Growth hormone secretion in primary adrenal Cushing’s syndrome is disorderly and inversely correlated with body mass index

Maarten O. van Aken,1 Alberto M. Pereira,1 Marijke Frölich,1 Johannes A. Romijn,1 Hanno Pijl,1 Johannes D. Veldhuis,2 and Ferdinand Roelfsema1

1Department of Endocrinology and Metabolic Diseases, Leiden University Medical Center, Leiden, The Netherlands; and 2Division of Endocrinology and Metabolism, Mayo Medical and Graduate Schools of Medicine, Mayo Clinic, Rochester, Minnesota

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Van Aken, Maarten O., Alberto M. Pereira, Marijke Frölich, Johannes A. Romijn, Hanno Pijl, Johannes D. Veldhuis, and Ferdinand Roelfsema. Growth hormone secretion in primary adrenal Cushing’s syndrome is disorderly and inversely correlated with body mass index. Am J Physiol Endocrinol Metab 288: E63–E70, 2005. First published August 24, 2004; doi:10.1152/ajpendo.00317.2004.—To evaluate the impact on the somatotropic axis of endogenous cortisol excess in the absence of primary pituitary disease, we investigated spontaneous 24-h growth hormone (GH) secretion in 12 adult patients with ACTH-independent hypercortisolism. Plasma GH concentration profiles (10-min samples) were analyzed by deconvolution to reconstruct secretion and approximate entropy to quantitate orderliness of the release process. Comparisons were made with a body mass index (BMI)-, age-, and gender-matched control group and an age- and gender-matched lean control group. GH secretion rates did not differ from BMI-matched controls but were twofold lower compared with lean subjects, mainly due to a 2.5-fold attenuation of the mean secretory burst mass ($P = 0.001$). In hypercortisolemic patients, GH secretion was negatively correlated with BMI ($R = -0.55$, $P = 0.005$) but not cortisol secretion. Total serum IGF-I concentrations were similar in the three groups. Approximate entropy (ApEn) was increased in patients with Cushing’s syndrome compared with both control groups (vs. BMI-matched, $P = 0.04$; vs. lean, $P = 0.001$), denoting more irregular GH secretion patterns. ApEn in patients correlated directly with cortisol secretion ($R = 0.77$, $P = 0.003$). Synchrony between cortisol and GH concentration series was analyzed by cross-correlation, cross-ApEn, and copulsatility analyses. Patients showed loss of pattern synchrony compared with BMI-matched controls, but copulsatility was unchanged. We conclude that hyposomatotropism in primary adrenal hypercortisolism is only partly explained (~30%) by increased body weight and that increased GH secretory irregularity and loss of synchrony suggest altered coordinate regulation of GH release.

CUSHING’S SYNDROME is characterized by increased cortisol secretion and is caused by ACTH-dependent cortisol excess (Cushing’s disease or the rare ectopic tumor ACTH production syndrome) or by ACTH-independent cortisol excess. The latter syndrome is caused by a unilateral adenoma (seldom a carcinoma) and less frequently by ACTH-independent bilateral macronodular adrenal hyperplasia (AIMAH). The latter syndrome is characterized by bilateral nodular enlargement of the adrenal glands and clinical and biochemical signs of cortisol excess with low or undetectable ACTH concentrations (25). The detrimental metabolic consequences of chronic cortisol excess are manifold and include loss of lean body mass, increased adiposity, bone loss, and repression of the thyrotropic, gonadotropic, and somatotropic axes. Indeed, the diminished growth hormone (GH) response to various stimuli, including insulin-induced hypoglycemia, GH-releasing hormone (GHRH), growth hormone secretagogues (GHS), and ghrelin, is well described in pituitary-dependent hypercortisolism (24, 30, 33, 46).

Because obesity, frequently a prominent feature of hypercortisolism, is accompanied by a decreased GH response to stimuli and diminished spontaneous GH secretion, it is mandatory that any comparison between the hypercortisolemic state and healthy subjects must include body mass index (BMI)-matched controls. In a previous study in patients with pituitary-dependent hypercortisolism, the 24-h GH secretion was negatively correlated to urinary cortisol excretion, and the GH secretion regularity was significantly decreased (17). Hypothetically, the GH secretory abnormalities could be the result of the presence of the pituitary adenoma itself, a tumoral product acting as a paracrine signal on the somatotrope, or the result of cortisol excess per se on the somatotropic axis.

