Cushing's Syndrome : hormonal secretion patterns, treatment and outcome.

Aken, M.O. van

Chapter 3

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 Roelfsema [1]

[1] Department of Endocrinology and Metabolic Diseases, Leiden University Medical Center, Leiden, the Netherlands and [2] Division of Endocrinology and Metabolism, Mayo Medical and Graduate Schools of Medicine, Mayo Clinic, Rochester,MN 55905.

American Journal of Physiology: Endocrinology and Metabolism 2005:288(1);E63-70

ABSTRACT

To evaluate the impact on the somatotropic axis of endogenous cortisol excess in the absence of primary pituitary disease, we investigated spontaneous 24-h 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 BMI-, age- and gender-matched control group and an age- and gender-matched group of lean controls. GH secretion rates did not differ from BMI-matched controls, but was 2-fold lower compared with lean subjects, mainly caused by 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 with cortisol secretion. Total serum IGF-I concentrations were similar in the 3 groups. Approximate entropy 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 were 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 suggests altered coordinate regulation of GH release.

INTRODUCTION

Cushing's syndrome is characterized by increased cortisol secretion and is caused by ACTH-dependent cortisol excess (Cushing's disease or the rare ectopic tumoral ACTH production syndrome) or by ACTH-independent cortisol excess. The latter syndrome is caused by an 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 GH response to various stimuli, including insulin-induced hypoglycemia, GHRH, growth hormone secretagogues (GHS) and ghrelin, is well described in pituitary-dependent hypercortisolism (24,30,33,46).

Since obesity, frequently a prominent feature of hypercortisolism, is accompanied by 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 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 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 (mean ± SE, [median]), BMI 25.6 ± 1.4 [24.1] kg/m^{2}. The age of the twelve control subjects, matched for age, gender and BMI was 45.3 ±3.7 [45] yr, BMI 26.6 ± 1.6 [24.6] kg/m^2 (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/m^2 (P=0.03 vs. patients) and their age 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 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 establishing the biochemical diagnosis of primary adrenal Cushing's syndrome, 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 7 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 three months before the study. All women had stable body weight for at least three months 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.

Table 1. Clinical characteristics of twelve patients with primary adrenal Cushing's syndrome.

patient	sex	age	diagnosis	UCE ¶ (nmol/24 h)	size of adrenal gland(s)	no. cortisol pulses/ 24 h	cortisol secretion /24 h
1	f	48	UAA	617	5 cm	19	3730
2	f	48	UAA	1017	2.8 cm	33	13720
3	f	43	UAA	300	3.5 cm	34	10060
4	f	21	UAA	2414	2.5 cm	24	10560
5	f	40	UAA	1677	2.0 cm	30	22330
6	m	58	UAA	490	4.8 cm	19	4420
7	f	25	UAA	1359	5.2 cm	31	8160
8	m	78	AIMAH	399	right 3 cm, left 2 cm	28	3660
9	f	41	AIMAH	1031	right 2.5 cm, left 3.4 cm	41	5190
10	f	48	AIMAH	641	right 2.5 cm, Left 5 cm	21	4390
11	f	50	AIMAH	407	right 2.8 cm, left 2 cm	34	9460
12	f	45	AIMAH	429	right 4.8 cm, left 4.1 cm	32	14280

UAA: unilateral adrenal adenoma. AIMAH: ACTH-independent macronodular adrenal hyperplasia. ¶ Urinary cortisol excretion: normal values < 220 nmol/24 h. The number of significant cortisol pulses and the cortisol secretion rate were determined with deconvolution analysis of the 24-hour cortisol concentration series.

Methods

Patients and control subjects were admitted to the hospital on the day of the study. An indwelling iv. 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 h. 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 h. Lights were turned off between 2200-2400 h. 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 were reported in a separate paper (submitted elsewhere); here we use only the 24 h secretion rates in regression analyses.

