

## Thyrotropin Secretion Patterns in Health and Disease

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**Thyroid hormones are extremely important for metabolism, development and growth during the life time. The hypothalamo-pituitary-thyroid (HPT) axis is precisely regulated for these purposes. Much of our knowledge of this hormonal axis is derived from experiments in animals and mutations in man. This review examines the HPT-axis particularly in relation to the regulated 24-hour serum TSH concentration profiles in physiological and pathophysiological conditions, including obesity, primary hypothyroidism, pituitary diseases, psychiatric disorders and selected neurological diseases. Diurnal TSH rhythms can be analyzed with novel and precise techniques, e.g. operator-independent deconvolution and approximate entropy. These approaches provide indirect insight in the regulatory components in pathophysiological conditions.**

### I. Introduction

**T**HE PITUITARY GLAND plays a key role in the regulation of many important hormonal systems, e.g., the corticotrope-adrenal complex, the thyrotrope-thyroid unit and the gonadotrope-gonadal system. In addition, other pituitary hormones act directly on various peripheral organs, e.g., growth hormone (GH), prolactin, oxytocin and vasopressin. After the important discovery of the radio-immunoassay by Solomon Berson and Rosalyn Yalow in 1959 (1), for which they received the Nobel Prize in 1977, investigators were able to measure hormones repeatedly over time in the blood of the human. Data seemed to indicate that each pituitary hormone had its specific secretion pattern, depending on age, gender, sleep-wake cycle and body composition. A further step in understanding how systemic hormone rhythms are regulated was the discovery that the hypothalamus is involved in the regulation of the pituitary gland by secreting distinct agonists and antagonists into the pituitary portal blood system, acting on specific cell clusters in the pituitary gland. The first of these isolated neuropeptides was the agonist thyrotropin-releasing hormone (TRH), TRH before 1970 (2). Somatostatin, an antagonist was discovered shortly thereafter. Conversely, peripheral (non-CNS) signals from the body, acting on the hypothalamus and the pituitary gland

modify pituitary hormone secretion, leading to complex secretion patterns in the blood.

Significant knowledge of pituitary hormone regulation has been obtained by analyzing 24-h hormone rhythms under various conditions, including fasting, changes in the wake-sleep cycle, puberty, menstrual cycle, age and in diseases such as acromegaly, Cushing's disease and polycystic ovary syndrome (3–5). New developments in experimental and analytical approaches include methods of modulating feedforward or feedback signals by drugs and construction of the endogenous dose-response curve (6–8). Earlier reviews on TSH have not encompassed these issues. The purpose of this review is to summarize the current knowledge of regulated 24-h secretion patterns of thyroid stimulating hormone, or thyrotropin (TSH) obtained during the last 3 decades. The goals are (i) to describe the TSH pattern in normal, healthy subjects, and analyze the influences of gender, age, body composition and sleep-wake cycle, and the effects of some drugs on this system; (ii) explore TSH rhythms in major endocrine disorders, including hypothyroidism, hypercortisolism, acromegaly and, prolactinoma, and also in the low T3 syndrome and diabetes mellitus; and (iii) highlight TSH patterns in selected nervous system disorders and major psychiatric illness. In addition, we identify syndromes and diseases in which the TSH rhythm remains to be studied in detail. To this end the functional aspects of the thyro-

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Abbreviations:

trophic axis will be discussed first briefly, before reviewing the literature on regulated TSH secretion.

### Ila. Thyroid hormone regulation

Precise regulation of circulating and intracellular concentrations of thyroid hormones (thyroxine and triiodothyronine) is essential for the control of many metabolic processes, heat production, physical development, weight maintenance, cell differentiation and growth (9). The prime stimulatory hormone for the thyroid is thyrotropin (TSH), with numerous secondary modulators, including insulin-like growth factor type I (IGF-I), inflammatory cytokines (IL-1, IL-6, and TNF- $\alpha$ ), and iodide availability (10). TSH in turn is under the stimulatory control of bilaterally represented paraventricular TRH neurons (TRH), the inhibitory neurotransmitters, dopamine and somatostatin, and negative feedback by thyroxine and triiodothyronine (11–13). The dose-dependent time-varying interplay among these regulators dictates 24-h TSH secretion patterns. Patterns are characterized by a day-night (nycthemeral) variation of serum TSH concentrations with superimposed (small) bursts. The active hypothalamic tripeptide TRH is processed from prepro-TRH in a subset of neurons in the paired paraventricular nuclei (PVN). TRH neurons project on the external median eminence, where nerve terminals secrete TRH into the portal blood-vessel system (14–16). TRH is essential for proper functioning of the TSH-thyroid complex. Thus, human TRH-receptor mutations and murine mutations of the TRH gene both lead to (central) hypothyroidism (17, 18). TRH neurons in the PVN receive direct neuronal input from the arcuate nucleus, including axons of cell bodies synthesizing proopiomelanocortin and cocaine-and-amphetamine-related transcript (POMC/CART) and agouti-related peptide and neuropeptide Y (AGRP/NPY). These metabotropic neurons, which express leptin receptors and are crucial for energy homeostasis and food intake, are intimately connected to hypophysiotropic TRH neurons of the PVN (19–21). In addition, TRH cells receive input from ascending catecholamine neurons of the brain stem (22).

Feedback signaling by thyroid hormones on TRH gene transcription and peptide synthesis is also important. Studies in the rat have shown that thyroid hormone feedback on TRH is regulated via local intracellular conversion of thyroxine (T4) into triiodothyronine (T3) by type 2 deiodinase in the tanycyte, a specialized glial cell, lining the third ventricle (20, 23–25). Thus deiodinase-2-receptor knockout mice have inappropriately elevated TSH concentrations in the face of increased T4 concentrations (26). Furthermore, the monocarboxylate-8 (MCT8) and MCT10 transporters are involved in the cellular uptake

and thereby translocation of T3 to the nuclear receptor in the PVN and other sites (27, 28). In the genetic absence of these transporters, mice have normal TSH levels in spite of high T3 concentrations. Patients suffering from comparable mutations were found in more than 45 families and they have severe psychomotor retardation. T4 and freeT4 levels are low, TSH levels normal to elevated and T3 and free T3 levels are high. The phenotype of this syndrome was first described by Allan, Herndon and Dudley (29–32).

Various factors regulate TSH secretion by the pituitary gland [Figure 1]. TSH synthesis and secretion are primarily controlled by the stimulatory action of TRH, as outlined above, and the negative feedback restraint by thyroid hormones (T4 and T3). Other factors, including leptin, dopamine and somatostatin modulate TSH as well as TRH release and/or synthesis (13, 19–21). Dopamine has dual actions, it inhibits TSH synthesis and release via dopamine-2 receptor (D2R) activation of the thyrotropes in the pituitary gland, and stimulates TRH secretion by cognate neurons in the paraventricular nucleus (33). An important metabolic signal, modulating the activity of the HPT axis is leptin, which exerts a stimulating effect on TRH synthesis and release, acting both directly on the thyrotropic part of the paraventricular nucleus and indirectly via the proopiomelanocortin/cocaine-and-amphetamine-related transcript (POMC/CART)-expressing neurons of the arcuate nucleus (20). Collectively, these pathways also inhibit appetite. A role for leptin was also suggested by studies in men. In adult subjects, the 24-h leptin and TSH rhythms were tightly coupled, and the secretion patterns are strikingly similar. However, in a homozygous leptin-deficient patient the TSH rhythm was disorganized, suggesting a functional role of leptin in the HPT axis (34). Comparable correlations between leptin and TSH profiles were obtained in short normal children (35).

Somatostatin directly inhibits TSH release by activation of the SST<sub>2</sub> and SST<sub>5</sub> receptor-subtypes expressed on the thyrotrope (13). Under the stimulatory action of TSH the thyroid gland secretes T4 and to a lesser extent T3. Most of the circulating T3 is derived from outer ring deiodination of T4 into T3 occurring in peripheral organs. Different types of deiodinating enzymes (D1, D2 and D3 deiodinases) are present in different organs, e.g., liver, brain and pituitary, and they are crucial for the production of the (locally) active hormone T3 and inactive metabolites (25, 31).

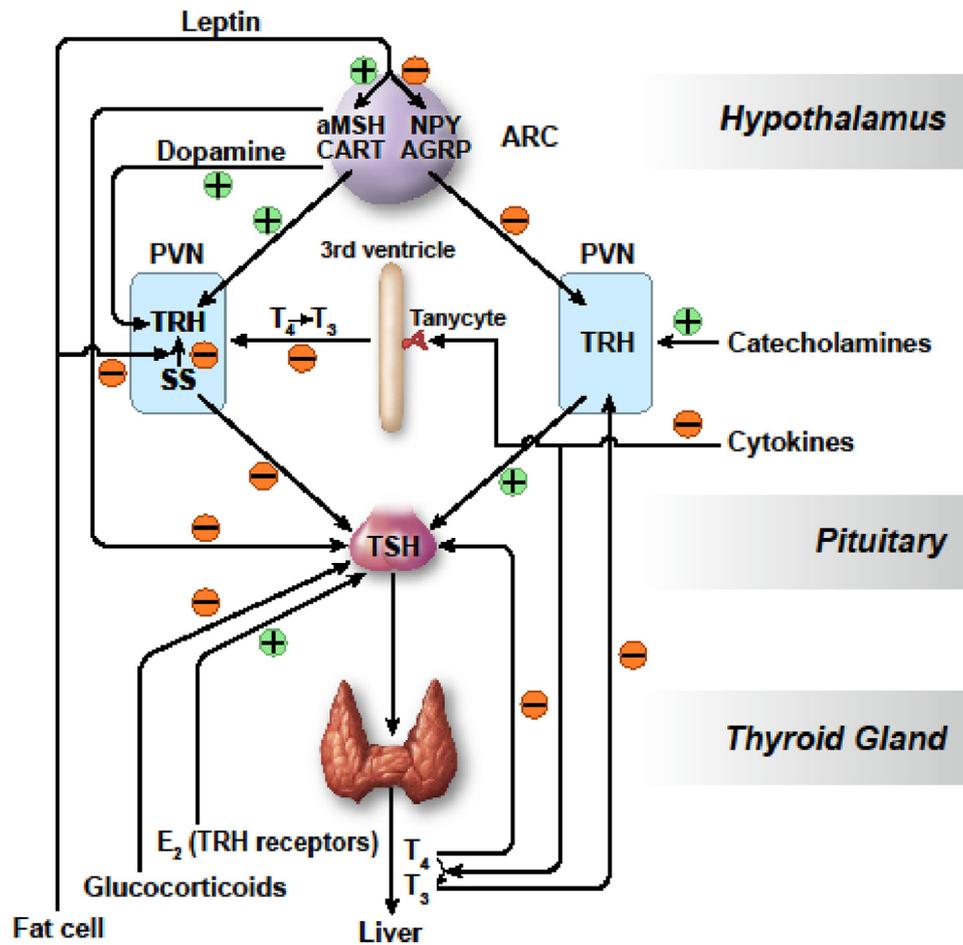
### Ilb. Analytical techniques used for the study of 24-h serum hormone profiles

#### *Pulse detection and secretion measurement programs.*

Early approaches to describing 24-h hormone patterns consisted of calculating the mean 24-h concentration, not-

Figure 1.