The present study aimed to explore the dynamics of spontaneous diurnal GH secretion in patients with Cushing’s syndrome, since these patients lack a pituitary adenoma but otherwise suffer from chronic endogenous cortisol excess. The prime issue is whether such patients display low-amplitude and/or disorderly GH secretion compared with BMI-matched controls, as we previously found in pituitary-dependent hypercortisolism (17).

SUBJECTS AND METHODS

Twelve patients with primary adrenal Cushing’s syndrome were studied. Mean age of the patients was 45.2 ± 4.2 (46.5) yr, and BMI was 25.6 ± 1.4 (24.1) kg/m² [mean ± SE (median)]. The age of the 12 control subjects matched for age, gender, and BMI was 45.3 ± 3.7 (45) yr, and BMI was 26.6 ± 1.6 (24.6) kg/m² ($P = 0.85$). In addition, another (historical) control cohort, matched for age and gender but otherwise with a perfectly normal BMI, was used as a lean reference group. The BMI in the latter group was 20.8 ± 0.4 (20.8) kg/m² ($P = 0.03$ vs. patients), and age was 42.2 ± 3.5 (39) yr. The diagnosis of primary adrenal Cushing’s syndrome was established by elevated 24-h urinary excretion of free cortisol, subnormal or absent suppression of plasma cortisol after administration of 1 mg of dexamethasone overnight, absent or subnormal suppression of urinary cortisol excretion during a low-dose dexamethasone test, and a low or undetectable
plasma ACTH concentration. After the biochemical diagnosis of primary adrenal Cushing’s syndrome was established, a CT scan or MRI scan of the adrenal glands was performed to identify the source of cortisol overproduction. After the present study was carried out, the patients underwent surgery, with resection of the abnormal adrenal gland(s), resulting in resolution of the Cushing’s syndrome. Histological diagnosis confirmed the presence of an adrenocortical adenoma in seven patients and macronodular hyperplasia in the remaining five patients. Clinical details are displayed in Table 1. Controls were recruited through advertising in local newspapers. None of the subjects was using any neuroactive drug (including oral contraceptives) for at least 3 mo before the study. All women had stable body weight for at least 3 mo before the study. The purpose, nature, and possible risks of the study were explained to all subjects, and written informed consent was obtained. The study protocol was approved by the ethics committee of the Leiden University Medical Center.

Methods

Patients and control subjects were admitted to the hospital on the day of the study. An indwelling intravenous cannula was inserted in a forearm vein at least 60 min before sampling began. Blood samples were withdrawn at 10-min intervals for 24 h, starting at 0900. A slow infusion of 0.9% NaCl and heparin (1 U/ml) was used to keep the line open. The subjects were free to ambulate but not to sleep during the daytime. Meals were served at 0900, 1230, and 1730. Lights were turned off between 2200 and 2400. No sleep monitoring by EEG was used. Plasma for GH and cortisol measurements was collected, centrifuged at 4°C for 10 min, and stored at −20°C until later analysis. The results of the cortisol data are not shown; here, we use only the 24-h secretion rates in regression analyses.

Assays

Plasma GH concentrations were measured in duplicate with the use of a sensitive time-resolved immunofluorometric assay (Wallac, Turku, Finland) specific for the 22-kDa GH protein. Human biosynthetic GH (Pharmacia & Upjohn, Uppsala, Sweden) was used as standard. Plasma IGF-I was determined by RIA (Incstar, Stillwater, MN) with a detection limit of 1.5 nmol/l and an interassay variation of 2% at 22 nmol/l. The interassay variation varied from 2 to 4% at 220 nmol/l. The purpose, nature, and possible risks of the study were explained to all subjects, and written informed consent was obtained. The study protocol was approved by the ethics committee of the Leiden University Medical Center.

