Thyrotropin Secretion Profiles Are Not Different in Men and Women

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Context: The hypothalamo-pituitary-thyroid axis in women may differ from that in men. Previous reports have shown an almost 2-fold increased response to TRH in females compared with males.

Objective: We analyzed TSH profiles in healthy men and women to delineate differences in the hypothalamo-pituitary-thyroid system.

Subjects and Intervention: The subjects, 24 men (mean age 44 ± 3 yr) and 22 women (mean age 42 ± 3 yr) underwent a 24-h study with blood sampling intervals of 10 min. Premenopausal women were investigated in the early follicular phase of the cycle.

Methods: Serum TSH concentration profiles were analyzed with a newly developed automated deconvolution program, approximate entropy, and cosinor regression.

Results: Basal and pulsatile TSH secretion, and also pulse frequency, hormone half-lives, and secretory mode were indistinguishable in the two genders. There were no differences in diurnal variation, and the times of maximal secretion coincided. Approximate entropy, reflecting secretory regularity, was not different between men and women. In women but not men, TSH secretion was dependent linearly on age.

Conclusions: TSH secretion is gender invariant and depends on age in women only. (***J Clin Endocrinol Metab*** **94: 3964–3967, 2009)**

The major regulators of TSH secretion are TRH, the inhibitory neurotransmitters dopamine and somatostatin, and negative feedback by T_4 and T_3 (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. Thyroid hormone secretion is tightly regulated and is essential for energy homeostasis and basal heat production (2). In this light it is not surprising that hypothalamic centers involved in the control of energy balance also exert effects on the paraventricular nucleus, which contains TRH-secreting neurons (3). One of the important metabolic signals modulating the activity of the hypothalamo-pituitary-thyroid axis is leptin, which exerts a stimulating effect on TRH synthesis and release, both directly and indirectly via proopiomelanocortin/cocaine-and-amphetamine-related transcript-expressing neurons of the arcuate nucleus (4).

Regulation of the hypothalamo-pituitary-thyroid axis may be different between men and women. Administra-

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Abbreviations: ApEn, Approximate entropy; BMI, body mass index.

tion of TRH causes a dose-dependent increase in serum TSH concentration, which is almost 2-fold higher in women than men (5). On the other hand, all studies in healthy individuals report (slightly) higher (free) T_4 concentrations in women than men (6, 7).

To test the hypothesis that TSH secretion differs in women and men over the 24-h cycle, especially during the nocturnal surge, we measured 24-h serum TSH profiles in healthy subjects over a wide age range. TSH secretion was quantitated using a new, operator-independent deconvolution method, and secretory regularity and diurnal variation were calculated with approximate entropy and cosinor regression.

Subjects and Methods

Subjects

Forty-six healthy subjects, 24 men [mean age 44 ± 2.5 yr, body mass index (BMI) 24.6 ± 0.8 kg/m^2] and 22 women (mean age 42 ± 3.1 yr, BMI 23.1 ± 0.7 kg/m^2) were recruited through advertisements in local newspapers. All subjects underwent full medical screening, including physical examination and standard laboratory chemistry. Special attention was given to the possible presence of (familial) thyroid disease and daily iodine intake. Acute or chronic disease, depression, head trauma, smoking, alcohol abuse, recent transatlantic flights, night-shift work, and use of medication were exclusion criteria.

Clinical protocol

The protocol was approved by the medical ethics committees of the universities and was performed according to the Helsinki declaration. Volunteers gave written acknowledgment of informed consent for participation. Premenopausal women were sampled in the early follicular stage of their menstrual cycle, and this was confirmed by estradiol and progesterone levels. Subjects were admitted to the clinical research centers of the universities at 0700 h, and 10-min sampling started at 0900 h. No daytime naps were allowed. 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 off between 2300 and 0730 h.

Assays

TSH concentrations were measured with a time-resolved immunofluorometric assay (Wallac Oy, Turku, Finland) calibrated against the World Health Organization second standard international reference preparation (80/558) human TSH. The limit of detection was 0.05 mU/liter and the interassay coefficient of variation was less then 5%. Free T_4 concentrations in the patients studied in Leiden were estimated using electrochemoluminescence immunoassay (Elecsys 2010, Roche Diagnostics Nederland BV, Almere, Netherlands) and in Amsterdam with a time-resolved immunofluorometric assay (Wallac Oy). The interassay coefficient of variation was 5–8% and the detection limit 2 pmol/liter.