## Simplified Ensemble Regulation of TSH Secretion



Schematic drawing of the regulation of the hypothalamus-pituitary-thyroid axis. Abbreviations: ARC arcuate nucleus, PVN paraventricular nucleus, NPY neuropeptide Y, CART cocaine-and amphetamine-regulated transcript, AGRP agouti-related protein, E<sub>2</sub> estradiol, SS somatostatin, - inhibition, + stimulation (unpublished line drawing).

ing time and magnitude of the maximal and minimal values and visually identifying assumed peaks or pulses. A general approach to identifying the acrophase (time of maximal value) is cosinor analysis, which essentially is the nonlinear fitting of a sinusoidal function to the data series (36). Disadvantages of this procedure are that the time of minimal concentration (nadir) is definitionally 12 h out of phase with the acrophase, which is rarely observed, and that only a small part of the physiological pattern is so explained. Other methods have also been applied, including Fourier transformation with a 24 h and a 12 h component and the robust baseline fitting of van Cauter (37, 38). In order to explain a greater proportion of short-term secretion variability over time, objective pulse-detection programs were developed.

A pulse is an abrupt increase and subsequent decrease

in the intensity (size or amplitude) of serially measured output. In principle, the size, shape and spacing of pulses may regular or variable and the underlying baseline process may be fixed or drift gradually. Generally, hormone secretion patterns are not strictly regular or, given the existence of random inputs from multiple sources, such as missing data, outliers, measurement variability, biological variability, nonuniformity of successive secretory-burst amplitudes, shapes, and inconsistent pulse-by-pulse stimulus sensitization or facilitation. Estimation of hormone pulsatility is thus obscured by observational uncertainties, host variables, and stochastic aspects of the biological process.

Early methods of pulse analysis used an empirical threshold approach. A brief increase in hormone concentrations greater than that explicable by the intraassay vari-

ability was defined as a pulse. Examples are the Santen and Bardin method (threshold is a 20% increase in any concentration), Cluster analysis (a critical two-sample t-statistic is required for accepting significant upstrokes and downstrokes in a peak), and the regional coefficient of variation method, which employs local sample variance to test for peaks (39, 40). Limitations of these fixed-criterion methods include variable false-positive and false-negative errors introduced by unequal sampling density (e.g., 5-min vs. 10-min intervals), nonuniform pulse shape (abrupt vs. slow increase in concentration), sample outliers, and variable assay precision.

Semiempirical methods use nadir and/or baseline estimates to specify superimposed pulsatility, but lack the combination of primary *in vivo* experimental validation and direct mathematical proof. Desade, Ultra, Detect and Pulsar discriminate baseline or nadir concentrations by numerical filtering, baseline detrending or line segmentation criteria (38, 41, 42). The first three techniques incorporate secretion estimates, thus representing a deconvolution approach. The performance characteristics and limitations of the empirical and semiempirical methods are quite similar and extensively discussed in several reviews on this subject (43, 44).

Modern requirements for optimal pulse analysis include 1) high discriminative accuracy of signal detection (> 90%); 2) direct empirical validation *in vivo*, *in vitro* and by computer simulation; 3) mathematical verification of reproducible parameter estimates; 4) robustness to sampling schedule and assay type; 5) low sensitivity to occasional outliers; 6) automated implementation; 7) quantitation of relevant endpoints like elimination kinetics, basal secretion, pulse signal size (amplitude or mass), duration, shape and number; and 8) utilization of relevant physiological knowledge of the system (3).

Deconvolution methods that use *a priori* half-life estimates were developed first historically (45–48). Assuming a known half-life makes the deconvolution problem of calculating secretion parameters much easier. Several non-parametric and parametric methods exist in this category, including Detect, Pulse4 and Deconv (41, 49, 50). A serious limitation of these methods is hormone kinetics (fast and slow half-lives and their relative amplitude contribution) must be estimated validly and precisely in the actual physiological or pathophysiological context under study (e.g., age, gender, intervention type, disease, and body composition). The use of incorrect half-lives or a single half-life may seriously affect correct identification of the number of significant pulses, pulse mass, pulse shape, and basal secretion.

More recently developed deconvolution methods simultaneously assess endogenous half-lives along with

pulse number and the other secretion parameters. Models often include the following simplifying assumptions to limit overparametrization and hence favor a unique valid solution: 1) half-life and distribution volume do not change during the observation interval; 2) basal secretion is arbitrarily time-invariant (a fixed value), slowly varying or some lower bound (e.g., 5%) of all sample secretion rates; 3) pulses are instantaneous secretion events (delta function) or finite duration bursts of symmetric (Gaussian) or asymmetric shape (Gamma function); 4) candidate pulses are defined first, and the secretion and elimination parameters are estimated conditional on each set of possible pulse times and 5) stochastic contributions (random effects) enter into the observations and biological dynamics.

Earlier deconvolution methods did not always incorporate objective ascertainment of pulse number and locations. Potential pulses were added by the analyst according to empirical criteria. A putative pulse was ultimately retained if the calculated amplitude (maximal secretion rate) or mass (integral) was significantly nonzero on post hoc testing of individual or joint 95% statistical confidence intervals. As no formal criteria are available, this approach may lead incorrect results (3, 51, 52). A significant improvement is the application of automated maximum likelihood estimation-based deconvolution techniques. All parameters, including shape parameters, elimination parameters, basal secretion and random variability are simultaneously calculated for pulse-timing set. This method has been extensively validated with *in vivo* experiments in men and animals, as well with simulation techniques (53–55). It should be realized that these expensive and time-consuming methods are not necessary for diagnostic or therapeutic purposes, but only for detailed, quantitative (patho)physiological studies.

#### **Model-free evaluation of endocrine networks.**

Aside from quantifying hormone secretion, estimating half-life etc., another powerful tool to investigate hormone secretion is Approximate Entropy (ApEn). ApEn is a scale- and model-independent univariate regularity statistic used to quantify the orderliness (subpattern consistency) of serial stationary (nontrending) measurements (56, 57). 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% (58). Higher ApEn defines reduced regularity of hormone secretion, which in general typifies puberty, aging, diminished negative feedback due to target-gland failure, fixed exogenous stimulation, and autonomous neuroendocrine tumors (3, 59, 60).

### **Spikiness.**

Spikiness is defined as the ratio of the SD of the first-differenced series (successive subtraction  $n - (n-1)$ , when  $n =$  number of samples) to the SD of the original series, as suggested by Pincus (61). The intent is to quantify sharp, brief, staccato-like unpatterned fluctuations, which would denote brief system instability. This is important, because inferences are often reported after omitting (rather than calibrating) momentary outlier-like spike events.

In summary, during the last 3 decades when 24-h TSH sampling studies were used to investigate time-dependent changes in serum hormone concentrations, pulse frequency and secretory characteristics in physiology and pathophysiology, sensitivity and precision of TSH assays, and comittant mathematical tools to quantify significant pulses, half-lives, pulse shape and mass and basal secretion have undergone great advancements. The consequences are that data and conclusions reported in earlier papers, using less sophisticated techniques, may differ from those in more recent publications. This may be illustrated by a report on hypothyroid patients in which two different techniques were used to analyze the same data. The modern deconvolution method detected almost twice the number of significant TSH pulses as Desade or Cluster. In addition, percentage pulsatile TSH secretion was much lower by the automated deconvolution in severely hypothyroid patients than that specified by Cluster or Desade (62, 63). Other important and relevant variables, such as pulsing regularity, secretion mode (shape of the pulse) and half-lives could not be calculated by Desade or Cluster, highlighting the limitations of older techniques. Such observations motivate the continuing use of serial blood sampling, and application of validated techniques, for more detailed understanding of the HPT axis in health and disease.

## **III. TSH secretion in the healthy human**

### **III a. Normal adult subjects**

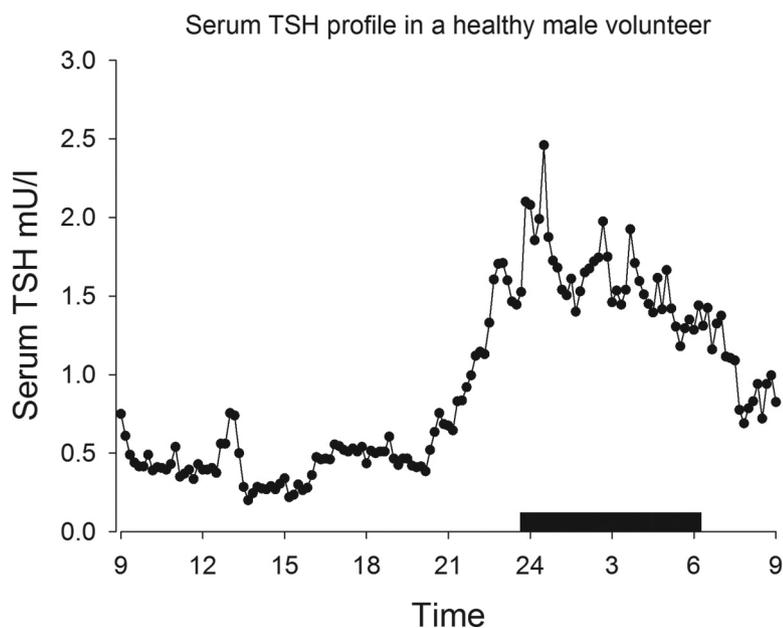
#### ***Circulating forms of thyrotropin.***

TSH is assembled and secreted as a heterodimer, consisting of a  $\alpha$ -chain, common to other pituitary glycoproteins, e.g., LH, FSH and chorionic gonadotropin, and a hormone-specific  $\beta$ -chain, responsible for binding to the receptor. The approximate molecular weight of TSH is 28 KD, but circulating forms have a higher molecular weight due to varying degrees of posttranslational glycosylation and sialylation of the  $\alpha$ - and  $\beta$ -chains, which in turn determine hormone half-life and biological potency (64). An important factor for TSH glycosylation in the pituitary is the strength of TRH signaling from the hypothalamus.