Calculations and Statistics

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 (53). Initial estimates of basal GH secretion rate were calculated to approximate the lowest 5% of all plasma GH concentrations in the time series. 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 pulses, as validated in simulations (42). The following four secretory and clearance measures of interest were estimated: 1) the number and locations of secretory events, 2) the amplitudes of secretory bursts, 3) the durations of randomly dispersed GH secretory bursts, and 4) the endogenous single-component subject-specific plasma half-life of GH. It was assumed that the GH distribution volume and half-life were time and concentration invariant. The following parameters were calculated: half-duration of secretory bursts (duration of the secretory burst at half-maximal amplitude), hormone half-life, burst frequency, amplitude of the secretory burst (maximal secretory rate attained within a burst), mass secreted per burst, basal secretion rate, pulsatile secretion rate (product of burst frequency and mean burst mass), and total secretion (sum of basal and pulsatile).

Approximate entropy. The univariate approximate entropy (ApEn) statistic was developed to quantify the degree of irregularity, or disorderliness, of a time series (42). Technically, ApEn quantifies the summed logarithmic likelihood that templates (of length m) of patterns in the data that are similar (within r) remain similar (within the same tolerance r) on the next incremental comparison and has been formally defined elsewhere (43). The ApEn calculation provides a single nonnegative number that is an ensemble estimate of relative process randomness, wherein larger ApEn values denote greater irregularity, as observed for ACTH in Cushing’s disease, GH in acromegaly, and prolactin in prolactinomas (43, 51, 52). Cross-ApEn (X-ApEn) quantifies joint pattern synchrony between two separate but parallel time series after standardization (z-score transformation) (44, 45). In the present analysis, we calculated X-ApEn between cortisol (leading) and GH, with r = 20% of the standard deviation of the individual time series and m = 1. This parameter choice affords sensitive, valid, and statistically well-replicated ApEn and X-ApEn metrics for assessing hormone time series of this length (44). ApEn and X-ApEn results are reported as absolute values and as the ratio of

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age, yr</th>
<th>Diagnosis</th>
<th>UCE, nmol/24 h</th>
<th>Adrenal Gland Size</th>
<th>No. of Cortisol Pulses/24 h</th>
<th>Cortisol Secretion/24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>48</td>
<td>UAA</td>
<td>617</td>
<td>5 cm</td>
<td>19</td>
<td>3,730</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>48</td>
<td>UAA</td>
<td>1,017</td>
<td>2.8 cm</td>
<td>33</td>
<td>13,720</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>43</td>
<td>UAA</td>
<td>300</td>
<td>3.5 cm</td>
<td>34</td>
<td>10,060</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>21</td>
<td>UAA</td>
<td>2,414</td>
<td>2.5 cm</td>
<td>24</td>
<td>10,560</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>40</td>
<td>UAA</td>
<td>1,677</td>
<td>2.0 cm</td>
<td>30</td>
<td>22,330</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>58</td>
<td>UAA</td>
<td>490</td>
<td>4.8 cm</td>
<td>19</td>
<td>4,420</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>25</td>
<td>UAA</td>
<td>1,359</td>
<td>5.2 cm</td>
<td>31</td>
<td>8,160</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>78</td>
<td>AIMAH</td>
<td>399</td>
<td>right 3 cm, left 2 cm</td>
<td>28</td>
<td>3,660</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>41</td>
<td>AIMAH</td>
<td>1,031</td>
<td>right 2.5 cm, left 3.4 cm</td>
<td>41</td>
<td>5,190</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>48</td>
<td>AIMAH</td>
<td>641</td>
<td>right 2.5 cm, left 5 cm</td>
<td>21</td>
<td>4,390</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>50</td>
<td>AIMAH</td>
<td>407</td>
<td>right 2.8 cm, left 2 cm</td>
<td>34</td>
<td>9,460</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>45</td>
<td>AIMAH</td>
<td>429</td>
<td>right 4.8 cm, left 4.1 cm</td>
<td>32</td>
<td>14,280</td>
</tr>
</tbody>
</table>

UAA, unilateral adrenal adenoma; AIMAH, ACTH-independent macronodular adrenal hyperplasia; F, female; M, male; UCE, urinary cortisol excretion (normal values <220 nmol/24 h). No. of significant cortisol pulses and cortisol secretion rate were determined with deconvolution analysis of the 24-h cortisol concentration series.
the absolute value to that of the mean of 1,000 randomly shuffled data series. Ratio values that approach 1.0 thus denote mean empirical randomness.

Copulsatility. Copulsatility between the cortisol and GH time series was quantified by hypergeometric (joint binomial) distribution (54). This program calculates the probability that hormone pulses in time series occur randomly. We used a time window of 40 min, with cortisol as leading hormone series. The position (time of maximal secretion rate within a pulse) and number of pulses were derived from the deconvolution analyses.

