrelationships among sleep architecture, nocturnal GH and cortisol secretory profiles, age, and gender have been studied extensively. In general, in aging men, the decrease in slow wave sleep is nearly maximal by age 40–50 yr and is closely associated with reduced nocturnal GH secretion, whereas REM sleep reaches its nadir by age 60–70, in association with augmented nocturnal release of cortisol. Similar, but less pronounced, changes in sleep architecture and neuroendocrine function occur in aging women, in whom the sleep-endocrine interrelationships are less tightly coupled (4, 51). In the present study, we did not record polysomnographic variables or actigraphy during the sleep period.

We detected no sex differences in the ApEn, or orderliness, of cortisol secretion in our elderly subjects. Similarly, van den Berg et al. (52) reported no differences in cortisol ApEn in relation to sex or age. We also found no sex differences in the ApEn for GH secretion, in contrast to prior reports that GH ApEn in women is higher than in age-matched young (35) and elderly (22) men. The latter two observations, in conjunction with increased GH ApEn after treatment of postmenopausal women (43) and young girls with gonadal dysgenesis/ Turner’s syndrome (29) with oral estrogen, suggest that estrogens increase the disorderliness and the amount of GH secreted. Conversely, a possible role of testosterone in influencing the regularity of GH secretion is suggested by a study of a cohort of men over a wide range of ages (18–63 yr) and body composition (BMIs 18–39 kg/m²), which showed that testosterone correlated negatively with ApEn (59). In this latter
study, Veldhuis et al. (59) found that serum testosterone was directly related to GH secretion rate, GH secretory burst mass, and mean serum GH concentration, which supports our findings of a direct relationship of free testosterone index with GH total basal secretion. Thus the similarity of the GH ApEn scores in the elderly women and men in the current study might relate to low estrogen contributing to an increase, and diminished testosterone leading to a decrease, in regularity of overnight GH secretory profiles.

We examined the joint pattern correlations between our cortisol and GH secretory profiles by cross-ApEn, a statistical measure of the synchrony of two data series (27). To our knowledge, there are no prior reports investigating the synchrony, by cross-ApEn, of two different hormone axes, except for luteinizing hormone (LH) and follicle-stimulating hormone or prolactin in older men (57). We found a greater cross-ApEn in women than men, consistent with a greater conditional irregularity or disorderliness of cortisol and GH release patterns in women (37). Whether the observed sex difference in the cross-ApEn in our elderly subjects results from estrogen acting as an endogenous stimulating signal for cortisol and GH release via a network mechanism, rather than affecting individual glandular secretory activity (38), remains to be determined. Roelfsema et al. (38) examined the synchrony of secretion between ACTH and cortisol in healthy men and women and found that cross-ApEn increased with age but did not differ by sex. Among other intra-axis studies to date, it appears that, with advancing age, many hormonal networks, such as LH-follicle-stimulating hormone, LH-prolactin, and LH-testosterone in men, exhibit increased cross-ApEn, suggesting a deterioration of coordinated neuroendocrine function (37, 55, 57). Finally, an increase in GH ApEn was recently observed in nonelderly patients with Cushing’s disease (62). Although the latter study did not directly examine the cross-ApEn of cortisol and GH, nor the influences of age or sex on such relationships, it raises the possibility that in healthy aged persons increased secretion of cortisol influences the orderliness of GH secretion. Obesity, particularly central adiposity, and age are potential confounders of the interrelationships of cortisol and GH secretion because of their known influences on both hormone axes. Our data revealed trends toward sex differences in the relationships of cortisol, but not GH, with BMI and waist circumference, which were not significant by comparison of slopes and ANOVA. Although most studies report direct relationships between cortisol and BMI in hypercortisolemic individuals, especially men (7, 28), others observe an inverse relationship in euadrenal persons, similar to our findings (47, 54). Within the limited age range studied, age was not a significant correlate of either cortisol or GH secretion.

The results of our multiple regression analysis suggest that the apparent interrelationships of cortisol and GH secretion in elderly women and men can be confounded by variations in body composition. Even though our measures of body composition were strongly interrelated, different modes of assessing body composition revealed different influences of cortisol on GH and vice versa, thereby suggesting that assessment methodology may alter the ability to predict the interrelationships of hormones in other studies.

When we looked for determinants of bihormonal synchrony, GH and not body composition was independently related to cross-ApEn in women, whereas cortisol was independently related to cross-ApEn in men, further suggesting that there may also be a significant interrelationship between secretion of cortisol and GH independent of body composition.

In conclusion, we observed greater integrated cortisol concentrations in healthy aged women vs. men, related primarily to an enhanced cortisol secretory mass/burst in women. Mean and integrated cortisol levels were positively associated with GH secretion in women and to a lesser extent in men. Although not further explained by the available literature, these gender differences might, in turn, reflect sex differences in body composition. We observed significantly higher cross-ApEn for cortisol and GH secretion in elderly women, suggesting relative disruption of the influence of cortisol on GH secretion, of GH on cortisol secretion, or of other factors (i.e., testosterone in men, estrogen in women, or body composition) on the coordinate secretion of both of these hormones. Further investigation is warranted to determine the mechanisms underlying the interactions between cortisol and GH secretion, their relationships with age and sex, and their possible joint impact on the physical frailty associated with the later stages of aging.

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