When TRH drive is decreased, for instance by hypothalamic damage, the biological activity of TSH is diminished, and conversely, increased TRH drive augments TSH bioactivity occurs in patients with thyroid hormone resistance (65, 66). Circulating forms of TSH are heterogeneous, caused by different degrees of glycosylation of the constituent subunits. In a series of elegant experiments Szkudlinski and colleagues concluded that sialylation of the  $\alpha$ -subunit positively determines TSH biological activity, measured by cAMP production in the Chinese Hamster Ovary (CHO) cell model and by T4 release in the rat, whereas increased sialylation of the  $\beta$ -subunit decreases TSH metabolic clearance in the rat (67). Persani found diurnal differences in TSH bioactivity assessed in the CHO model. The ratio of bioactivity to immunoactivity (B/I) was higher during the day than night, although not present in 4 of 7 subjects (68). In subsequent studies they established an inverse relationship between the B/I ratio and degree of sialylation. Particularly, in patients with primary hypothyroidism the degree of sialylation was increased, but the B/I ratio was similar during the day and night (69). The degree of glycosylation of TSH probably does not interfere with the immunological TSH assay, although no detailed data are available, but it may impact TSH clearance in men. Only limited data on TSH kinetics are available in animals and they cannot be applied confidently to humans (70–72). However, in normal healthy subjects we could not demonstrate differences in half-lives during the day and night (73). Moreover, in severely hypothyroid patients, we found a decreased endogenous TSH half-life, where animal data would predict an increased half-life (62). Therefore, kinetic studies with different TSH isoforms are needed in humans.

#### ***Normal serum TSH patterns.***

After the introduction of sensitive TSH assays, several groups studied the diurnal variation of serum TSH, generally by comparing mean levels during selected parts of the 24-h cycle or by Fourier analysis (74–82). Lowest TSH concentrations are observed during the afternoon, followed by a rise during the evening and a maximum in the first part of the night [Figure 2]. After the onset of sleep, TSH levels decrease, which is prevented by keeping the subject awake (83, 84) [Figure 3]. In addition to this nycthemeral pattern, repeated variably sized bursts of TSH release occur during the 24-h period as quantitated by multifrequency Fourier analysis (82), Cluster analysis or Desade (63, 82, 85–87). During the nocturnal TSH rise, burst frequency and amplitude increase as quantified with Cluster, Desade and Matlab-based deconvolution (53, 88). Nocturnal TSH pulses, quite the opposite of noctur-

**Figure 2.**

The 24-h serum TSH concentration profile in a healthy male volunteer aged 34 and with a BMI of 23 kg/m<sup>2</sup> (unpublished figure from data of Ferdinand Roelfsema).

nal GH pulses, correlate with reduced delta wave activity (slow wave deep sleep) (89).

#### **TSH bursts.**

Common to the secretory patterns of other pituitary hormones, TSH secretion is partially pulsatile and partial basal. Although large differences exist among different hormones, TSH secretion most resembles that of prolactin. How TSH pulses are generated is not known, in contrast to ACTH, GH and LH. A very elegant study by Samuels et al. convincingly demonstrated that continuous iv TRH infusions for 48 h at different doses does not alter the intrinsic TSH pulse frequency in normal men or in T4-replaced hypothyroid patients. Rather, TSH pulse amplitude is selectively amplified, more so in the substituted hypothyroid patients than in healthy subjects, since the latter group responded to increased TSH with enhanced T3 levels (90). Infusion experiments with somatostatin and dopaminergic drugs did also not lead to decreased pulse frequency (91, 92). Finally, infusions with naloxone inhibit TSH secretion, especially the TSH amplitude during the nocturnal surge, without impact on pulse frequency. T3 levels decreased by 21%. These experiments suggest that endogenous opioids have significant influence on TSH secretion, especially during the nocturnal surge (93). Collectively, these studies suggest that the TSH 'pulse generator' of which the anatomical and functional details are not known, is a robust and stable system under dif-

ferent conditions, in contrast to LH pulsing. However there is a small temporal increase in pulse frequency along with the marked increase in pulse amplitude during the nocturnal TSH surge, which mediates the diurnal variation alluded to above. Whether there is also a contribution to this surge by increased basal TSH secretion is uncertain since most analyses assume time-invariant basal TSH output.

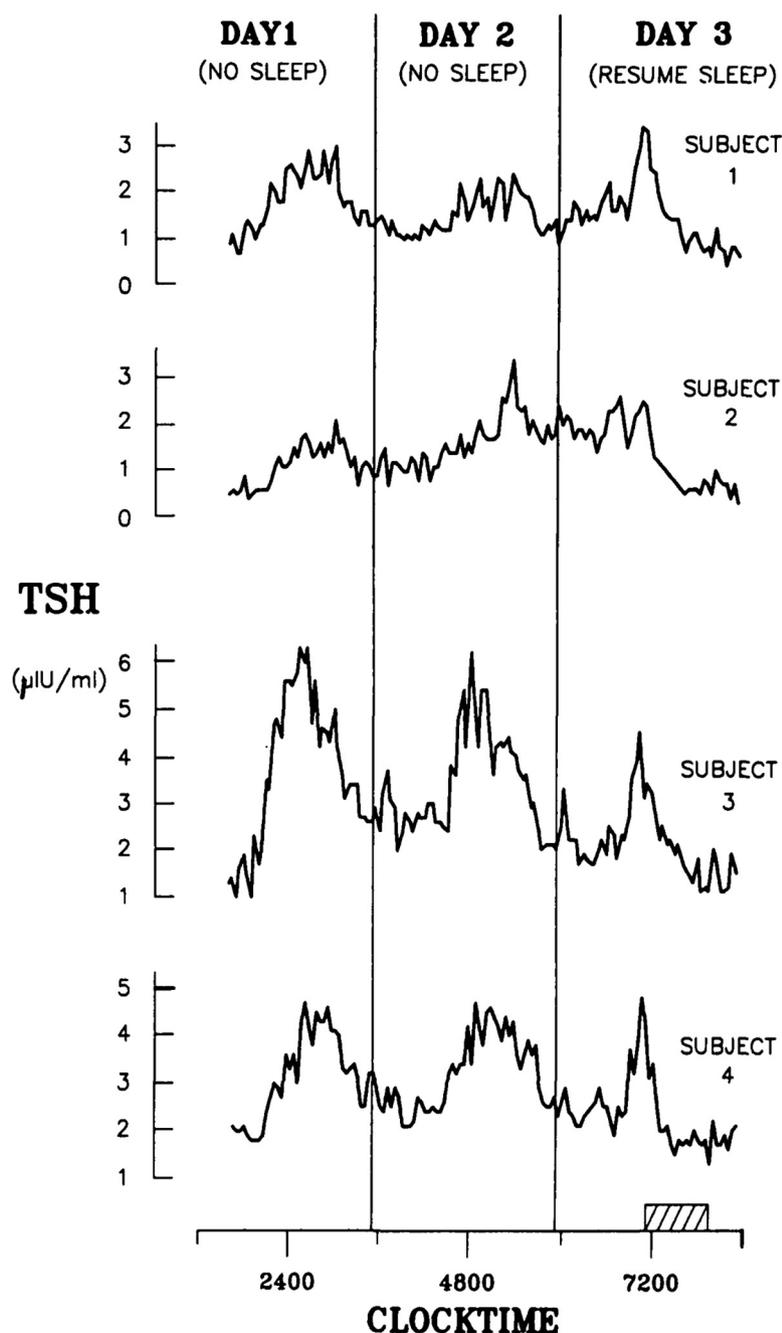
#### **Influence of gender.**

In the studies discussed above no gender differences were noted, although the size of the groups was generally small, thus limiting the statistical power. In a recent study analysis of 46 healthy individuals (22 men and 24 women, with ages ranging from 25 to 64 yr) pulsatile TSH secretion was gender-independent as assessed by way of a recently developed operator-independent deconvolution method applied to 10-min TSH samples over 24 h [Figure 4] (3, 53, 54, 73).

On the other hand in some, but not all studies in which pituitary TSH reserve was investigated with a near-maximal TRH dose, a larger increment was noted in women than men (94). Population surveys on using single sample TSH levels in healthy individuals have yielded conflicting results, viz higher TSH levels in women than in men (95, 96) or no gender difference (97, 98). Nonetheless, thyroid hormone levels (T4 and free T4) in healthy subjects are (slightly) higher in women than men (95–98), the exact basis for which is not known.

#### **24-h production rate of thyrotropin.**

TSH production rates in the human have also been measured with isotopic techniques. By single injection, calculated mean daily TSH secretion was 165 mU (99) and by isotopic equilibrium 104 mU (100). Operator-independent deconvolution analysis yielded the following results in 46 men and women: daily basal (nonpulsatile) secretion 12.5 ± 1.1 mU/liter distribution volume, daily pulsatile secretion 18.1 ± 1.7 mU/liter distribution volume, and daily total secretion 30.6 ± 2 mU/liter distribution volume (73). Deconvolution data are expressed as mass secreted per liter distribution volume. Estimating the latter as 6% of body weight (like the plasma volume) would yield a mean daily TSH production rate of 116 mU, a value close to data reported with other techniques mentioned above.