Statistical analysis. Results are expressed as means ± SE. Comparison between groups was done with one-way ANOVA, followed post hoc by Tukey’s honestly significantly different test to contrast means. Derived measures (deconvolution and ApEn) were transformed logarithmically before analysis to limit dispersion of variance. In addition, (stepwise) linear regression was applied to evaluate the relation between relevant variables. Cross-correlation analysis was applied to test for significant time-lagged (linear) synchrony between successive serum concentrations of cortisol and GH, considered pairwise, as described previously (54). Calculations were carried out with Systat (release 11; Systat Software, Richmond, CA). Differences were considered significant for $P < 0.05$.

RESULTS

Daily Plasma GH in Patients and BMI-Matched Controls

Secretion profiles of the 24-h plasma GH concentration series of the patients are shown in Fig. 1. Deconvolution of the GH profiles revealed no differences in basal GH secretion rate, secretory burst half-duration, burst amplitude, burst mass, half-life, basal secretion, pulsatile secretion, and total secretion between the patients and the BMI-matched controls (Table 2). GH was secreted in a predominantly pulsatile fashion in

![Fig. 1. Growth hormone (GH) concentration profiles of hypercortisolemic patients obtained by 10-min blood sampling for 24 h (1 mU/l = 0.38 μg/l). Sampling started at 0900 (24-h clock). BMI, body mass index.](http://ajpendo.physiology.org/)
patients and in BMI-matched controls, as displayed in Fig. 1. In healthy lean controls, GH secretion was 2-fold higher than in patients and was accomplished by a 2.5-fold increase in burst mass \( (P = 0.001) \) at similar pulse frequency. Total serum IGF-I concentrations were similar in the groups: patients, 16.5 ± 3.4; BMI-matched controls, 16.8 ± 0.8; and lean controls, 20.1 ± 2.2 nmol/l \( (\text{ANOVA}, P = 0.44) \).

**GH and Meals**

The influence of meals on GH concentrations was analyzed by comparing the mean of 10 serial samples preceding lunch and dinner in patients and body weight-matched controls and mean GH in the samples after the start of lunch and dinner during 90 min. In patients, the mean GH decrease after lunch was 0.48 mU/l \( (P = 0.03) \) and in controls 1.16 mU/l \( (P = 0.006) \). The mean GH decrease after dinner was 1.51 mU/l in patients \( (P = 0.04) \) and 2.19 mU/l in controls \( (P = 0.02) \). The mean GH decreases in patients and controls were statistically similar.

**ApEn**

ApEn in patients was increased, denoting an irregular secretion pattern: patients 0.7386 ± 0.044 vs. BMI-matched controls 0.5271 ± 0.0455 \( (P = 0.04) \) and vs. lean controls 0.4492 ± 0.050 \( (P = 0.001) \). The ApEn ratio was 0.5102 ± 0.015 in patients, 0.4250 ± 0.021 in body weight-matched controls \( (P = 0.016) \), and 0.3820 ± 0.024 in lean controls \( (P = 0.0002) \).

**Factors Influencing GH Secretion**

In a stepwise linear regression analysis, the 24-h GH secretion in patients and BMI-matched controls was significantly negatively correlated with BMI \( (R = -0.55, P = 0.005) \), as displayed in Fig. 2. However, other parameters, including cortisol secretion rate, free urinary cortisol excretion, age, estradiol, gender, and duration of cortisol excess (in patients only), were nonsignificant predictors. Thus the variation in total GH secretion was explained by BMI for 30%. In addition, ApEn was significantly and positively correlated \( (R = 0.77, P = 0.003) \) with the cortisol secretion rate, as displayed in Fig 3, but not with BMI \( (R = 0.03) \).