Calculations and statistics

Deconvolution analysis

Each hormone concentration time series was analyzed using a recently validated deconvolution method (8). The automated Matlab program (The Mathworks, Inc., Natick, MA) first detrends the data and normalizes concentrations to the unit interval (0, 1). Second, successive potential pulse-time sets, each containing one fewer burst, are created by a smoothing process (a nonlinear adaptation of the heat diffusion equation). Third, a maximum-likelihood expectation deconvolution method estimates all secretion and elimination rates simultaneously for each candidate pulse-time set. 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 (minutes), slow half-life (minutes), 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, minutes).

Approximate entropy (ApEn)

ApEn is a scale-and model-independent univariate regularity statistic used to quantitate the orderliness (subpattern consistency) of serial stationary measurements (9). 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 greater than 90%. Reduced pattern regularity typifies hormone secretion in puberty and aging, during diminished negative feedback or fixed exogenous stimulation, and by autonomous neuroendocrine tumors (10).

Diurnal rhythmicity

Nyctohemeral variation of TSH concentrations was determined by a nonlinear unweighted least-squares cosine regression.

Statistics

Data are presented as mean ± SEM unless otherwise specified. Comparisons of means of groups were made with the Student's *t* test. Relations between variables were explored with regression techniques. Statistical calculations were performed with Systat software, version 11 (Systat Inc., San Jose, CA). Significance level was set at 0.05.

Results

Mean serum TSH concentration profiles are displayed in Fig. 1 and show slightly higher levels during the night in men than women. Mean 24-h TSH concentration in women was 1.42 ± 0.13 mU/liter and in men 1.51 ± 0.12 mU/liter ($P = 0.61$) and correlated with free T_4 concentration ($R = 0.51$, $P = 0.0007$).

Pulse frequency (17.8 ± 1.2 per 24 h in men and 16.3 ± 1.1 in women), rapid and slow hormone half-lives estimated with a slow fraction of 0.32 (21.3 ± 1.5 and 102 ± 3.9 min in men and 17.8 ± 1.2 and 104 ± 4.3 min in women), secretory burst wave-form (mode) (16.9 ± 1.7 min in men and 21.5 ± 2.9 min in women), basal secretion (11.6 ± 1.7 mU/liter in men and 13.4 ± 1.4 mU/liter in women), and pulsatile secretion (19.7 ± 2.8 mU/liter in men and 16.3 ± 1.8 mU/liter in women) as well as total 24-h secretion (30.7 ± 2.6 mU/liter in men and 29.7 ± 3.1

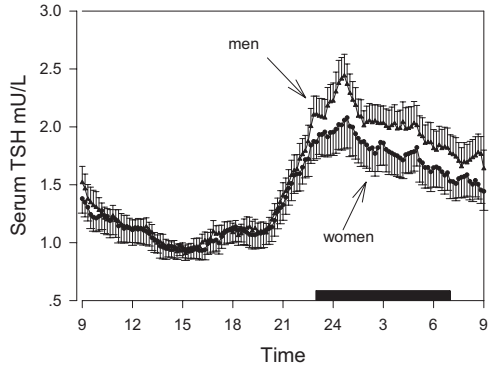

FIG. 1. Twenty-four-hour serum TSH concentration time series in 24 healthy men and 22 healthy women. Blood samples were taken every 10 min for 24 h. Blood sampling started at 0900 h. Lights were off between 2300 h until 0730 h next morning. Data are shown as the group mean and SEM.

mU/liter in women) were similar in both genders. Deconvolution analysis, restricted to the dark period also did not disclose a gender difference (*viz.* total TSH secretion during this period was 14.8 mU/liter in women and 15.7 mU/liter in men, $P = 0.66$). The increase in TSH secretion during the dark phase was caused by amplified basal secretion (5.40 ± 0.35 mU/liter to 9.46 ± 0.77 mU/liter, $P < 0.0001$) and increased pulsatile secretion (3.08 ± 0.39 mU/liter and 5.79 ± 0.44 mU/liter, $P < 0.0001$) without any significant change in secretion mode, pulse frequency, or hormone half-lives, and the increase was also gender independent.

The regularity of subordinate patterns of TSH as assessed by ApEn was similar in men and women (0.819 ± 0.042 *vs.* 0.866 ± 0.0048, respectively) and was age and BMI invariant.