**Figure 3.**

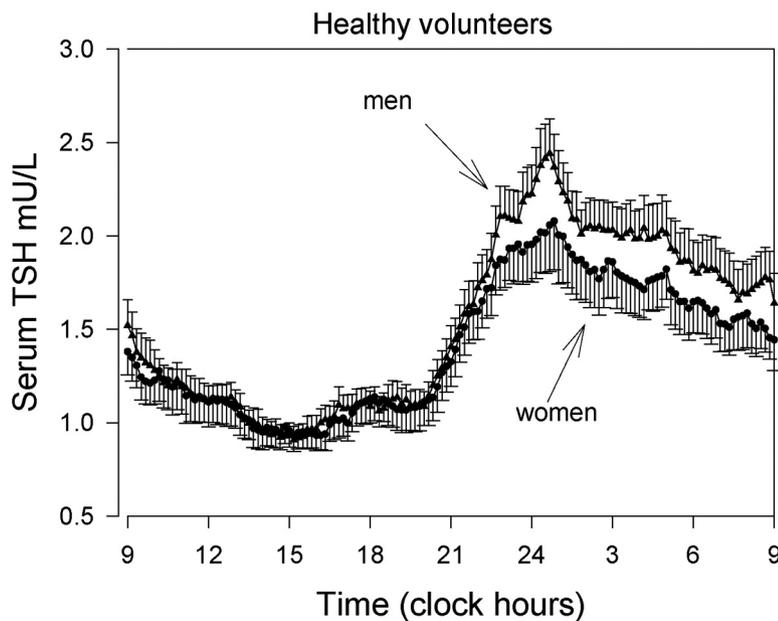
The effect of 64-h sleep deprivation on serum TSH profiles in 5 healthy adult men. The figure shows a significant alteration of the daytime TSH waveform in response to sleep loss, compatible with the existence of an inhibitory effect in early nightly sleep on TSH release. Reproduced with permission of The Endocrine Society from reference 84.

#### **Influence of age.**

Age correlates positively, although weakly, with total TSH secretion in healthy women ( $R = 0.44$ ,  $P = .04$ ) (73). In another study TSH secretion was investigated in 6 young men and 6 old healthy elderly nonobese male volunteers, aged 69–83 y. Cluster analysis, primarily a pulse

detection program, disclosed that TSH secretion remained pulsatile with unchanged frequency, but levels were slightly higher in the elderly, while nocturnal TSH surge was diminished (101). However, two other studies reported decreased TSH levels at increased age. In one study, a group of older men aged 67–84 was compared with 20–27 y-old young men. The older cohort had a decreased 24-h mean and cosine amplitude along with diminished TSH response to TRH administration, but normal release of T4 and T3 (102). A comparable result was found in 10 elderly male subjects, who had lower mean nocturnal TSH levels than 10 young subjects (75). Collectively, these outcomes can be harmonized by postulating a gender x age interaction in TSH regulation with declining TSH in men and rising TSH in women. Literature inferences in epidemiological studies on *thyroid* function in humans in relation to age are also not unanimous. The NHANES III study found a significant correlation between age and a single measurement of TSH in both genders (95), but other studies reported no influence of age (96–98). A timely review on this issue suggests the use of age- and race-specific reference limits (103). Some recent data also support this view (104). By excluding subjects with antithyroid antibodies, the NHANES III study found that racial differences in TSH levels were not determined by thyroid antibodies, at least among black, white and Mexican Americans. Furthermore women had lower 2.5 and 50th percentiles than males. Albeit based on upon repeated sampling over 24-h, it is possible that our study on TSH secretion was under-

powered. One hundred subjects or more with a BMI in the relevant range and ages from 20–80 yr may be required to establish firmly the impact of age, gender and BMI on TSH secretion, as was done in comparable studies on ACTH-

**Figure 4.**

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. Reproduced with permission of The Endocrine Society from reference 73.

cortisol secretion and GH secretion in healthy subjects (105, 106).

#### **Obesity.**

Recently, the association between a single TSH measurement within the normal range and adiposity was reviewed. The authors collected 29 studies, which fulfilled their inclusion criteria (107). Almost all studies were cross sectional studies, and 2 studies longitudinal. Twelve studies were population-based studies. Eighteen studies showed a positive relationship between measures of adiposity and serum TSH. One of the two longitudinal studies found a positive association between BMI and serum TSH only in nonsmokers (108) and the other a positive relation between body weight and TSH in men and women (109). In clinical practice, there is hardly a problem about the TSH level in these subjects, because they fall within clinically normal limits. A summary of the thyrotropic axis in aging is depicted in Figure 5.

Intensive blood sampling studies in obese premenopausal women demonstrated a 1.6 fold higher diurnal TSH secretion compared with normal-weight controls, which was proportional to circulating leptin concentration (110). Amplified TSH secretion was reversible after weight loss (111). Short-term treatment with bromocriptine also normalizes augmented TSH secretion in overweight adults, suggesting that deficient dopaminergic D2-

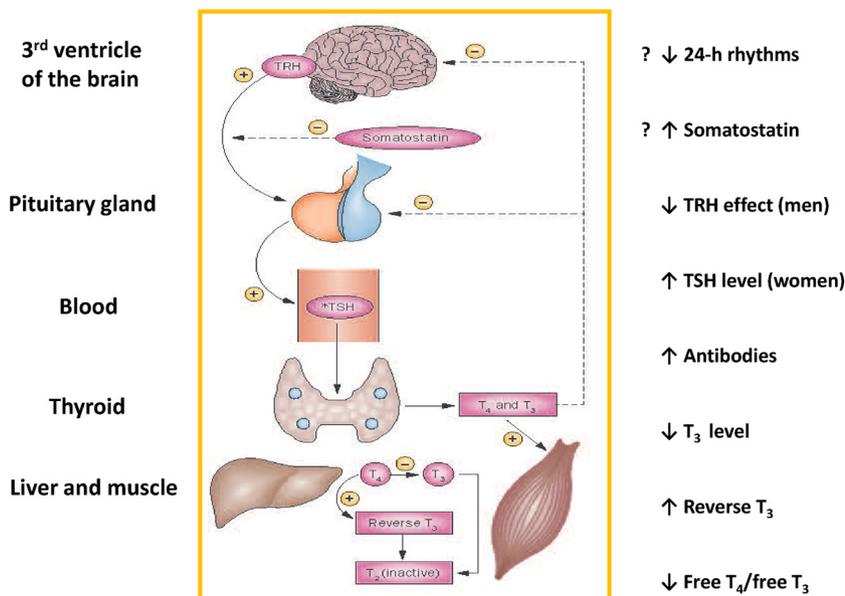
receptor neurotransmission contributes to disruption of the HPT axis (112). This mechanism might also be operative in the mild hypersecretion of prolactin and decreased GH secretion found in obese women (113–115). Such data are not available for obese men.

#### **Timing of the acrophase.**

The acrophase (time of maximal concentration) of the diurnal rhythm in TSH concentrations occurred at 0300 a.m., ranging from 24.00 – 0530 h in the same 46 persons. This estimate from 10-min data is comparable to results reported by others usually with less frequent sampling (73–76, 78, 80, 81, 85–87, 116, 117).

#### **Influence of sleep.**

The first report on the effect of sleep on TSH secretion appeared in 1976 (79). These investigators sampled blood every 20 or 30 min during 24–48 h in healthy young subjects. The TSH assay had a detection limit of 0.2 mU/L. Sleep was monitored electroencephalographically. All subjects had the nocturnal TSH increase, with a maximum between 2100 and 0100 h. After the start of sleep, TSH levels decreased, which was prevented by staying awake. Reversal of the sleep-wake cycle induced a shift of the rhythm with a higher amplitude during the first awake night, and lower amplitude during the second sleep night. In another study, sleep was prevented for 64 h. The 24-h rhythm persisted, but the TSH peak became wider, and again the TSH decrease was observed after sleep (84). Other studies have confirmed these observations (83, 118–120). Periods of slow-wave sleep ( $\delta$  wave sleep) were especially associated with the decline in TSH levels (120, 121). Slow-wave activity anticipated plasma TSH levels by 10–20 min, and TSH profiles correlated negatively with  $\delta$  activity. Thyroid hormone levels remained unchanged, suggesting that central control mechanisms are involved in the modulation of the sleep-wake state and the regulation of TSH secretion (121). Additional studies revealed that the decrease in  $\delta$  activity caused diminished secretion not only of TSH but also of cortisol, while an increase in  $\delta$  activity was related to increased GH and PRL secretion (89). The influence of a stable shift of the sleep-wake cycle was studied in night workers. One study showed that the core body tempera-

**Figure 5.****TRH-TSH Axis in Aging**

Human thyrotropic axis in ageing. Upward arrows denote increases in the signaling factor or effector with aging, and downward arrows denote decreases observed with aging. The plus and minus signs denote stimulation and inhibition, respectively. The interrupted lines signify negative feedback or inhibition, and the continuous lines positive feedforward or stimulation. \*: The hormone (TSH) value is increased by obesity, and decreased by systemic illness and undernutrition, especially in ageing individuals. Veldhuis JD, Nature in press 2013, Pituitary function in ageing.

ture and TSH rhythms remained synchronized, while exhibiting a phase delay of 6.5 h (122). Another study by the same authors compared TSH, cortisol, PRL and GH rhythms in night workers with those of day-active controls. Sleep latency was shorter in night workers, but otherwise all sleep parameters were similar in the two groups. During sleep cortisol levels in night workers were higher, while TSH levels lower, but GH and PRL did not differ. During work (in day-active subjects 0900–1700 h, in night workers 2200–0600 h) cortisol levels were lower in night workers, while during night PRL and TSH were higher in night workers. This study demonstrated that even after 2 y the biological adaptation is not complete (123). Several studies explored the influence of exercise or day time naps on TSH phase resetting. Nocturnal exercise for 3 h is capable of inducing a phase delay of TSH, which is larger when exercise is performed in the early night (124, 125). Day-time naps in darkness also induces TSH and melatonin phase shifts in the human, and the direction is time-dependent: in the morning a phase delay is observed while naps in the evening cause a phase advance (126). The importance of these studies is the potential application to readjustment of circadian rhythms, for instance after

transmeridian flights. Only one report examined both TSH pulsatility and diurnal rhythmicity during sleep deprivation and recovery. TSH pulse frequency did not change during these manipulations nor did the time of the nocturnal increase. However, TSH pulse amplitude rose during sleep withdrawal, and fell during the recovery phase, almost abolishing the diurnal rhythm (127).