Assays

Plasma GH concentrations were measured in duplicate using a sensitive time-resolved immunofluorometric assay (Wallac, Inc., Turku, Finland), specific for the 22-kDa GH protein. Human biosynthetic GH (Pharmacia & Upjohn Inc., Uppsala, Sweden) was used as standard calibrated against WHO-IRP 80-505, with a detection limit of 0.03 mU/L and an intra-assay variation coefficient of 1.6-8.4% at plasma values between 0.25-40 mU/L (to convert mU/L to μg/L divide by 2.6). All samples from any subject were run in the same assay.

The serum IGF-I was determined by RIA (Incstar Corp., Stillwater, MN.) with a detection limit of 1.5 nmol/L and an interassay variation coefficient of less than 11%. Plasma cortisol concentrations were measured by RIA (Sorin Biomedica, Milan, Italy). The detection limit of the assay was 25 nmol/l. The interassay variation varied from 2 – 4 % at the concentrations obtained in this study.

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 (12). 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 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 next incremental comparison and has been formally defined elsewhere (43). The ApEn calculation provides a single non-negative number, which 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 PRL 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 cross-ApEn between cortisol (leading) and GH, with r=20% of the SD of the individual time-series and m=1. This parameters choice affords sensitive, valid and statistically well-replicated ApEn and cross-ApEn metrics for assessing hormone time-series of this length (44). ApEn and cross-ApEn results are reported as absolute values and as the ratio of the absolute value to that of the mean of 1000 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 the 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 the mean ± SEM. Comparison between groups was done with one-way ANOVA, followed post hoc by Tuckey's honestly significantly different (HSD) 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 pair wise, as described previously (54). Calculations were carried out with Systat (release 11, Systat Software Inc, 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 patients and in BMI-matched controls as displayed in the figure. In healthy lean controls GH secretion was two-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 nmol/L, BMI-matched controls 16.8 ± 0.8, and lean controls 20.1 ± 2.2 (ANOVA, P = 0.44).

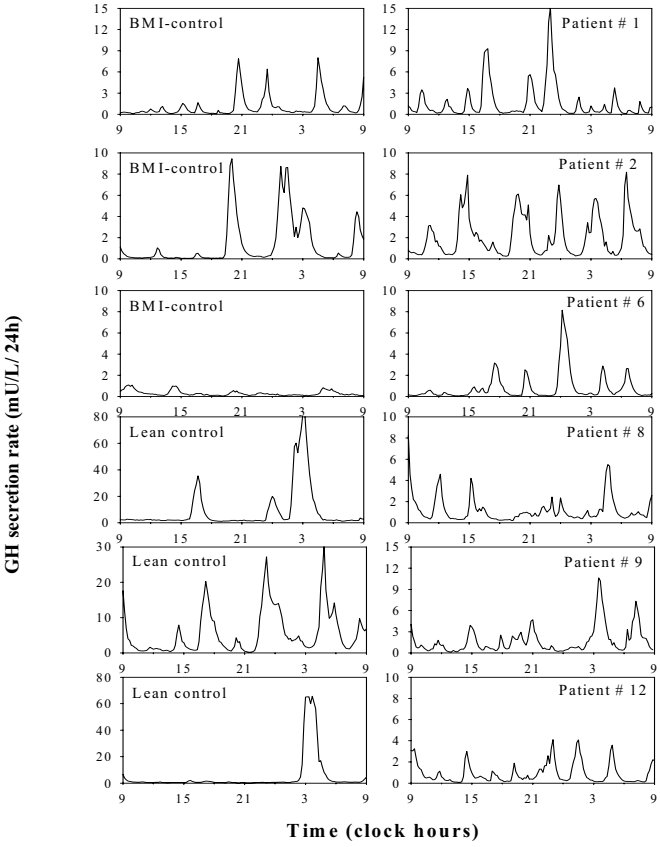

Fig 1.GH concentration profiles of hypercortisolemic patients, obtained by 10-min blood sampling for 24 h. Sampling started at 0900 h, One mU/L=0.38 µg/L.