**Relation Between Cortisol and GH Secretion**

Pattern synchrony between cortisol and GH was quantified by X-ApEn in patients and BMI-matched controls. X-ApEn in patients was 1.648 ± 0.113 and in controls was 1.004 ± 0.050 \( (P < 0.0001) \). The ApEn ratios were 0.8682 ± 0.054 and 0.6134 ± 0.026, respectively \( (P < 0.0001) \), denoting diminished pattern synchrony in patients. Conventional linear cross-correlation between cortisol (leading) and GH concentrations revealed a negative correlation in control subjects \( [\text{median} -0.30, 95\% \text{ confidence interval (CI)} \ -0.15 \text{ to } -0.39] \) and a mean time lag of 30 min \( (95\% \text{ CI } 0 \text{ to } 65 \text{ min}) \), indicating opposite changes in cortisol concentrations, followed by those of GH. Five of the patients had a positive correlation. Median correlation coefficient was \( -0.09, \) with a 95% CI of \( -0.15 \text{ to } +0.16 \). The mean time lag was 75 min, with a 95% CI of \( 37\text{ to } 100 \text{ min} \). Copulsatility of cortisol and GH pulses was statistically highly significant in all patients and in 10 of 12 control subjects \( (P \text{ values between } 10^{-3} \text{ and } 10^{-13}) \).

**Unilateral Versus Bilateral Adrenal Pathology**

BMI, IGF-I, and age were comparable in these subgroups. No differences were found in GH secretion parameters, as estimated by deconvolution, ApEn, and synchrony estimates of GH and cortisol.

**DISCUSSION**

In this investigation of primary adrenal cortisol excess, the 24-h GH secretion was comparable to that of BMI-matched

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**Table 2. Secretory parameters of 24-h GH plasma concentration series in 12 patients with ACTH-independent hypercortisolism and control groups**

<table>
<thead>
<tr>
<th></th>
<th>Patients ((n = 12))</th>
<th>BMI-Matched Controls ((n = 12))</th>
<th>Lean Controls ((n = 12))</th>
<th>(P) Value vs. Matched C</th>
<th>(P) Value vs. Lean C</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal secretory rate, mU/l, min(^{-1})</td>
<td>0.0056 ± 0.0012 ((0.0044))</td>
<td>0.0067 ± 0.0009 ((0.0062))</td>
<td>0.0116 ± 0.0035 ((0.0074))</td>
<td>NS</td>
<td>NS</td>
<td>0.13</td>
</tr>
<tr>
<td>Half-life, min</td>
<td>14.7 ± 0.6 ((15.4))</td>
<td>14.2 ± 0.6 ((14.6))</td>
<td>14.7 ± 0.6 ((14.8))</td>
<td>NS</td>
<td>NS</td>
<td>0.78</td>
</tr>
<tr>
<td>Secretory burst half-duration, min</td>
<td>28.2 ± 2.2 ((27.0))</td>
<td>25.5 ± 1.7 ((26.6))</td>
<td>27.1 ± 1.8 ((27.7))</td>
<td>NS</td>
<td>NS</td>
<td>0.62</td>
</tr>
<tr>
<td>No. of secretory bursts/24 h</td>
<td>20.5 ± 0.9 ((21))</td>
<td>17.7 ± 1.4 ((17))</td>
<td>17.3 ± 1.2 ((17.5))</td>
<td>0.10</td>
<td>0.06</td>
<td>0.13</td>
</tr>
<tr>
<td>Mean burst interval, min</td>
<td>71 ± 3 ((65))</td>
<td>83 ± 7 ((80))</td>
<td>86 ± 8 ((84))</td>
<td>0.13</td>
<td>0.07</td>
<td>0.15</td>
</tr>
<tr>
<td>Secretory burst amplitude, mU/l, min(^{-1})</td>
<td>0.178 ± 0.025 ((0.172))</td>
<td>0.310 ± 0.047 ((0.296))</td>
<td>0.490 ± 0.057 ((0.462))</td>
<td>0.11</td>
<td>0.00009</td>
<td>0.0001</td>
</tr>
<tr>
<td>Basal secretion mU/l, 24 h(^{-1})</td>
<td>8.1 ± 1.7 ((6.3))</td>
<td>9.6 ± 1.4 ((9.1))</td>
<td>16.7 ± 5.1 ((10.7))</td>
<td>0.90</td>
<td>0.15</td>
<td>0.13</td>
</tr>
<tr>
<td>Pulsatile secretion, mU/l, 24 h(^{-1})</td>
<td>102 ± 13 ((102))</td>
<td>134 ± 26 ((120))</td>
<td>229 ± 36 ((190))</td>
<td>0.69</td>
<td>0.006</td>
<td>0.006</td>
</tr>
<tr>
<td>Total secretion, mU/l, 24 h(^{-1})</td>
<td>110 ± 13 ((112))</td>
<td>143 ± 27 ((127))</td>
<td>245 ± 40 ((200))</td>
<td>0.71</td>
<td>0.007</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Data are means ± SE, with the median value in parentheses; 1 mU/l = 0.38 µg/l BMI, body mass index; GH, growth hormone; C, controls; NS, not significant. Statistical comparisons were made by ANOVA, followed post hoc by Tukey’s honestly significantly different test.
healthy controls, and IGF-I concentrations were similar. However, the regularity of the GH secretory process and the pattern synchrony of cortisol and GH in the patients were clearly diminished.

