CLINICAL STUDY

Diminished and irregular TSH secretion with delayed acrophase in patients with Cushing’s syndrome

Ferdinand Roelfsema, Alberto M Pereira, Nienke R Biermasz, Marijke Frolich, Daniel M Keenan, Johannes D Veldhuis, and Johannes A Romijn

Department of Endocrinology and Metabolic Diseases, Leiden University Medical Center, 2333 ZA Leiden, The Netherlands; 1Department of Statistics, University of Virginia, Charlottesville, Virginia 22904, USA and 2Endocrine Research Unit, Mayo Medical and Graduate Schools, Clinical Translational Research Center, Mayo Clinic, Rochester, Minnesota 55901, USA

(Correspondence should be addressed to F Roelfsema; Email: f.roelfsema@lumc.nl)

Abstract

Context: The hypothalamus–pituitary–thyroid axis in Cushing’s syndrome may be altered. Previous reports have shown diminished serum TSH concentration and decreased response to TRH.

Objective: We analyzed serum TSH profiles in relation to cortisol profiles in patients with hypercortisolism of pituitary (n = 16) or primary-adrenal origin (n = 11) and after remission by pituitary surgery (n = 7) in order to delineate aberrations in the hypothalamus–pituitary–thyroid system.

Intervention: Patients and controls (n = 27) underwent a 24-h blood sampling study. Serum TSH and cortisol were measured with precise methods, and data were analyzed with a deconvolution program, approximate entropy (ApEn), and cosinor regression.

Results: Pulsatile TSH secretion and mean TSH pulse mass were diminished during hypercortisolism, independently of etiology (P < 0.001). TSH secretion was increased in patients in remission only during daytime due to increased basal secretion (P < 0.01). Pulse frequency and half life of TSH were similar in patients and controls. TSH ApEn (irregularity) was increased in patients with hypercortisolism (P < 0.01), but was normal in cured patients. Cross-ApEn between TSH and cortisol, a measure of pattern synchrony loss, was increased in active disease, indicating (partial) loss of secretory synchrony. The TSH rhythm was phase delayed in hypercortisolemic patients, but normal in cured patients (P < 0.01). Free thyroxine levels were decreased only in pituitary-dependent hypercortisolism compared with controls (P = 0.003). Total 24-h TSH correlated negatively and linearly with log-transformed cortisol secretion (R = 0.43, P = 0.001).

Conclusion: Cortisol excess decreases TSH secretion by diminishing pulsatile release, whereas surgically cured patients have elevated nonpulsatile TSH release. Diminished TSH secretory regularity in active disease suggests glucocorticoid-induced dysregulation of TRH or somatostatinergic/annexin-1 control.

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Introduction

The major regulators of TSH secretion are TRH, the inhibitory neurotransmitters dopamine and somatostatin, and negative feedback by thyroxine (T₄) and triiodothyronine (T₃) (1). The interplay among these regulators in time dictates the TSH secretion pattern, which is characterized by a diurnal variation of serum TSH concentrations with superimposed (small) bursts. The 24-h secretion profile of TSH has been well described in various pathophysiological conditions, including hyperthyroidism, hypothyroidism, obesity, fasting, and nonthyroidal illness (2–5). Thyroid hormone secretion is tightly regulated, and is essential for energy homeostasis and basal heat production (6). From this perspective, it is not surprising that hypothalamic centers involved in the control of energy balance also exert effects on the paraventricular nucleus (PVN), which contains TRH-secreting neurons (7). One of the important metabolic signals modulating the activity of the hypothalamic–pituitary–thyroid (HPT) axis is leptin, which exerts a stimulating effect on TRH synthesis and release, both directly and indirectly via the proopiomelanocortin/cocaine- and amphetamine-related transcript expressing neurons of the arcuate nucleus (8). Glucocorticoid administration to healthy subjects diminishes the TSH increase to TRH and decreases spontaneous TSH secretion (9, 10). In Cushing’s disease, the TSH response to TRH administration is decreased (11, 12).