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

	Patients (n=12)	BMI-matched controls (n=12)	Lean controls (n=12)	P-value *vs* matched C	P-value *vs* lean C	ANOVA
Basal secretory rate (mU/L/min)	0.00563 ± 0.0012 [0.0044]	0.0067 ± 0.0009 [0.0062]	0.0116 ± 0.0035 [0.0074]	NS	NS	0.13
Half-life (min)	14.7 ± 0.6 [15.4]	14.2 ± 0.6 [14.6]	14.7 ± 0.6 [14.8]	NS	NS	0.78
Secretory-burst half duration (min)	28.2 ± 2.2 [27.0]	25.5 ± 1.7 [26.6]	27.1 ± 1.8 [27.7]	NS	NS	0.62
No. of secretory bursts/24 h	20.5 ± 0.9 [21]	17.7 ± 1.4 [17]	17.3 ± 1.2 [17.5]	0.10	0.06	0.13
Mean burst interval (min)	71 ± 3 [65]	83 ± 7 [80]	86 ± 6 [84]	0.13	0.07	0.15
Secretory burst amplitude (mU/L/min)	0.178 ± 0.025 [0.172]	0.310 ± 0.047 [0.296]	0.490 ± 0.057 [0.462]	0.11	0.00009	0.0001
Basal secretion (mU/L/24h)	8.1 ± 1.7 [6.3]	9.6 ± 1.4 [9.1]	16.7 ± 5.1 [10.7]	0.90	0.15	0.13
Pulsatile secretion (mU/L/24h)	102 ± 13 [102]	134 ± 26 [120]	229 ± 36 [190]	0.69	0.006	0.006
Total secretion (mU/L/24h)	110 ± 13 [112]	143 ± 27 [127]	245 ± 40 [200]	0.71	0.007	0.007

Statistical comparisons were made by ANOVA, followed by *post hoc* Tuckey's HSD test. Data are expressed as mean ± SE. The median value is shown in brackets. One mU/L =0.38 µg/L.

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 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 diner was 1.51 mU/L in patients (P = 0.04) and in controls 2.19 mU/L (P = 0.02). The mean GH decreases in patients and controls were statistically similar.

Approximate entropy

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 in patients was 0.5102 ± 0.015, 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 non-significant 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).

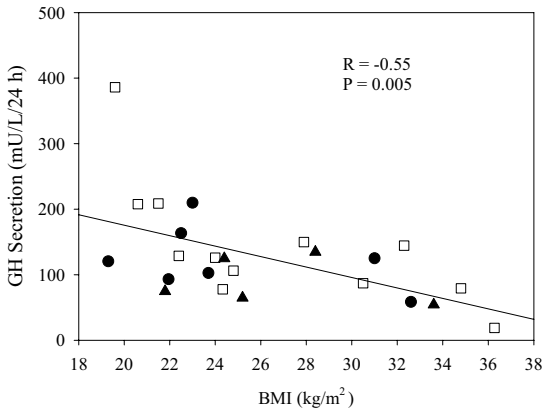

Fig 2. Linear regression of GH secretion rate per 24 h, as estimated by deconvolution of the serum GH profiles, on BMI. Closed symbols reflect hypercortisolemic patients (circles unilateral adenoma, triangles bilateral nodular hyperplasia) and open squares BMI-matched controls.

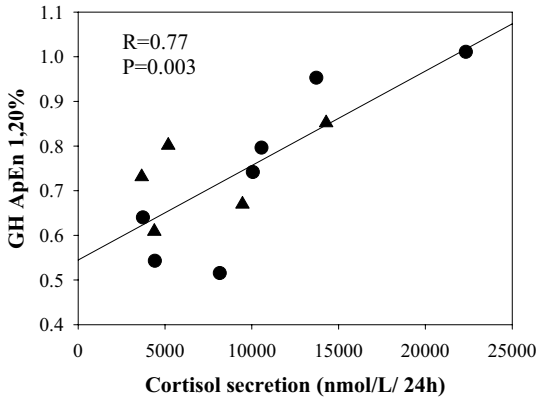

Fig 3. Relation between cortisol secretion rate and ApEn in patients. Patients with a unilateral adenoma are shown as circles and patients with bilateral hyperplasia as triangles.