Stimulated GH release is severely restricted in Cushing’s syndrome, and either no increase or only a small increment is noted after administration of GHRH, GH-releasing peptide (GHRP; hexarelin and GHRP-2), and ghrelin (2, 20, 30, 33). Because most of the GH stimulation studies in Cushing’s syndrome lack body weight-matched controls, the specificity of this finding might be questioned. GH release after reduction of the endogenous somatostatin tonus is also greatly diminished in hypercortisolism, e.g., by pretreatment with pyridostigmine or arginine infusion or after abrupt cessation of an intravenous infusion with somatostatin (13, 29, 32). Collectively, these results could point to a (reversible) defect of the pituitary gland, i.e., the somatotrop cell. Indeed, repeated GHRH administration in the hypercortisolemic state leads to potentiation to this hormone (31). Furthermore, administration of acipimox caused a sevenfold increase in GH release after GHRH administration, accompanied by a threefold decrease in circulating free fatty acids, and almost doubling of spontaneous 24-h GH secretion (28). Finally a hypocaloric diet for 3 days resulted in a fourfold GH increase after GHRH injection (34).

Similarities with experimental results in obesity are distinct, since it is well established that GHRH-stimulated GH release is diminished in obesity and increases during caloric restriction and after weight loss (14). Spontaneous 24-h GH secretion is severely restricted in the overweight human and increases or normalizes after weight reduction and during acipimox treatment (23, 41). In other studies, both BMI and abdominal visceral fat mass predict irregular (disorderly) GH release (12, 50). The basis for this inferred feedback alteration in GH secretion is not known (14).

Reports on spontaneous GH secretion in Cushing’s syndrome, as studied with 24-h blood sampling protocols, are scarce. In one such contribution, Magiakou et al. (35) studied 15 patients with hypercortisolism (14 pituitary-dependent patients and 1 with primary bilateral nodular hyperplasia), of whom 6 were prepubertal. They described severely depressed GH secretion compared with normal-weight controls, mainly caused by decreased pulse amplitude, but with unchanged pulse frequency (35). The intriguing observation was that the expected restoration of GH secretion after curative pituitary surgery failed to occur, notwithstanding significant weight loss and normalization of BMI in 50% of the patients who had preoperatively increased values. These observations suggest that (visceral) obesity is an important determinant of GH secretion in Cushing’s syndrome, irrespective of its etiology; apparently, however, after pituitary surgery, other factors play (or still play) a role in the diminished GH secretion.

We established a significant negative relationship between BMI and GH secretion in pituitary-independent hypercortisolism and in the matched controls. BMI, however, explained only 30% of the variability in GH, suggesting that other mechanisms likely contribute to the observed hyposomatotropism, as discussed above. It is unfortunate that we had no data on visceral fat mass in our patients and controls, because most likely, a higher correlation coefficient would have been found. Nevertheless, we did not find a relation between the degree of cortisol excess and GH secretion rate, as was previously found in our laboratory (17) for pituitary-dependent hypercortisolism. A conspicuous difference in clinical presentation between the two forms of the syndrome was the very high cortisol secretion rate in some of the (male) Cushing’s disease patients, which could explain the divergent results.