To test the hypothesis that endogenously regulated TSH secretion is inhibited by chronic cortisol excess throughout the 24-h cycle, including the nocturnal surge, we measured 24-h serum TSH and cortisol levels.
concentration profiles in patients with hypercortisolism and healthy control subjects. We also studied patients in long-term surgical remission, because TSH profiles have not been studied in such patients, and it is not known whether abnormalities in TSH secretion eventually normalize upon recovery of hypercortisolism. Hormone secretion rates were calculated using a new, operator-independent deconvolution method. Furthermore, we determined the relationship between TSH and cortisol secretion rates by regression analysis and pattern synchrony of the two hormones by cross-approximate entropy (ApEn).

**Subjects and methods**

**Subjects**

We recruited 16 consecutive patients (11 females and 5 males) with pituitary-dependent hypercortisolism (mean age 31.6 years, range 17–56 years; mean body mass index (BMI) 28.6 kg/m², range 20.5–38.9 kg/m²). The diagnosis of pituitary-dependent hypercortisolism was based on increased 24-h urinary excretion of free cortisol, subnormal or absent suppression of serum cortisol after administration of 1 mg dexamethasone overnight, subnormal suppression of urinary cortisol excretion during a low-dose dexamethasone test, suppression of serum cortisol by 190 nmol/l or more during a 7-h i.v. infusion of dexamethasone in a dose of 1 mg/h, positive immunosuppressing for ACTH of the adenoma and clinical cortisol dependency during several months after adenomectomy. We also included 11 consecutive patients (nine females and two males), with unilateral or bilateral adenoma (mean age 44.9 years, range 20–78 years; mean BMI 25.1 kg/m², range 19.3–33.6 kg/m²). The diagnosis of primary adenoma Cushing’s syndrome was based on increased urinary excretion of free cortisol, absence or diminished dexamethasone suppression of urinary cortisol excretion and serum cortisol, a low or undetectable plasma ACTH concentration, the presence of a unilateral or bilateral adrenal adenoma on computed tomography (CT) or magnetic resonance imaging scanning, histological confirmation of the adenoma and clinical remission after surgery. Furthermore, we included seven patients (four females and three males) in long-term remission of pituitary-dependent hypercortisolism (mean age 34 years, range 27–44 years, mean BMI 25.6 kg/m², range 20.6–33.3 kg/m²). The mean remission duration after transsphenoidal pituitary surgery was 6.8 years, range 4–11 years. None of these surgically treated patients used hormonal replacement therapy at least 2 years before and at the time of the blood sampling study, and all had a normal 24-h urinary excretion of cortisol and normal dexamethasone overnight cortisol suppression test. In four of these seven patients, the ACTH-adrenal reserve function was investigated by either the insulin tolerance test or CRH test. In all four patients, the stimulated cortisol concentration exceeded 0.55 μmol/l, pointing to a normal ACTH-adrenal reserve function. Twenty-seven control subjects (13 females and 14 males) were recruited through advertisements in local newspapers. The mean age was 42.9 years, range 29–64 years and BMI 25.6 kg/m², range 19.3–33.6 kg/m². Before participation, all control subjects underwent medical screening, including medical history taking, physical examination, standard laboratory hematology, blood chemistry, and urine tests. Acute or chronic disease, depression (present or in history), head trauma, smoking, alcohol abuse, recent transatlantic flights, night shift work, body weight change prior to the study (> 5 kg in 3 months), recent blood donation or participation in another clinical trial (< 3 months), and use of medication (including oral contraceptives) were exclusion criteria for participation.

The protocol was approved by the Medical Ethics Committee of the Leiden University Medical Center and was performed according to the Helsinki declaration. All patients and volunteers gave written acknowledgement of informed consent for participation.