Relation between cortisol and GH secretion

Pattern synchrony between cortisol and GH was quantified by cross-ApEn in patients and BMI-matched controls. X-ApEn in patients was 1.648 ± 0.113 and in controls 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 (median -0.30, 95% confidence interval (CI) -0.15 to -.0.39), and a mean time lag of 30 min (95% CI 0-65 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 to +0.16. The mean time lag was 75 min, 95% CI 37-100 min. Co-pulsatility of cortisol and GH pulses was statistically highly significant in all patients and in 10 of 12 control subjects (P-values between 10^{-3} to 10^{-13}).

Unilateral vs. 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 BMI-matched 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, GHRP (hexarelin and GHRP-2) and ghrelin (2, 20, 30, 33). Since 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 pre-treatment with pyridostigmine, arginine infusion, or after abrupt cessation of an iv infusion with somatostatin (13, 28,34). Collectively, these results could point to a (reversible) defect of the pituitary gland, i.c. the somatotropic cell. Indeed, repeated GHRH administration in the hypercortisolemic state leads to potentiation to this hormone (29). Furthermore, administration of acipimox caused a 7-fold increase in GH release after GHRH administration, accompanied by a 3-fold decrease in circulating FFA's, and almost doubling of spontaneous 24 h GH secretion (31). Finally a hypocaloric diet for 3 days resulted in a 4-fold GH increase after GHRH injection (32).

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 studied

15 patients with hypercortisolism (14 pituitary-dependent and one with primary bilateral pigmented nodular hyperplasia), of whom 6 patients 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 the 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, but apparently 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 we previously found for pituitary–dependent hypercortisolism (17). 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 (non-pulsatile) secretion this observation is compatible with heightened somatostatin inhibition (3), decreased hypothalamic GHRH secretion, a defect in the GHRH/GH secretagogue receptor signalling or direct non-receptor-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 (one year or more) it takes for restoration of GH secretion in most of the (adult) patients after surgical cure of Cushing's syndrome (18, 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 mechanism at a point distal to the formation of cyclic AMP and Ca ion entry (48). However, the same mediator has also a centrally stimulatory effect on GH (40). Finally, leptin might also be involved in the GH regulation. Circulating leptin concentrations 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 somatotropic axis (8). Also observations in patients with homozygous and heterozygous leptin gene mutations indicate that GH secretion is correlated with adiposity (39). Finally, r-metHuleptin administration in healthy lean men did not prevent fasting-induced augmentation of GH pulsatility or 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 expression, 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 adenomas (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 (nonpulsatile) secretory patterns in neurohormone time series, which in turn mirrors feedforward and feedback adjustments driven by (patho) physiological changes in interglandular communication. 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 disorderly GH release due to endogenous adrenal cortisol excess with presumptively normal premorbid hypothalamo-pituitary function.

The 24-hour 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 mid-luteal phase-women and in children (1, 6). The inverse relationship might be explained by the known ability of glucocorticoids to suppress GH secretion, possibly via heightened somatostatinergic tone (14). In patients the correlation between the two hormones was smaller, and even positive in 5 subjects. Indeed, abolishment of the cortisol-GH correlation can be induced by fasting in adult healthy women, while a positive correlation is seen in children with congenital adrenal hyperplasia under glucocorticoid substitution therapy (1, 6). Changes of cortisol patterns as observed during the stress of caloric deprivation, and by definition non-physiological glucocorticoid substitution therapy lead to desynchronization of hormone secretion patterns, as we now also described for endogenous primary adrenal hypercorticism. The loss of inter-axis synchrony in our patients is corroborated by (lag-independent) cross-ApEn analysis. Disruption of pattern synchrony of GH and cortisol is also seen during fasting in adult women. Interestingly and not previously 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, thus 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 cortisol-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 syndrome exhibit moderate hyposomatotropism, as demonstrated by decreased GH pulsatile secretion, which is only partly (~ 30%) explained by adiposity. This observation in combination with disruption of GH pattern regularity and synchrony points to impaired net peptidyl-drive of orderly somatotrope secretion.