Compared with lean controls, our patients had a 50% reduction in pulsatile GH secretion, exclusively caused by secretory burst amplitude decrement. In the absence of a significant change in basal (nonpulsatile) secretion, this observation is compatible with heightened somatostatin inhibition (3), decreased hypothalamic GHRH secretion, a defect in the GHRH/GH secretagogue receptor signaling, or direct nonreceptor-related GH inhibition. Experimental evidence, mainly obtained in the rat, has demonstrated that high doses of glucocorticoids decrease the expression of hypothalamic GHRH mRNA and increase that of somatostatin (11, 14, 27). On the other hand, dexamethasone increased mRNA of the GHRH receptor and the GH secretagogue receptor, which certainly explains the dexamethasone potentiation of GH release after GHRH in the human and in the rat (26, 37, 49) but not the diminished GH response to GHRH/GHS during chronic glucocorticoid excess. Accordingly, the amount and duration of cortisol excess appear to be important.

Other mechanisms might limit GH secretion in chronic hypercortisolism. For instance, in the rat, dexamethasone administration decreased mitosis and increased apoptosis of pituitary cells (38). If such a mechanism is also present in the human somatotrope, this might (partly) explain the extended time (1 year or more) it takes for restoration of GH secretion in most of the (adult) patients after surgical cure of Cushing’s syndrome (16, 21, 49). Nonetheless, permanent damage to the somatotrope appears to be the rule rather than exception in childhood-onset Cushing’s disease after surgery and radiation treatment (4). Another mechanism potentially relevant for the inhibitory effect of glucocorticoids on GH secretion is via the action of annexin 1. This peptide is a mediator of the anti-inflammatory actions of glucocorticoids and has significant effects on cell growth, differentiation, apoptosis, membrane fusion, endocytosis, and exocytosis (22). This peptide, widely distributed in the body, is also present in the folliculostellate cells in the pituitary gland, but not in the pituicytes, and exerts its GH-suppressing effect on the somatotrope via a paracrine

Fig. 3. Relation between cortisol secretion rate and approximate entropy (ApEn) in patients. •, Patients with a unilateral adenoma; ▲, patients with bilateral hyperplasia.
mechanism at a point distal to the formation of cAMP and Ca
ion entry (48). However, the same mediator also has a centrally
stimulatory effect on GH (40). Finally, leptin might also be
involved in the GH regulation. Circulating leptin concentra-
tions in Cushing’s syndrome are disproportionately increased
compared with BMI-matched healthy controls (7, 15, 36).
Short-term fasting in Cushing’s syndrome did not restore
normal leptin levels, and GH secretion remained blunted (19).
However, several recent clinical studies suggest that a direct
role for leptin in GH regulation is rather limited. In morbidly
obese patients treated by biliopancreatic diversion, changes in
insulin levels predicted changes in leptin levels and the soma-
totropic axis (8). Also, observations in patients with homozy-
gous and heterozygous leptin gene mutations indicate that GH
secretion is correlated with adiposity (39). Finally, recombi-
nant methionyl human leptin administration in healthy lean
men did not prevent fasting-induced augmentation of GH
pulsatility or a decline in free IGF-I levels but restored, in part,
total IGF-I levels (5).

GH concentration fell after meals in patients and in controls.
Theoretically, one might expect a diminished inhibitory action
in patients because of decreased hypothalamic GHRH expres-
sion and increased somatostatin expression, as discussed above
(11, 14, 27). The differences between patients and controls
were not significant (P values < 0.60), suggesting that lack of
power was not responsible.

A conspicuous and specific observation was the decreased
regularity of GH secretion measured using ApEn, as previously
described in patients with ACTH-producing pituitary adeno-
mas (17). The degree of irregularity of GH release in patients
with adrenal cortisol excess was significantly greater than that
estimated in obese controls. The ApEn statistic quantitates the
relative orderliness or reproducibility of subordinate (nonpul-
satile) secretory patterns in neurohormone time series, which
in turn mirrors feedforward and feedback adjustments driven by
(patho)physiological changes in interglandular communica-
tion. The validity of ApEn to this end has been established in
theoretical and experimental contexts (9, 55, 56). In view of the
unchanged IGF-I feedback signal in the patients, decreased
regularity of GH secretion could reflect impaired coordinate
control of GH secretion by somatostatin, GHRH, and ghrelin
and/or altered pituitary responsiveness to these peptides (9,
10). Available data do not address the reversibility of disor-
derly GH release due to endogenous adrenal cortisol excess
with presumptively normal premorbid hypothalamo-pituitary
function.