**Clinical protocol**

Premenopausal women were sampled in the early follicular stage of their menstrual cycle. Subjects were admitted to the Clinical Research Center at 0700 h. A cannula for blood sampling was inserted into an antecubital vein, which was attached to a 3-way stopcock and kept patent by a continuous 0.9% NaCl infusion (500 ml/24 h). Blood samples for basal parameters were withdrawn and 24-h blood sampling started at 0900 h. Blood was collected at 10-min intervals for determination of TSH and cortisol concentrations. Subjects remained recumbent during the blood sampling period, except for bathroom visits. Daytime naps were not allowed. Well-being and vital signs were recorded at regular time intervals (hourly). Meals were served according to a fixed time schedule and consumed within limited time periods. Lights were switched off at 2300 h, and care was taken not to disturb subjects during withdrawal of blood samples, while they were sleeping. No electroencephalogram sleep registration was performed. Lights were switched on and subjects were awakened at 0730 h in the morning.

**Assays**

TSH concentrations were measured with a time-resolved immunofluorometric assay (Wallac, Turku, Finland) calibrated against the WHO 2nd standard International Reference Preparation (IRP) (80/558) hTSH. The limit of detection was 0.05 μU/l and the interassay coefficient of variation (CV) was < 5%.
Cortisol was assayed by high-sensitivity (25 nmol/l) solid-phase RIA (Sorin Biomedical, Milan, Italy). Intra- and interassay CV were 5.1 and 6.4% respectively. No samples were undetectable in either assay. Free T4 (fT4) concentrations were estimated using electrochemiluminescence immunoassay (Elecys 2010, Roche Diagnostics Nederland BV) and total T3 concentration with Abbott AXSYM (Abbott Park).

Calculations and statistics

Deconvolution analysis Hormone concentration time series were analyzed using a recently validated deconvolution method (13, 14). First, the automated Matlab program detrends the data and normalizes concentrations to the unit interval [0, 1]. Secondly, successive potential pulse-time sets, each containing one fewer burst, are created by a smoothing process (a nonlinear adaptation of the heat diffusion equation). Thirdly, a maximum likelihood expectation deconvolution method estimates all secretion and elimination rates simultaneously for each candidate pulse-time set. The deconvolution model specifies basal secretion (β0), two half-lives (α1, α2), an accumulation process and weak interpulse length dependency for secretory-burst mass (η0, η1), random effects on burst mass (σA), procedural and measurement error (σe), and a three-parameter secretory-burst waveform (β1, β2, β3). Model selection is performed to distinguish among the candidate pulse-time sets using the Akaike information criterion. Observed interpulse intervals are described by a two-parameter Weibull process (more general form of a Poisson renewal process). The parameters (and units) are frequency (number of bursts per unit time, lambda of Weibull distribution), regularity of interpulse intervals (unitless gamma of Weibull), fast half-life (min), slow half-life (min), basal and pulsatile secretion rates (concentration per unit time), mass secreted per burst (concentration), and waveform mode (time delay to maximal secretion after burst onset, min).

Approximate entropy ApEn, is a scale- and model-independent univariate regularity statistic used to quantify the orderliness (subpattern consistency) of serial stationary measurements (15). Mathematical models and feedback experiments establish that pattern orderliness monitors feedback and/or feedforward interactions within an interlinked axis with high sensitivity and specificity, both >90%. Cross-ApEn (X-ApEn) is a scale- and model-independent two-variable regularity statistic used to quantify the relative pattern synchrony of coupled time series. Clinical experiments establish that changes in two-hormone synchrony monitor feedback and/or feedforward adaptations within an interlinked axis with high sensitivity and specificity (16). Lower X-ApEn scores denote greater pattern synchrony between (coordinate control of) the interlinked signals. Reduced regularity of hormone secretion typifies puberty, aging, diminished negative feedback due to target-gland failure, fixed exogenous stimulation, and autonomous neuroendocrine tumors (17, 18).

Diurnal rhythmicity Nyctohemeral variation of TSH concentrations was determined by a nonlinear unweighted least-squares cosine regression. Ninety-five percent statistical confidence intervals were determined for the 24-h cosine amplitude (50% of the zenith–nadir difference), mesor (rhythmic mean), and acrophase (clock time of maximal value).