REFERENCES

1. Bergendahl M, Iranmanesh A, Pastor C, Evans WS and Veldhuis JD. Homeostatic joint amplification of pulsatile and 24-hour rhythmic cortisol secretion by fasting stress in midluteal phase women: concurrent disruption of cortisol-growth hormone, cortisol-luteinizing hormone, and cortisol-leptin synchrony. *J Clin Endocrinol Metab* 85: 4028-4035, 2000.
2. Burguera B, Muruais C, Penalva A, Dieguez C and Casanueva FF. Dual and selective actions of glucocorticoids upon basal and stimulated growth hormone release in man. *Neuroendocrinology* 51: 51-58. 1990.
3. Calabresi E, Ishikawa E, Bartolini L, Delitala G, Fanciulli G, Oliva O, Veldhuis JD and Serio M. Somatostatin infusion suppresses GH secretory burst number and mass in normal men: a dual mechanism of inhibition. *Am J Physiol Endocrinol Metab* 270: E975-E979, 1996.
4. Carroll PV, Monson JP, Grossman AB, Besser GM, Plowman PN, Afshar F and Savage MO. Successful treatment of childhood-onset Cushing's disease is associated with persistent reduction in growth hormone secretion. *Clin Endocrinol* 60:169-174, 2004.
5. Chan JL, Heist K, DePaoli AM, Veldhuis JD and Mantzoros CS. The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short-term starvation in healthy men. *J Clin Invest* 111:1409-1421, 2003.
6. Charmandari, 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.
7. Cizza G, Lotsikas AJ, Licinio J, Gold PW and Chrousos GP. Plasma leptin levels do not change in patients with Cushing's disease shortly after correction of hypercortisolism. *J Clin Endocrinol Metab* 82: 2747-2750, 1997.
8. De Marinis L, Bianchi A, Mancini A, Gentilella R, Perrella M, 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 composition. J *Clin Endocrinol Metab* 89:174-180, 2004.
9. Fahry LS and Veldhuis JD. Joint pituitary-hypothalamic and intrahypothalamic autofeedback construct of pulsatile growth hormone secretion. *Am J Physiol Regul Integr Comp Physiol* 285: R1240-R1249, 2003.
10. Fahry LS and Veldhuis JD. Putative GH pulse renewal: periventricular somatostatinergic control of an arcuate-nuclear somatostatin and GH-releasing hormone oscillator. *Am J Physiol Regul Integr Comp Physiol* 286:R1030-R1042, 2004.
11. Fife SK, Brogan RS, Giustina A and Wehrenberg WB. Immunocytochemical and molecular analysis of the effects of glucocorticoid treatment on the hypothalamic-somatotropic axis in the rat. *Neuroendocrinology* 64:131-138, 1996.
12. Friend K, Iranmanesh A and Veldhuis JD. The orderliness of growth hormone (GH) release process and the mean mass of GH secreted per burst are highly conserved in individual men on successive days. *J Clin Endocrinol Metab* 81:3746-3753, 1996.
13. Giustina A, Bossoni S, Bodini C, Ferrari C, Pizzocolo G, Scalvini T, Schettino M and Wehrenberg WB. Pyridostigmine enhances even it does not normalize the growth hormone responses to growth hormone-releasing hormone in patients with Cushing's disease. *Horm Res* 35:99-103, 1991.
14. Giustina A and Veldhuis JD. Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. *Endocr Rev* 19:717-797, 1998.
15. Groote Veldman R, Frölich M, Pincus SM, Veldhuis JD and Roelfsema F. Hyperleptinemia in women with Cushing's disease is driven by high-amplitude pulsatile, but orderly and eurhythmic, leptin secretion. *Eur J Endocrinol* 144:21-27, 2001.
16. Groote Veldman R, Van den Berg G, Pincus SM, Frölich M, Veldhuis JD and Roelfsema F. Increased episodic release and disorderliness of prolactin secretion in both micro- and macroprolactinomas. *Eur J Endocrinol* 140: 192-200, 1999.