The 24-h concentration profiles allowed an appraisal of
possible coordinate secretion of cortisol with GH. In normal
subjects, we found a reciprocal relationship between these two
hormones, as previously demonstrated in midluteal-phase
women and in children (1, 6). The inverse relationship might
be explained by the known ability of glucocorticoids to sup-
press GH secretion, possibly via heightened somatostatinergic
tone (14). In patients, the correlation between the two hor-
mones was smaller and even positive in five subjects. Changes of
toilet patterns as observed during the stress of caloric deprivation
and, by definition, nonphysiological glucocorticoid substitu-
tion therapy lead to desynchronization of hormone secretion
patterns, as we now also described for endogenous primary
adrenal hypercorticism. The loss of interaxis synchrony in our
patients is corroborated by (lag-independent) cross-ApEn anal-
ysis. Disruption of pattern synchrony of GH and cortisol is also
seen during fasting in adult women. Interesting, and not pre-
viously reported, was the loss of synchrony between GH and
cortisol in 15 patients with ACTH-dependent hypercorticism
(45, 47). In these patients, cross-ApEn was 1.640 ± 0.068,
greatly elevated to a similar degree as the adrenal form of
hypercorticism (P = 0.000013 vs. controls, and P = 0.99 vs.
adrenal hypercorticism). Collectively, these results indicate
that endogenous hypercorticism leads to disruption of corti-
rol-GH synchrony, irrespective of its cause. Notwithstanding
the obvious loss in synchrony, copulsatility of cortisol and GH
remained strong. This finding is somewhat surprising, since
tumoral cortisol secretion in patients with adrenal adenoma is
ACTH independent, and could therefore indicate that cortisol
feedback is involved in the temporal timing of GH pulses. At
present, no other data in literature are available to support this
hypothetical view.

In summary, patients with primary adrenal Cushing’s syn-
drome exhibit moderate hyposomatotropism, as demonstrated
by decreased GH pulsatile secretion, which is only partly
(~30%) explained by adiposity. This observation in combina-
tion with disruption of GH pattern regularity and synchrony
points to impaired net peptide drive of orderly somatotrope
secretion.

REFERENCES
Homeostatic joint amplification of pulsatile and 24-hour rhythmic cortisol
secretion by fasting stress in midluteal phase women: concurrent disrup-
tion of cortisol-growth hormone, cortisol-luteinizing hormone, and corti-
Dual and selective actions of glucocorticoids upon basal and stimulated
Veldhuis JD, and Serio M. Somatostatin infusion suppresses GH secre-
tory burst number and mass in normal men: a dual mechanism of
4. Carroll PV, Munson JP, Grossman AB, Besser GM, Ploeman PN,
Afshar F, and Savage MO. Successful treatment of childhood-onset
Cushing’s disease is associated with persistent reduction in growth hor-
5. Chan JL, Heist K, DePaoli AM, Veldhuis JD, and Mantzoros CS.
The role of falling leptin levels in the neuroendocrine and metabolic adaptation
2003.
6. Charmandarei E, Pincus SM, Mathews DR, Johnston A, Brook CGD,
and Hindmarsh PC. Oral hydrocortisone administration in children with
classic 21-hydroxylase deficiency leads to more synchronous joint growth
hormone and cortisol secretion. J Clin Endocrinol Metab 87: 2238–2244,
2002.
leptin levels do not change in patients with Cushing’s disease shortly after
correction of hypercortisolemia. J Clin Endocrinol Metab 82: 2747–2750,
1997.
Giampietro A, Porcelli T, Tilaro L, Fusco A, Valle D, and Tacchino
RM. Growth hormone secretion and leptin in morbid obesity before and
after biliopancreatic diversion: relationships with insulin and body com-
9. Fahry LS and Veldhuis JD. Joint pituitary-hypothalamic and intrahy-
thalamic autoregulatory feedback control of pulsatile growth hormone secretion.
23. Lacroix A, Ndiaye N, Tremblay J, and Hamet P. 

24. Hughes NR, Lissett CA, and Shalet SM. 


28. Fife SK, Brogan RS, Giustina A, and Wehrenberg WB. 

29. Fahry LS and Veldhuis JD. 

30. Voshol PJ, Meinders AE, and Pijl H. 


44. Taylor AD, Christian HC, Morris JF, Flower RJ, and Buckingham JC. Evidence from immunoneutralization and antisense studies that the