**Other factors modifying TSH secretion.**

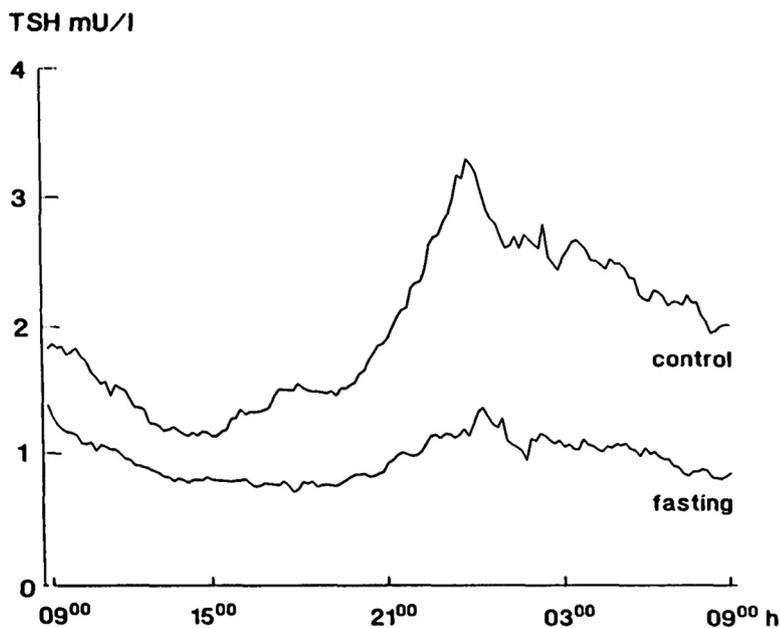
Various experimental and clinical conditions are associated with altered TSH levels, including administration of somatostatin, dopaminergic drugs, and prolonged fasting. Dopamine and somatostatin infusions in healthy subjects acutely diminish spontaneous secretion as long as the drug is infused. Inhibitory actions are likely via the D2-receptor and SST2 and SST5 receptors on the thyrotropes (91, 128–131). Prolonged fasting also diminishes TSH secretion by reducing burst amplitude, and blunts the nocturnal increase (132–136) [Figure 6]. These

changes may be mediated by the concomitant decrease in circulating leptin, because the fasting-associated decrease in TSH is prevented by leptin administration (137, 138).

Few data exist on TSH-ApEn in normal subjects. In normal subjects aged 20 to 65 y, TSH ApEn is age- and gender-invariant, in contrast to age- and sex-dependent GH and LH secretion patterns (139–141). In obese women, TSH secretion is enhanced, but unexpectedly ApEn was not increased, suggesting normal feedback (110).

**Thyroid hormones**

Various studies have explored whether thyroid hormones exhibit a diurnal rhythm. The conclusions of these studies are not unequivocal. Azukizawa could not establish significant diurnal rhythms for T<sub>4</sub> and T<sub>3</sub>, and changes in thyroid hormone concentrations could be explained by changes in serum protein levels (74). Another explanation offered is that the degree of glycosylation of TSH differs between day and night, leading to biologically less active TSH during the night (68). Other studies have established significant diurnal rhythms for T<sub>4</sub> and T<sub>3</sub> (78,

**Figure 6.**

Plasma TSH concentrations during normal food intake and during the last 24 h of a 60-h fast. Each curve represents the mean of eight subjects. Reproduced with permission of The Endocrine Society from reference 132.

82). In addition, a recent large study described a closely time-lagged relationship between TSH and  $ft_3$  (142). However, one careful study in young men who underwent two 10-min blood sampling studies over 24 h, either with or without a normal sleep period, under constant enteral feeding, could not establish a relation between TSH and  $ft_4$  or  $ft_3$  (119). Collectively, some publications suggest a relation between TSH and thyroid hormone secretion, but others not, which may be caused by different experimental conditions and analytical tools to explore mutual relations.

The suprachiasmatic nucleus (SCN) harbors the master biological clock (143). Lesions of the SCN in rats disrupt  $T_4$  and  $T_3$  rhythms, but the TSH rhythm persists at diminished amplitude (144). Neuroanatomical studies have disclosed a functional connection between the SCN and the thyroid gland, which can explain the loss of thyroid hormone rhythms in the presence of a TSH rhythm, although of diminished amplitude. This pathway can thus modulate thyroid hormone secretion in concert with TSH (144).

#### **Receptor polymorphisms of TSH and deiodinases.**

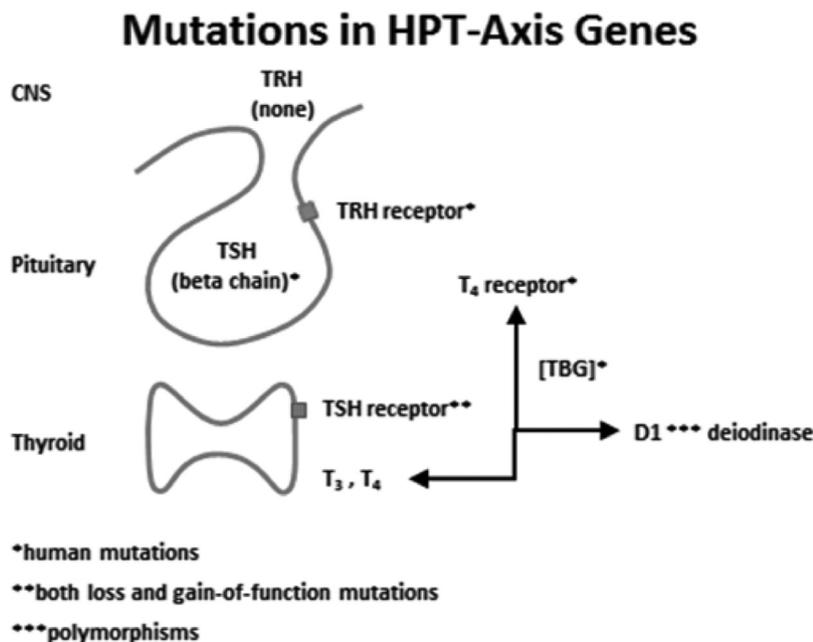
Gain-of-function mutations of the TSH receptor (TSHR) are well known, which result in the phenotype of toxic adenoma or multinodular goiter (145). Germline loss-of-function mutations of the TSH receptor are asso-

ciated with TSH resistance (see Section V) and hypothyroidism. To date, three germline TSH receptor polymorphisms have been described, Asp36His, Pro52Thr, and Asp727Glu (146). The TSHR-Glu727 allele is associated with lower serum TSH, without affecting  $ft_4$ , suggesting possibly enhanced TSH-receptor activity (147, 148). The other two, less frequent polymorphisms are not associated with changes in TSH or iodothyronine levels. The TSHR is not only expressed in the thyroid gland, but also in adipose tissue, orbital tissue, brain, lymphocytes and bone. Recent clinical studies have demonstrated the association of the Asp727Glu polymorphism and hip bone mineral density (BMD) (149) and insulin resistance (150).

Two polymorphisms have been described for the D1 deiodinase, which is present in the liver, kidney and thyroid stimulating the conversion of  $T_4$  to  $T_3$  (151). One polymorphism leads to a decreased  $T_3/rT_3$  ratio, especially in the elderly, who cannot fully compensate via increased  $T_3$  production by muscle type 2 deiodinase. Polymorphisms of the D2 or D3 deiodinases do not lead to altered circulating hormone levels, but decreased intracellular availability may lead to increased insulin resistance (152). TSH profiles in healthy subjects have not been related to these polymorphisms, but the impact is probably not large. Known gene mutations in the HPT axis are schematically shown in **Figure 7**.

#### **III b. Pregnancy**

During pregnancy major changes in the endocrine system occur. One of the early changes is the enhanced renal iodide clearance thus increasing the iodine requirement. If iodine availability is low, the relative lack may lead to increased TSH secretion, a high serum  $T_3/T_4$  ratio, increased serum thyroglobulin concentration, and finally goiter formation in mother and child (153, 154). During the first trimester human chorionic gonadotropin (hCG) increases temporarily. This glycoprotein consists of an  $\alpha$ -subunit (common to LH, FSH and TSH) and a noncovalently associated hormone-specific  $\beta$ -subunit. This hormone possesses an intrinsic, although weak, thyroid stimulating activity (155). Desialylated and deglycosylated hCG are superagonists for the human thyroid follicle

**Figure 7.**

Schematic drawing of mutations in the hypothalamo-pituitary-thyroid axis genes (unpublished figure).

(156). During the period of peak secretion of hCG, the T<sub>4</sub> level rises while that of TSH decreases (155). During pregnancy T<sub>4</sub> and T<sub>3</sub> levels continue to increase, due to increased secretion of thyroxine binding globulin (TBG) under the influence of estrogens and diminished plasma clearance (157). The increase occurs early in pregnancy and by 16–20 wk plasma concentrations have doubled (153). Increased TBG levels and expanded plasma volume lead to an enlarged T<sub>4</sub> pool. During normal pregnancy with sufficient iodine intake, the required increase in T<sub>4</sub> (and T<sub>3</sub>) secretion by the thyroid is met without difficulty, but in women without a functional thyroid gland after surgery and radioactive iodine treatment for thyroid cancer, or other forms of hypothyroidism, higher thyroxine doses are mandatory.

There is one study available on TSH secretion in pregnancy. Four women were studied late in pregnancy (weeks 34–38) and two others early in pregnancy (weeks 11 and 17). All women showed a clear diurnal rhythm with highest levels attained in the late evening or early night (158) but the sampling frequency was insufficient for pulsatility analysis.