17. Groote Veldman R, Frölich M, Pincus SM, Veldhuis JD and Roelfsema F. Growth hormone and prolactin are secreted more irregularly in patients with Cushing's disease. *Clin Endocrinol* 52:625-635, 2000.
18. Groote Veldman R, Frölich M, Pincus SM, Veldhuis JD and Roelfsema F. Apparently complete restoration of normal daily adrenocorticotropin, cortisol, growth hormone, and prolactin secretory dynamics in adults with Cushing's disease after clinically successful transsphenoidal adenomectomy. *J Clin Endocrinol Metab* 85:4039-4046, 2000.
19. Grottolli S, Gauna C, Tassone F, Aimaretti G, Corneli G, Wu Z, Strasburger CJ, Dieguez C, Cassanueva FF, Ghigo E and Maccario M. Both fasting-induced leptin reduction and GH increase are blunted in Cushing's syndrome and in simple obesity. *Clin Endocrinol* 58:220-228, 2003.
20. Hotta M, Shibasaki T, Masuda A, Imaki T, Sugino M, Demura H, Ling N and Shizume K. Effect of human growth hormone-releasing hormone on GH secretion in Cushing's syndrome and non-endocrine disease patients treated with glucocorticoids. *Life Sci* 42:979-984, 1988.
21. Hughes NR, Lissett CA and Shalet SM. Growth hormone status following treatment for Cushing's syndrome. *Clin Endocrinol* 51:61-66, 1999.
22. John CD, Christian HC, Morris JF, Flower RJ, Solito E and Buckingham JC. Annexin 1 and the regulation of endocrine function. *Trends in Endocrinol Metab* 15:103-109, 2004.
23. Kok P, Buijs MM, Kok SW, van Iersel IHAP, Frölich M, Roelfsema F, Voshol PJ and Meinders AE, Pijl H. Acipimox enhances growth hormone secretion in obese women. *Am J Physiol Regul Integr Comp Physiol* 286:R693-R698, 2003.
24. Krieger DT and Glick SM. Growth hormone and cortisol responsiveness in Cushing's syndrome. Relation to a possible central nervous system etiology. *Am J Med* 52: 25-40, 1972.
25. Lacroix A, Ndiaye N, Tremblay J and Hamet P. Ectopic and abnormal hormone receptors in adrenal Cushing's syndrome. *Endocr Rev* 22:75-110, 2001.
26. Lam KS, Lee MF, Tam SP and Srivastava G. Gene expression of the receptor for growth hormone-releasing hormone is physiologically regulated by glucocorticoids and estrogen. *Neuroendocrinology* 63: 475-480, 1996.
27. Lam KS and Srivastava G. Gene expression of hypothalamic somatostatin and growth hormone-releasing hormone in dexamethasone-treated rats. *Neuroendocrinology* 66: 2-8, 1997.
28. Leal-Cerro A, Pereira JL, Garcia-Luna PP, Astorga R, Cordido F, Casanueva FF and Dieguez C. Effect of enhancement of endogenous cholinergic tone with pyridostigmine on growth hormone (GH) responses to GH-releasing hormone in patients with Cushing's syndrome. *Clin Endocrinol* 33:291-295, 1990.
29. Leal-Cerro A, Pumar A, Villamil F, Astorga R, Dieguez C and Casanueva FF. Growth hormone releasing hormone priming increases growth hormone secretion in patients with Cushing's syndrome *Clin Endocrinol* 38:399-403, 1993.
30. Leal-Cerro A, Pumar A, Garcia-Garcia E, Dieguez C and Casanueva FF. Inhibition of growth hormone release after combined administration of GHRH and GHRP-6 in patients with Cushing's syndrome. *Clin Endocrinol* 41: 649-654, 1994.
31. Leal-Cerro A, Jimenez LM, Astorga R, Fernandez-Lopez I, Dieguez C and Casanueva FF. Acute pharmacological reduction of plasma free fatty acids enhances the growth hormone (GH)-releasing hormone-mediated GH secretion in patients with Cushing's syndrome. *J Clin Endocrinol Metab* 82:3165-3168, 1997.
32. Leal-Cerro A, Venegas E, Garcia-Pesquera F, Jimenez LM, Astorga R, Dieguez C and Casanueva FF. Enhanced growth hormone (GH) responsiveness to GH-releasing hormone after dietary restriction in patients with Cushing's syndrome. *Clin Endocrinol* 48:117-121, 1998.
33. Leal-Cerro A, Torres E, Soto A, Dios E, Deghenghi R, Arvat E, Ghigo E, Dieguez C and Casanueva FF. Ghrelin is no longer able to stimulate growth hormone secretion in patients with Cushing's syndrome but instead induces exaggerated corticotrophin and cortisol responses. *Neuroendocrinology* 76:390-396, 2002.
34. Leal-Cerro A. Soto A, Martinez MA, Alvarez P, Isidro L, Casanueva FF, Dieguez C and Cordido F. Effect of withdrawal of somatostatin plus growth hormone (GH)-releasing hormone as a stimulus of GH secretion in Cushing's syndrome. *Clin Endocrinol* 57: 745-749, 2002.