All subjects had a significant diurnal TSH rhythm. The mesor (1.54 ± 0.11 mU/liter in men and 1.36 ± 0.13 mU/liter in women) and amplitude (0.60 ± 0.07 mU/liter in men and 0.51 ± 0.06 mU/liter in women) were similar in both sexes. The acrophases almost coincided in men and women (0254 h ± 19 min in men, 0300 h ± 11 min in women).

Twenty-four hour TSH secretion correlated positively with age in women but not men (Fig. 2). Fasting morning serum free T_4 concentrations were 14.5 ± 0.7 nmol/liter in women and 14.4 ± 0.4 nmol/liter in men (P = NS).

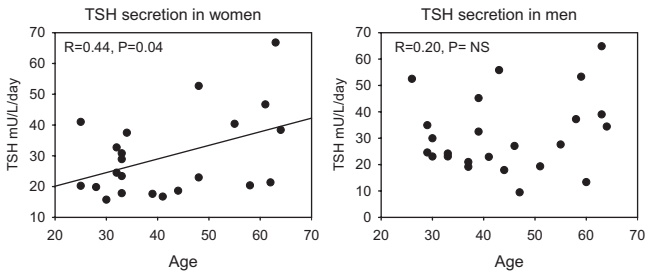

FIG. 2. Relationship between the daily TSH secretion rate and age in healthy women (*left panel*) and men (*right panel*).

No differences between the two centers were present with respect to the gender distribution, outcome of TSH deconvolution analysis, or T_4 concentrations.

Discussion

This study indicates that 24-h TSH secretion in adults is gender invariant. Furthermore, there were no differences in the diurnal rhythms, and the phase setting of TSH secretion was almost identical in both sexes. In addition, the secretory regularity was similar in men and women. Finally, TSH secretion was positively related to age in women but not men.

So far as we are aware, this is the first large clinical study to assess the influence of gender on spontaneous diurnal TSH secretion in healthy subjects. Previous 24-h blood sampling studies, with limited number of subjects analyzed with different less advanced statistical tools, did not disclose or investigate gender differences (11–14). Population survey studies on TSH levels in healthy individuals yielded conflicting results, *viz.* higher TSH levels in women than men (6, 8) or no gender differences (15, 16). On the other hand, thyroid hormone levels (T_4 and free T_4) in healthy subjects report (slightly) higher thyroid hormone concentrations in women (6, 7, 15, 16). It is not surprising that for the present study with the limited number of subjects, this gender difference was not demonstrable.

Other clinical studies report a larger increase in TSH after TRH in women than men (5). Mechanisms involved in this amplified TSH response include increased sensitivity of TSH to TRH, decreased hypothalamic somatostatin secretion, or diminished dopamine tonus in the female gender. Diminished central somatostatin tonus is in accordance with the observation of a higher GH secretion rate in women than men (17). On the other hand, the physiological relevance of the TRH test may be questioned by the pharmacological nature of the dose. The lack of the gender difference in spontaneous TSH secretion under physiological conditions suggests that the physiological balance between stimulatory and inhibitory effects on TSH secretion is gender invariant. This conclusion is corroborated by the finding of comparable ApEn in males and females, which monitors neuroendocrine feedback and feedforward signaling strength in humans (9, 10). In contrast to these finding, blood sampling studies in the rat have shown less TSH secretion by female than male rats, lower T_4 levels, and higher T_3 concentrations (18). The latter study also demonstrated that the diurnal TSH variation is lost after lesioning of the suprachiasmatic nucleus and that functional connections between this nucleus and the thyroid gland exist, which independently regulate thyroid hormone secretion, and therefore also feedback onto TSH.

A remarkable finding in this study was the relation between age and TSH secretion in women. The National Health and Nutrition Examination Survey III study found a correlation between age and TSH in both genders (6), but other studies reported no influence of age (8, 15, 16). Although the influence of body composition on diurnal TSH secretion has not been studied extensively, in two analyses obese women secreted more TSH than normal-weight, age-matched controls. This difference decreased after weight reduction or bromocriptine administration (19, 20). To date TSH secretion in obese men has not been studied similarly. In addition, we did not investigate the influence of the menstrual cycle *per se* on TSH secretion. Thus, our conclusions apply more particularly to women having a low estradiol concentration.

In summary, this study in healthy control subjects did not disclose a gender effect on TSH concentrations, pulsatile or basal secretion, pattern regularity, or diurnal release A significant positive covariate of TSH secretion was age in women.

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