Statistical analysis Data are presented as mean ± S.E.M., unless otherwise specified. Comparisons of means were made using ANOVA. Post-hoc testing was done by using selected contrasts according to the a priori hypothesis. Relationships between variables were analyzed by linear regression techniques. Logarithmic transformation of the data was applied when required. Statistical calculations were performed with Systat software, version 11 (Systat Inc., San Jose, CA, USA). Significance level was set at 0.05.

Results

Mean serum TSH concentration profiles of the patients and controls are displayed in Fig. 1, showing diminished TSH concentrations in the two hypercortisolemic patient groups and increased concentrations in the patients in remission. Individual TSH profiles in a representative patient of each group and a control subject, showing the overall pattern with its secretory bursts are shown in Fig. 2.
Results of the deconvolution analysis are displayed in Table 1. Because there were no gender differences in TSH secretion parameters calculated by deconvolution, ApEn and cosinor regression, comparisons between patient groups and controls were made only by disease classification. Pulse frequency was similar in all patient groups, and cortisol excess had no effect on estimated TSH half-lives. In addition, the secretion burst shape (waveform) was similar in patients with active disease and those in remission and controls, as indicated by the mode. The regularity of interburst intervals was also unchanged, as demonstrated by the Weibull gamma results. Pulsatile (and total) TSH secretion was decreased to a similar degree in both forms of hypercortisolism, and this was mediated by a diminished pulse mass. In contrast, TSH secretion was increased in patients in remission due to amplified basal (nonpulsatile) secretion (Table 1).

Detailed results of the deconvolution analysis of daytime and nighttime TSH secretion are shown in Fig. 3. In all patient groups and control subjects, TSH secretion was larger during the night than during daytime. Furthermore, daytime TSH secretion was diminished in patients with pituitary-dependent hypercortisolism and primary adrenal hypercortisolism (P=0.006) compared with healthy controls and increased in patients after successful pituitary surgery (P=0.002). TSH secretion during nighttime was decreased in hypercortisolemic patients compared

Figure 2 Representative serum TSH and cortisol profiles in one patient with pituitary-dependent hypercortisolism, one patient with a cortisol-secreting adrenal adenoma, one patient after curative pituitary surgery, and one healthy control volunteer.
with controls (P < 0.0001) but unchanged in cured patients (P = 0.13).

The regularity of subordinate patterns of TSH as assessed by ApEn was decreased in patients with active disease (Table 2). Secretory regularity of cortisol was greatly diminished in patients with active disease, but in patients in remission ApEn was similar to that of controls. X-ApEn between cortisol (leading) and TSH was increased in patients with active disease, denoting loss of pattern synchrony of the hormone secretion. Cortisol-TSH X-ApEn and TSH ApEn in controls and hypercortisolemic patients were decreased, respectively. A significant negative linear regression was found between the serum-\( \mathrm{T}_4 \) concentration and log-transformed cortisol secretion (\( R = -0.53, P = 0.0017 \)). \( \mathrm{T}_4 \) levels were decreased in patients with hypercortisolism (P.Cushing 1.41 ± 0.14 nmol/l, adrenal adenoma 1.38 ± 0.07 nmol/l, controls 1.60 ± 0.04 nmol/l, P = 0.02), and normal in surgically treated patients (1.76 ± 0.23 nmol/l, P = 0.53).

**Discussion**

This study indicates that cortisol excess, independently of cause, blunts 24-h TSH secretion by reducing pulsatile secretion via decreased pulse amplitude. TSH pulse frequency and interpulse variability remained unchanged in hypercortisolemic patients. However, TSH secretory regularity was decreased (increased ApEn) in patients with hypercortisolism. Furthermore,
a significant diurnal TSH rhythm was present in all patients with hypercortisolism, but with a diminished mesor (mean) and amplitude and a delayed acrophase (time of maximum). In contrast, patients in remission after selective pituitary surgery exhibited amplified TSH secretion during daytime due to increased basal secretion. Secretory regularity of TSH was diminished in hypercortisolemic patients and normal in patients cured by transphenoidal surgery. These observations reveal alterations in the hypothalamus–pituitary–thyroid axis in active Cushing’s syndrome.