35. Magiakou MA, Mastorakos G, Gomez MT, Rose SR and Chrousos GH. Suppressed spontaneous and stimulated growth hormone secretion in patients with Cushing's disease before and after surgical cure. *J Clin Endocrinol Metab* 78:131-137, 1994.
36. Masuzaki H, Ogawa Y, Hosoda K, Miyawaki T, Hanaoka I, Hiraoka J, Yasuno A, Nishimura H, Yoshimasa Y, Nishi S and Nakao K. Glucocorticoid regulation of leptin synthesis and secretion in humans: elevated plasma leptin levels in Cushing's syndrome. *J Clin Endocrinol Metab* 82: 2542-2547, 1997.
37. Miller TL and Mayo KJ. Glucocorticoids regulate pituitary growth hormone-releasing hormone receptor messenger ribonucleic acid expression. *Endocrinology* 138: 2458-2465, 1997.
38. Nolan LA, Kavanagh E, Lightman SL and Levy A. Anterior pituitary cell population control: basal cell turnover and the effects of adrenalectomy and dexamethasone treatment. *J Neuroendocrinol* 10:207-215, 1998.
39. Ozata M, Dieguez C and Casanueva FF. The inhibition of growth hormone secretion presented in obesity is not mediated by the high leptin levels: a study in human leptin deficiency patients. *J Clin Endocrinol Metab* 88: 312-316, 2003.
40. Philip JG, John CD, Cover PO, Morris JF, Christian HC, Flower RJ and Buckingham JC. Opposing influences of glucocorticoids and interleukin-1 on the secretion of growth hormone and ACTH in the rat in vivo: role of hypothalamic annexin 1. *Br J Pharmacol* 134: 887-895, 2001.
41. Pijl H, Langendonk JG, Burggraaf J,Frölich M, Cohen AF, Veldhuis JD and Meinders AE. Altered neuroregulation of GH secretion in viscerally obese premenopausal women. *J Clin Endocrinol Metab* 86:5509-5515, 2001.
42. Pincus SM. Approximate entropy as a measure of system complexity. *Proc Natl Acad Sci USA* 88:2297-2301, 1991.
43. Pincus SM and Goldberger AL. Physiological time-series analysis: what does regularity quantifies? *Am J Physiol* 266: H1643-H1656, 1994.
44. Pincus SM, Mulligan T, Iranmanesh A, Gheorghiu S, Godschalk M and Veldhuis JD. Older males secrete luteinizing hormone more irregularly, and jointly more asynchronously than younger males. *Proc Natl Acad Sci USA 93*: 14100-14105, 1994.
45. Roelfsema F, Pincus SM and Veldhuis JD. Patients with Cushing's disease secrete adrenocorticotropin and cortisol jointly more asynchronously than healthy subjects. *J Clin Endocrinol Metab* 83:688-692, 1998.
46. Smals AE, Pieters GF, Smals AG, Benraad TJ and Kloppenborg PJ. Human pancreatic growth hormone releasing hormone fails to stimulate human growth hormone both in Cushing's disease and in Cushing's syndrome due to adrenal adenoma. *Clin Endocrinol* 24:401-407, 1986.