### IIIc. Neonates

TSH secretory dynamics have been studied in neonates. Shortly after birth, TSH levels are increased in the infant compared with the mother. TSH concentrations rise to peak values at 30 min (159, 160) and then decrease rapidly

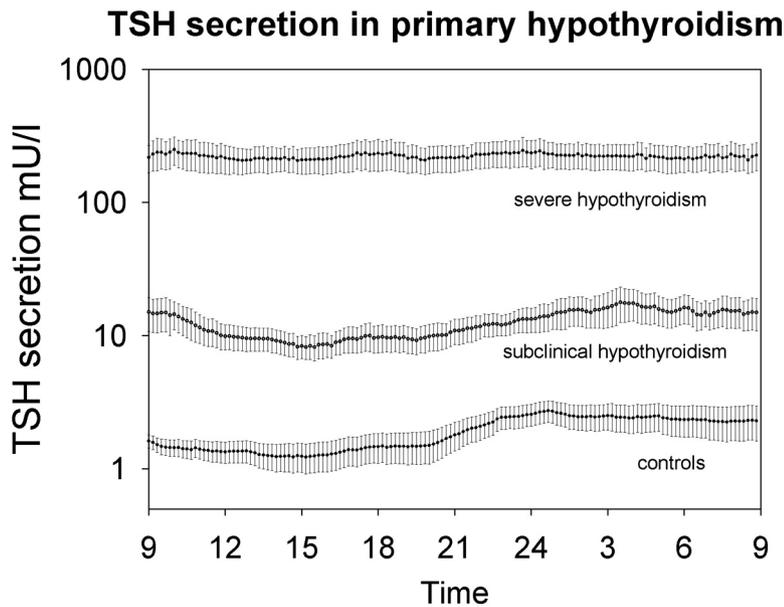
over 3–4 h, and more gradually for 1–2 d. Protein-bound iodine, representing mainly thyroxine, is increased after 4 h and maximal at 24 h (159). In another study, T<sub>3</sub> levels increased five-fold from 47 ng/dl at birth to 227 ng/dl at 2 h, but an early rise of T<sub>4</sub> was less marked, increasing from 15.3 to 17.9 μg/dl (159, 160). In addition, the authors noted an increase of mean PRL from 165 ng/ml to 214 ng/ml at 30 min. Comparable observations were reported by the group of Similä (161). The mechanism involved in the postnatal TSH surge is unknown, and may be caused by acute release of stored TSH by TRH (like PRL), or disinhibition of release by decreasing dopamine. A logical explanation for the TSH rise shortly after birth might be the cold-induced TSH surge. However, keeping the newborn at 99–103°F immediately after birth did not prevent the early acute release of

TSH (159), suggesting that another potent stimulus such as physical stress is involved. On the other hand cooling warm infants resulted in a decrease in mean rectal temperature of 3.3°F and increased TSH by 38%, demonstrating that cold surges-induced TSH secretion can be induced in the newborn.

At birth, TSH secretion is characterized by basal and pulsatile secretion (162). In one study, the TSH interpulse interval was 133 min and the estimated half-life 75 min. Three days later, both basal and pulsatile TSH secretion diminished, the latter by decreased pulse amplitude. Prenatal administration of TRH, 2 h before elective caesarian section increased TSH and T<sub>3</sub> levels compared with controls without interfering with the postnatal TSH rise (163). When TRH was administered 12 h before birth, TSH and T<sub>3</sub> levels were similar to those in controls, but T<sub>4</sub> levels were not measured.

The HPT axis in the neonate responds to various conditions as in the adult, including suppression of TSH by neonatal hyperthyroidism or prenatal administration of glucocorticoids and elevation of TSH by cooling of the body (159). Furthermore, dopamine administration to the hypothyroid neonate suppresses TSH (164).

Postnatal screening for hypothyroidism is important for preventing permanent brain damage. A consistent and optimal time window should be chosen for thyroid function measurements, depending on the choice of T<sub>4</sub>, TBG,

**Figure 8.**

Twenty-four hour serum TSH-concentration time series in 8 patients with severe hypothyroidism, 8 patients with mild hypothyroidism and 38 healthy control subjects. Data are shown as mean and SEM on a logarithmic scale. Reproduced with permission of The Endocrine Society from reference 62

fT4 and TSH. Especially in premature and sick children the diagnosis of hypothyroidism (primary or central) may be difficult. In any case, false negative results should be avoided (165, 166). Table 1 summarizes the main physiological factors discussed, viz. age, obesity, pregnancy and infancy. These factors variously influence T4, T3 and TSH as noted.

#### IV. Hypothyroidism

##### IVa. TSH Secretion in primary hypothyroidism

Increased basal serum TSH concentrations in primary hypothyroidism were recognized with the introduction of TSH bioassays and immunoassays in clinical practice. The diurnal secretion pattern has been studied by several groups. Clinical data show increased levels throughout 24-h day-night cycle, and often, but not always, an absent

diurnal variation by comparing mean day and mean night levels (63, 85, 167–169). The number of TSH bursts in hypothyroidism remains unchanged, but pulse amplitude increases in proportion to the severity of the condition. In the study by Adriaanse and her colleagues, mild (subclinical) hypothyroidism shared characteristics with normal controls, i.e., intact diurnal secretion pattern, increased pulse amplitude and increased pulse frequency during the nocturnal surge (63). We have reanalyzed TSH secretion profiles in severe and mild hypothyroidism using an objective deconvolution technique. TSH pulsatility was maintained, with an unchanged burst frequency, but total secretion is increased by ten-fold in subclinical hypothyroidism and by 200-fold in severe disease [Figure 8]. The mechanism comprised combined amplification of basal and pulsatile secretion

(62). In this investigation, 6 out of 8 patients with severe hypothyroidism still retained a statistically significant diurnal rhythm, demonstrated with cosinor analysis, although with a small relative amplitude and with a time delay of the acrophase. As in euthyroid subjects, infusions of dopamine or somatostatin decrease TSH levels in hypothyroidism (130, 170) as does treatment with thyroid hormone, which also restores the weak or absent diurnal TSH rhythm (171, 172). The latter observation suggests that the suprachiasmatic nucleus output is itself thyroid-hormone dependent.

Hypothyroidism is associated with increased TSH ApEn, denoting increased irregularity, likely due to the increased TRH signaling and decreased feedback via thyroid hormones (62). In subclinical hypothyroidism when thyroxine levels are still in the normal range, TSH ApEn is

**Table 1.** Thyroid hormone and TSH levels in human physiology

	T4(fT4)	T3(ft3)	TSH	Nocturnal surge	Rhythm study	References
Age	= (F), =	↓ (F), ↓	↑ =, ↓ (M)	↓ =	yes	73,75,95– 98,101–104
Gender	↑ (F)		↑ =	↑	yes	95–98
Obesity	=	=	↑ =	↑	yes	107–110
Pregnancy	= (fT4)	↑	↓	?	no	153,155,158
Neonates	↑	↑	↑	?	partial	159–162

F and M between parentheses: men or women, ↑ increased, ↓ decreased, = no change

unchanged, consistent with the concept that T4 feedback dictates secretory orderliness. TSH secretion in severe primary hypothyroidism is marked by increased spikiness, denoting unpredictable, brief, sharp elevations in TSH concentrations (62). The same anomaly applies to TSH-secreting tumors (173). The mechanism is not known, but in principle might reflect enhanced thyrotrope electrophysiological sensitivity.

#### IVb. Central hypothyroidism

The other important cause of hypothyroidism is secondary hypothyroidism, caused by pituitary diseases, including primary adenomas, other (para)pituitary masses, sequelae from pituitary surgery and irradiation, Sheehan's syndrome, trauma, and infiltrative diseases like sarcoidosis. Tertiary hypothyroidism is caused by hypothalamic damage by cranial irradiation, craniopharyngioma, or trauma. Collectively, secondary and tertiary hypothyroidism is often referred to as central hypothyroidism. Pure hypothalamic disease is rare, but pituitary disorders frequently have a hypothalamic component. Clinically, T4 levels are diminished, but the serum TSH concentration may vary from subnormal to mildly increased levels. Other abnormalities, although not always present, include subnormal TSH increase and attendant free T4 and T3 increases after TRH administration. The seeming paradox of increased or normal immunoreactive TSH with decreased T4 is explained by diminished biological activity of TSH, caused by increased sialylation of the  $\beta$ -subunit of TSH. In a TSH bioassay, sera from patients with central hypothyroidism (pituitary and hypothalamic) had decreased bioactivity, which correlated with the lower T3 response to TRH (174). Explanations for increased sialylation are diminished TRH signaling on the thyrotrope and decreased feedback by diminished T4 (175, 176).

Few TSH profile studies exist in patients with (sporadic) central hypothyroidism. A study in 16 patients with hypothalamic-pituitary diseases, in which two parts of 4 h each of the 24-h cycle were sampled and compared, showed the absence of the nocturnal TSH increase in 6 subjects. These patients also had subnormal T4 and T3 levels (177). Comparable results were obtained in 52 children with diverse hypothalamic-pituitary diseases (178). Samuels and coworkers also studied the TSH profile in central hypothyroidism and hypogonadism (5 patients with hypothalamic-pituitary lesions, i.e., craniopharyngioma, sarcoidosis and Rathke's cleft cyst). Common to all patients was the loss of the nocturnal increase in pulse amplitude, estimated by Cluster analysis (179, 180). Later, this study was repeated in a larger group of subjects (181). In this patient cohort of 19 patients with hypothalamic-pituitary disease, 6 patients had moderately de-