As far as we are aware, this is the first large study to assess spontaneous TSH secretion in patients with active Cushing’s syndrome and after successful treatment compared with healthy controls. Another TSH profile study in pituitary-dependent hypercortisolism, comprising three patients, reported decreased pulsatile TSH secretion with different analytical techniques (19). Our findings are also in keeping with previous reports documenting decreased efficacy of TRH in inducing TSH release in patients with hypercortisolism and in healthy subjects after glucocorticoid administration, and suggesting a relationship between the degree of glucocorticoid excess and impairment in the TSH response (10–12). Indeed, a single dose of glucocorticoids (1–2 mg dexamethasone) causes an acute decrease in pulsatile TSH production in healthy men (9). Even a mild elevation of serum cortisol concentrations (about 32%) induced by timed cortisol infusions decreases pulsatile TSH secretion by 50% (20).

Circulating TSH concentrations are the net result of concerted influences of prior and ongoing hormone secretion, distribution, and elimination. TSH half-lives were similar in patients and controls, and therefore, the present analysis suggests that unless the distribution volume changed, cortisol directly or indirectly modulates TSH release by the thyrotropes. More particularly, TSH secretion was diminished in hypercortisolism, irrespective of etiology and normal in patients in remission.

Various factors regulate TSH secretion by the pituitary gland. TSH synthesis and secretion are primarily controlled by the stimulatory action of TRH and the negative feedback restraint by thyroid hormones (T4 and T3), whereas other factors, including leptin, dopamine, and somatostatin modulate release (1, 6–8, 21). Dopamine has dual actions on TSH secretion. It inhibits TSH synthesis and release through D2R activation of the thyrotropes in the pituitary gland and, in addition, stimulates TRH secretion by cognate neurons in the PVN (22).

| Table 2 Approximate entropy and diurnal properties of serum cortisol and TSH concentration series in patients with hypercortisolism, patients after curative treatment, and healthy control volunteers. |
|---------------------------------|-------------------|-------------------|-------------------|-------------------|
| | P.Cushing | Adrenal adenoma | Pituitary surgery | Controls |
| | (n=16) | (n=11) | (n=7) | (n=27) |
| ApEn cortisol (ApEn) | 1.450 ± 0.058§ | 1.463 ± 0.109§ | 1.039 ± 0.085 | 0.896 ± 0.031 | <0.0001 |
| ApEn TSH (ApEn) | 1.128 ± 0.049§ | 1.175 ± 0.060† | 1.027 ± 0.111 | 0.889 ± 0.045 | 0.002 |
| X-ApEn (mU/l) | 1.746 ± 0.077§ | 1.777 ± 0.105§ | 1.347 ± 0.097 | 1.124 ± 0.039 | <0.0001 |
| Mesor (mU/l) | 0.72 ± 0.10§ | 0.64 ± 0.12‡ | 2.07 ± 0.41‡ | 1.33 ± 0.10 | <0.0001 |
| Amplitude (mU/l) | 0.13 ± 0.01§ | 0.15 ± 0.04‡ | 0.67 ± 0.15 | 0.52 ± 0.05 | <0.0001 |
| Acrophase (h, min) | 0605 ± 75§ | 0710 ± 80† | 0236 ± 30 | 0240 ± 15 | 0.0015 |

Data are given as mean and s.e.m. Logarithmic transformation of the data was performed when the variation of the groups was not homogenous. Significance levels versus controls: †P < 0.01; ‡P < 0.001; §P < 0.0001. P.Cushing: Morbus Cushing.
Several in vitro studies in the last decades have suggested that part of the inhibitory effect of glucocorticoids on TSH secretion may be mediated via a direct effect on the pituitary gland (12, 23). Subsequent studies have identified a role for annexin 1 (lipocortin 1), a protein produced by the pituitary folliculostellate cell, which acts as a paracrine mediator of acute regulatory effects of glucocorticoids, not only for TSH secretion but also for the secretion of ACTH, prolactin, and luteotropic hormone (24–27). Glucocorticoid-suppressive effects on TSH secretion in the human, either in acute experiments in volunteers or in patients suffering from chronic glucocorticoid excess, are fully in agreement with these experimental studies, particularly because a direct pituitary-inhibitory effect would not necessarily change the pulse frequency or half-life of TSH.