47. Tamura H, Kamegai J, Sugihara H, Kineman RD, Frohman LA and Wakabayashi I. Glucorticoids regulate pituitary growth hormone secretagogue receptor gene expression *J Neuroebdocrinol* 12:481-485, 2000.
48. Taylor AD, Christian HC, Morris JF, Flower RJ and Buckingham JC. Evidence from immunoneutralization and antisense studies that the inhibitory actions of glucocorticoids on growth hormone release in vitro require annexin 1 (lipocortin 1). *Br J Pharmacol* 131:1309-1316, 2000.
49. Tzanela M, Karavitaki N, Stylianidou C, Tsagarakis S and Thalassinos NC. Assessment of GH reserve before and after successful treatment of adult patients with Cushing's syndrome. *Clin Endocrinol* 60:309-314, 2004.
50. Vahl N, Jorgensen JO, Skjaerback C, Veldhuis JD, Orskov H and Christiansen J.Abdominal adiposity rather than age and sex predicts the mass and pattern regularity of growth hormone secretion in mid-life healthy adults. *Am J Physiol Endocrinol Metab* 272: E1108-E1116, 1997.
51. Van den Berg, Pincus SM, Veldhuis JD, Frölich M and Roelfsema F. Greater disorderliness of ACTH and cortisol release accompanies pituitary-dependent Cushing's disease. *Eur J Endocrinol* 136:394-400, 1997.
52. Van den Berg G, Pincus SM, Frölich M, Veldhuis JD and Roelfsema F Reduced disorderliness of growth hormone release in biochemically inactive acromegaly after pituitary surgery. *Eur J Endocrinol* 138:164-169, 1998.

53. Veldhuis JD, Carlson ML and Johnson ML. The pituitary gland secretes in bursts: appraising the nature of glandular secretory impulses by simultaneous multiple-parameter deconvolution of plasma hormone concentrations. *Proc Natl Acad Sci USA* 84: 7686-7690, 1987.
54. Veldhuis JD, Johnson ML, Faunt LM and Seneta E. Assessing temporal coupling between two, or among three or more, neuroendocrine pulse trains: cross-correlation analysis, simulation methods, and conditional probability testing. *Methods Neurosci* 20:336-376, 1994.
55. Veldhuis JD, Straume M, Iranmanesh A, Mulligan T, Jaffe C, Barkan A, Johnson ML and Pincus S. Secretory process regularity monitors neuroendocrine feedback and feedforward signalling strength in humans. Am J Physiol Regul Integr Comp Physiol 280:R721-R729, 2001.
56. Veldhuis JD, Johnson ML, Veldhuis OL, Straume M and Pincus SM. Impact of pulsatility on the ensemble orderliness (approximate entropy) of neurohormone secretion. Am J Physiol Regul Integr Comp Physiol 281:R1975-R1985, 2001.