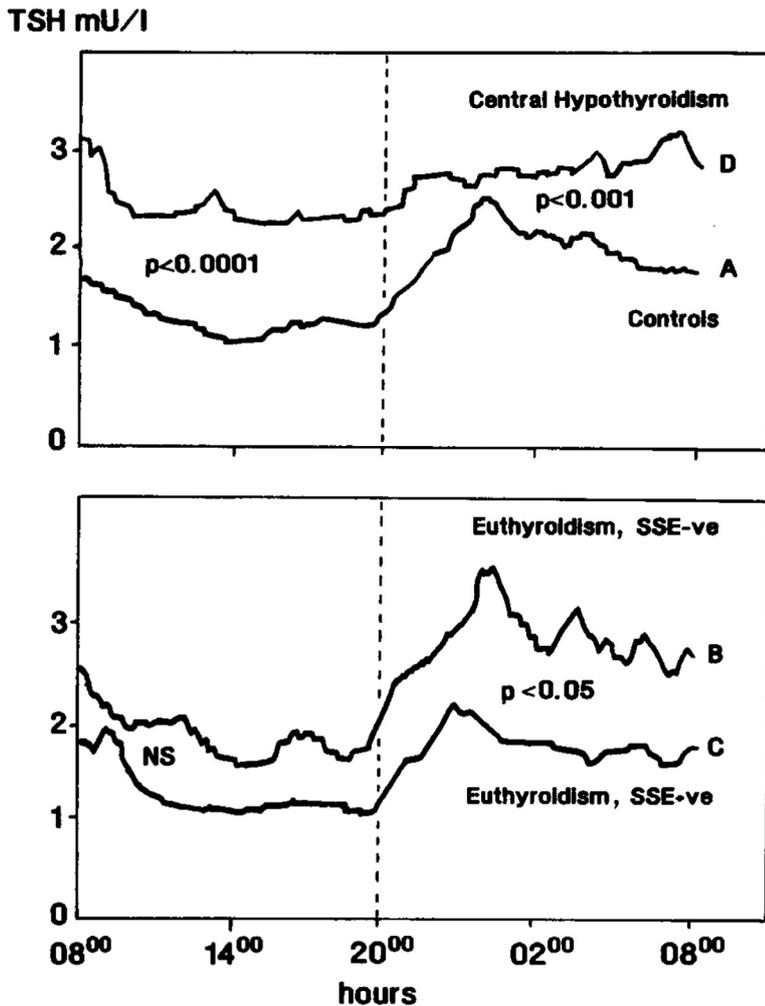
creased T4 levels and slightly raised TSH concentrations. The majority had a nonfunctioning adenoma or prolactinoma. The nocturnal TSH rise was absent in patients with a suprasellar extension of the adenoma and in patients with decreased T4 levels. Patients in the latter category also had other pituitary deficiencies. The nocturnal rise correlated with the TSH response to TRH administration, but not with serum T4 concentrations (181) [Figure 9].

Another cause of hypothalamo-pituitary damage is brain irradiation of malignant brain tumors in children. Darzy and colleagues performed a 24-h blood sampling study in 37 adult survivors of childhood cancer. The patients had mildly elevated basal TSH levels and slightly larger TSH response after TRH injection than controls. Furthermore there was damping of the amplitude of the 24-h rhythm. Only patients with concomitant GH deficiency had a diminished or abolished nocturnal TSH rise without hypothyroidism, suggesting that more extensive hypothalamic impairment attenuates nocturnal TSH secretion (182). Although not proven, the nighttime decline in somatostatin release inferred from GH elevations combined with adequate TRH or GHS drive may contribute to overnight TSH elevation (183).

#### IV c. Rare forms of central hypothyroidism

Central hypothyroidism can be caused by congenital disorders associated with mutations of pituitary transcription-factor genes, causing various defects in pituitary differentiation, e.g., POU1F1, PROP1, HESX1, LHX3, LHX4 and LEPR, thereby leading to insufficiency of one or more pituitary functions (184, 185). These patients have undetectable or low to normal serum TSH concentrations. No detailed 24-h TSH profiles are available, although circadian properties of TSH are retained (186). Recently, loss-of-function mutation in *IGSF1* (an immunoglobulin superfamily) in 11 unrelated families was identified as the cause of central (pituitary) hypopituitarism and testicular enlargement (187). Another rare disorder leading to central hypothyroidism is inactivating mutation of the TRH receptor-1 (17). The 9 y-old patient so reported had subnormal T4 and inappropriately low TSH. Both TSH and prolactin were unresponsive to TRH stimulation. Comparable cases were recently described in another family. The male proband was diagnosed with central hypothyroidism at age 11 because of growth retardation, lethargy and fatigue. FT4 was decreased to 0.36 ng/dl, TSH was low normal at 0.6 mU/L, and PRL and TSH responses to TRH were subnormal. His sister was diagnosed with hypothyroidism at the age of 33 y. The defect was caused by a C-to-T transition at position 49 of the TRH-receptor gene, leading to a premature stop codon at position 17 and the synthesis of a TRH receptor that

Figure 9.



Plasma TSH profiles in controls and patients with pituitary disease, divided into patients with suprasellar extension of the tumor (SSE+ve) and without (SSE-ve). Hypothyroid and SSE+ve patients do not display the nocturnal TSH increase. Reproduced with permission of The Endocrine Society from reference 181

lacked the whole transmembrane domain (186). Unfortunately, 24-h TSH profiles were not studied in these unique patients.

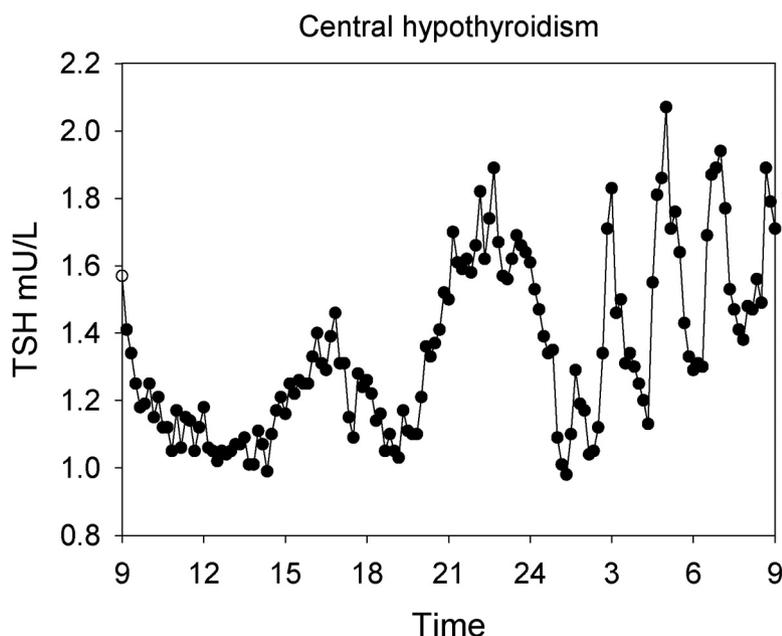
The TSH profile is relevant for establishing whether TSH pulsatility, whether or not decreased in absolute levels strictly depends upon the endogenous TRH signal. Conversely, studies by Samuels and her colleagues have demonstrated that continuous infusions of TRH for prolonged times (up to 48 h) with different doses do not influence pulse number, but only amplitude, implying that TRH is not required for TSH pulse generation per se, as discussed in Section III (90). Analogously, patients with an inactivated GHRH-receptor still exhibit GH pulsatility, although with greatly diminished amplitude (188). TRH-receptor deficient mice have low concentrations of TSH, prolactin and thyroid hormones. Although the diurnal

TSH rhythm has not been studied one would expect a deficient or absent rise of TSH in the dark period (189). Whereas no patient has yet been described with an isolated TRH-peptide mutation, this transgene has been constructed in mice (190).

Other more common forms of central hypothyroidism are inactivating mutations of the unique  $\beta$ -chain of TSH and 8 different mutations have been described (191, 192). Patients present with low T4 and T3 levels and low or immeasurable TSH levels. No detailed TSH secretion studies have been reported in patients with TSH  $\beta$ -chain mutations. Comparable changes in mean levels of thyroid hormones, but with elevated TSH concentrations were observed in families with loss-of-function mutations of the thyrotropin receptor (193). Some families have been described in whom resistance to TSH was not caused by mutations of TSH or the TSH receptor (194). Finally, drug-associated central hypothyroidism was reported in 27 patients with cutaneous T-cell lymphoma treated with bexarotene, a retinoid receptor agonist. Mean TSH dropped from 2.2 mU/l to 0.05 mU/L, and fT4 from 12.9 pmol/L to 5.8 pmol/L. TSH suppression was related to the bexarotene dose. Nine-

teen patients developed clinical signs of hypothyroidism. In vitro, the drug suppressed TSH promoter activity, TSH mRNA synthesis and TSH secretion (195). Additional studies in patients with thyroid cancer on a fixed thyroid hormone substitution dose showed that bexarotene also amplifies thyroid hormone metabolism by nondeiodinase pathways (196).

Unexplained central (secondary) hypothyroidism also exists in the clinical literature. We have studied a male patient, born in 1936, suffering from familial central hypothyroidism, not caused by a TRH receptor-1 or TSH  $\beta$ -chain mutation. At the time of diagnosis in 1956, the PBI was 3.2  $\mu$ g/dl (normal values between 4–8  $\mu$ g/dl) and thyroid uptake of radioactive iodine was 31%. The latter increased to 54% after Ambinon administration (a bovine TSH preparation produced by Organon, The Nether-

**Figure 10.**

Serum TSH profile in a male patient with familial central hypothyroidism 5 wk after withdrawal of thyroxine replacement. This patient had no gene mutation of the TRH receptor or  $\beta$ -subunit of TSH (unpublished figure).

lands), while PBI increased from 2.8 to 11.5  $\mu\text{g}/\text{dl}$ . Four of 13 nephews, but not two brothers, also had biochemically and clinically proven secondary hypothyroidism, as did a male grandchild. Later studies in the patient showed a subnormal increase of low basal prolactin and TSH after iv TRH administration, but a normal GH and cortisol increase during insulin-induced hypoglycemia. Furthermore, MRI of the pituitary gland and hypothalamus was normal. The TSH secretion profile was studied 6 wk after withdrawal of thyroxine as shown in **Figure 10**. At that time the FT4 level was 10.9 pmol/L and the TT3 1.0 nmol/L. The pattern is characterized by several broad bursts in the daytime and five rapid brief TSH bursts at night. Cosinor analysis demonstrated normal, although low amplitude TSH rhythm with a normal acrophase. Deconvolution analysis revealed normal mean secretory burst size, frequency and regularity. The only difference with healthy controls was the rather short half-life of the slow component, i.e., 69 min, although still within the normal range. This result might indicate the presence of modified TSH glycoprotein, but the precise cause the mild, although clinically relevant central hypothyroidism has not been disclosed. So far as we know this the only patient with a genetic abnormality causing central hypothyroidism, in whom a detailed TSH secretion study was performed.