Somatostatin directly inhibits TSH secretion by activation of the SST2 and SST5 receptor subtypes expressed on the thyrotrope (28). Several animal studies have demonstrated that glucocorticoids rapidly increase hypothalamic somatostatin mRNA and somatostatin release (29–31). Thus, increased somatostatin signaling to the thyrotripe during amplified glucocorticoid effects could inhibit TSH secretion. There is also experimental evidence that glucocorticoids can modulate TRH release by the PVN, which contains glucocorticoid receptor-immunoreactive TRH neurons (32–34). Glucocorticoid administration induces long-lasting inhibition of TRH secretion in rats and the expression of TRH mRNA (35, 36).

Short-term high-dose dexamethasone administration in man decreases serum T3 and increases rT3 concentrations, an effect attributed to the inhibition of type I deiodinase in the liver (37). Whether hypercortisolism in the human (or experimental animal) can affect the other types of tissue-specific deiodinase has not been studied in detail. The decreased serum T3 values that we observed in the hypercortisolemic patients suggest decreased peripheral tissue conversion of T4 into T3, but unfortunately we have no data on serum rT3 concentrations. In addition, it is not known whether possibly altered intrapituitary thyroid hormone metabolism may lead to diminished TSH secretion (38, 39). The relative contribution of each of these mechanistic pathways to the inhibitory effect of glucocorticoids on TSH secretion is unknown.

Secretory process regularity as measured by ApEn monitors neuroendocrine feedback and feedforward signaling strength in humans (15, 16). On these grounds, increased TSH secretory restraint, as inferred here in hypercortisolemic patients should lead to decreased ApEn, i.e. increased pattern regularity. Conversely, more irregular hormone secretion patterns arise commonly from tumors, as demonstrated by highly irregular ACTH and cortisol secretion in pituitary-dependent hypercortisolism, irregular GH secretion in GHRH-secreting neuroendocrine tumors, and GH and TSH secretion in active acromegaly (40–42). Feedback signaling by tumor-driven cortisol patterns was irregular, as quantified by grossly elevated cortisol ApEn. Therefore, we hypothesize that endogenous somatostatin/annexin-1 release, putatively induced by irregular cortisol secretion, might also be less well coordinated with respect to pituitary TSH release. X-ApEn analysis supports, but does not directly prove this reasoning.

A remarkable finding of the diurnal TSH rhythm in our patients was the delayed acrophase during hypercortisolism, which was normal in patients after pituitary surgery. However, from this isolated result, it is not justified to conclude that the circadian pacemaker was reset by hypercortisolism per se, without simultaneously investigating other robust rhythms, for instance the 24-h temperature rhythm under constant environmental conditions.

An unexpected finding was increased daytime TSH secretion in treated patients. In patients with Addison’s disease, spontaneous TSH secretion is twofold amplified during acute withdrawal of hydrocortisone replacement and decreased twofold after 5 days of high-dose glucocorticoid replacement (43). At the same time, we should point out that we cannot exclude the possibility that some of the patients in the latter small cohort suffered from subclinical hypocortisolism.

In the face of diminished TSH secretion during hypercortisolism, one would expect decreased serum T4 concentrations. Indeed, levels were decreased in patients with hypercortisolism, although in all cases serum fT4 concentration levels remained within normal limits as reported in other studies (11, 12, 19). A possible explanation for the relatively normal T4 concentrations during hypercortisolism is that the biological activity of TSH was increased by altered posttranslational processing of the oligosaccharide chains of the TSH molecule (44).
In summary, this study in euthyroid patients with untreated hypercortisolism discloses a negative correlation between specific dynamic features of TSH secretion and cortisol production. We propose that increased endogenous somatostatin and intrapituitary annexin-1 feedback are mechanisms responsible for this observation, although this view certainly does not exclude other considerations, such as decreased TRH action and secretion or increased dopaminergic outflow.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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