In summary, many factors affect TSH dynamics in (sec-

ondary) hypothyroidism: Table 2. Additional rare forms of central (hypothalamo-pituitary) hypothyroidism arise from genetic defects in thyrotrope development, TRH receptor, TSH subunits and their processing as well as from reversible drug effects. Treatment is the same as that of other causes of secondary hypothyroidism.

#### V. Thyroid hormone resistance syndromes

Multiple steps are required for secreted thyroid hormone to exert its effects on target tissues, including active transport across the cell membrane, intracellular metabolism and hormone activation, cytosolic and nuclear processing, association with receptors and interaction with coregulators (197). The first recognized defect involved the thyroid hormone-receptor  $\beta$  gene (TR $\beta$ ), discovered in 1967, and became known as 'resistance to thyroid hormone' (RTH) (198). Recently, the authors have broadened the definition of RTH to include all clinical forms that lead to reduced intracellular effectiveness of T3 (199). The phenotype of this autosomal recessive defect varies within and between families. Clinical features are goiter, tachycardia, emotional disturbances, including the attention-deficit hyperactivity disorder (ADHD), mental retardation, and hearing loss. These heterogeneous features putatively reflect tissue-specific alterations in thyroid-hormone action. Biochemically the syndrome is characterized by elevated serum T4 and T3, usually high rT3 and inappropriately elevated TSH concentrations due to diminished feedback signaling (200–202). The response of TSH to TRH is normal or exaggerated, and the bioactivity of TSH is increased, explaining the frequent occurrence of goiter (203). Twenty-four-h TSH dynamics were studied in one postmenopausal patient. The circadian TSH rhythm was preserved although at a much higher mean level. Bromocriptine treatment diminished TSH secretion and normalized thyroid hormone levels (204). Recently, two new mutations in the thyroid hormone-receptor alpha gene were described. The first report was of a 6 y-old girl, exhibiting classical features of severe hypothyroidism, including growth retardation. The fT4 level was decreased, but fT3 level normal to elevated, resulting in decreased fT4/fT3 ratio. TSH was within normal limits (1.04 mU/l).

**Table 2.** Thyroid hormone and TSH levels in hypothyroidism

	T4(fT4)	T3(fT3)	TSH	Nocturnal surge	Rhythm study	References
Overt primary HT	↓	↓	↑	↑ =	yes	62,63,167–169
Subclinical primary HT	=	=	↑	↑	yes	62,63
Central HT	= ↓ (mild)	=	= ↑ (mild)	= ↓	yes	177–181
Irradiation	=	ND	↑ (mild)	=, ↓	yes	182
TRH receptor mutation	↓	↓	↓	?	no	17,186
Post-TRH receptor mutation	↓	↓	↑	=	yes	Unpublished
β-chain TSH mutation	↓	↓	↓	?	no	192
TSH receptor mutation	↓	↓	↑	?	no	193
NTI	= ↓	↓	= ↑	↓	yes	221–225

↑ increased, ↓ decreased, = no change. Central: includes pituitary and hypothalamic diseases, the receptor defects are all inactivating mutations, NTI non-thyroidal illness, HT hypothyroidism. ND not determined.

Since the syndrome was caused by a heterozygous dominant-negative nonsense mutation of the gene encoding the thyroid hormone receptor alpha, the basis for low free T4 level is not so clear (205). Similar clinical findings were reported in a Greek girl and her father. She was diagnosed with growth retardation and hypothyroidism. Treatment with thyroxine caused an initial catch-up growth, but GH replacement had no influence on the growth rate. The girl had low-normal fT4 and high total T3 levels, but TSH was in the normal range. Similar biochemical findings were present in the father, who also had a short stature (-3.77 SDS). The genetic basis in these siblings was a frame-shift mutation (F397fs406X) (206). Pulsatile TSH and diurnal TSH rhythms in this condition have not yet been studied.

In contrast to early concepts of passive diffusion, the hydrophobic thyroid hormones are actively transported across the cell membrane. The most specific transporters are the monocarboxylate transporters (MCT), especially MCT8 and MCT10 (24, 29). Mutations of the MCT8 (*SLC16A2*) gene lead to the syndrome in boys, originally described indirectly in 1944 by Allan, Herndon and Dudley, of severe neurological deficits, cognitive impairment and inability to speak (32, 207). Biochemically the syndrome is characterized by high serum T3 and low rT3 levels, subnormal fT4 and normal to slightly raised TSH. This leads to severe hypothyroidism of the brain, but in other organs with sufficient other transporters hyperthyroidism occurs, such as the liver. Recently, the thyroid hormone analog diiodothyropropionic acid (DITPA), which does not require the MCT8 transporter and acts as

T3, has been used in 4 children suffering from this syndrome. Biochemically they showed a favorable, but incomplete response (208). Apparently, successful treatment should begin early in pregnancy. Detailed studies on resultant TSH secretion pattern are not available. Finally, reduced sensitivity to thyroid hormone can be caused by an inactivating mutation of the selenocysteine insertion sequence-binding protein-2 (SBP2) gene, required for biosynthesis of selenoproteins like the deiodinases (209, 210). This rare disorder in children is characterized by short stature and delayed bone age and puberty. Laboratory findings are high-normal to raised T4, normal or slightly diminished T3, high rT3 and slightly elevated or normal TSH. In addition, these patients may also suffer from azoospermia, axial muscle dystrophy, increased photosensitivity, and increased insulin sensitivity. No detailed studies of the circadian TSH rhythm or TSH pulsatility are available in these patients.

In summary (Table 3), mutations of thyroid hormone transporters, receptors, and interconverting enzymes tend to elevate TSH, albeit not always markedly. The clinical presentation often includes growth failure involving the skeleton and brain.

## VI. Other thyroid disorders

Increased T3 and T4 blood levels decrease TSH secretion as shown in the elegant studies by Brabant (211). Infusion of thyroid hormone diminishes the nocturnal TSH increase, although this effect requires several hours to develop. The pattern of TSH secretion remains pulsatile.

**Table 3.** Syndromes of reduced sensitivity to thyroid hormone

	Gene	ft4	ft3	rT3	TSH	Rhythm study	References
RTH	TR $\beta$	↑↑	↑ =	↑↑	=	no ↑ (mild)	198,200–202
RTH	TR $\alpha$	=, ↓	=, ↑	↓	=	no	205,206
THCTD	MCT8	↓	↑↑	↓	=	no ↑ (mild)	28,29,32,207
THMD	SBP2	↑	↓	↑↑	=	no ↑ (mild)	209,210

↑ increased, ↓ decreased, = no change. RTH resistance to thyroid hormone, THCTD thyroid hormone cell transporter defect, THMD thyroid hormone metabolism defect.

Addition of iopodate, which prevents the conversion of T4 in T3, fully abolishes the inhibitory effect of T4. Manifest hyperthyroidism, but not subclinical hyperthyroidism, suppresses TSH secretion below the detection limit of most TSH assays (212, 213). Patients with nontoxic nodular goiter generally exhibit pulsatile TSH secretion with the nocturnal increase, although TSH concentrations at night are diminished (212, 214). In another report no differences between patients and controls were present (215). These disparate findings may be related to the degree of autonomy of the thyroid gland or possibly T3/T4 ratios, but such clinical details are not mentioned in these reports.

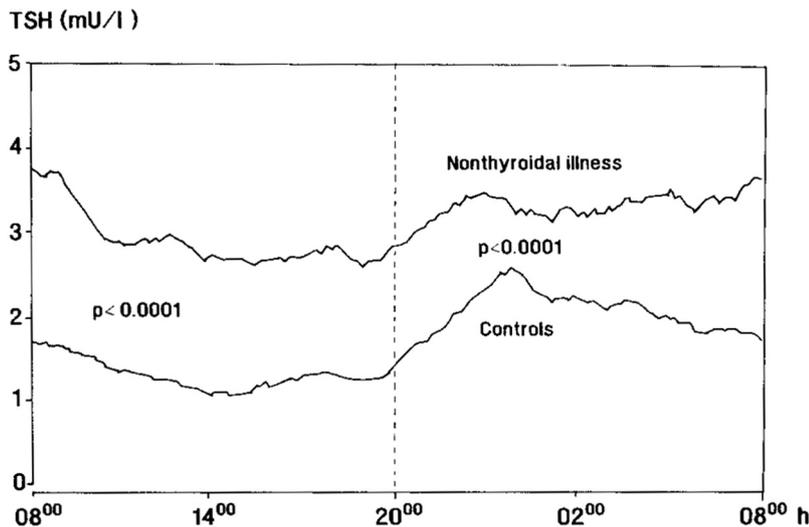
### VII. Non-thyroidal illness

Acute and especially severe illness profoundly affects the hypothalamic-pituitary-thyroid axis. Systemic illness, but also major surgery, generally leads to reversible decrease in T3 and increase in rT3, hence the term ‘low T3 syndrome’ (216). In prolonged critical illness in intensive care units, T4 and ft4 also decrease in proportion to the severity of the condition, together with a decrease in TSH. The mechanisms involved in the abnormal thyroid hormone concentrations are multiple, and include diminished TRH drive of TSH, reduced thyroxine uptake by the liver, enhanced intracellular metabolism and decreased secretion by the thyroid gland (217–220). The various basal and clinical aspects of the syndrome were reviewed by Boelen and her colleagues (220). In a study by Adriaanse and colleagues in 1993, twenty-one patients with different nonsurgical diseases were included, of whom 11 had a subnormal T3 and raised rT3 [Figure 11]. All 21 patients underwent 24-h of blood sampling study at 10-min intervals. The 10 patients with low T3 had mildly mean elevated TSH (3.5 mU/L versus 1.7 mU/L in controls), caused by increased pulse amplitude (assessed by Cluster and Desade) with unchanged pulse frequency. There was no nocturnal TSH increase in 4 patients despite mildly increased daytime levels, and normal ft4 levels (221). Patients using

drugs interfering with thyroid hormone levels were excluded and none was in a catabolic state, which may explain the mild nature of abnormalities in only half of the patients. In contrast, patients with severe illness treated in the intensive care unit (ICU) show more profound abnormalities. In 8 severely ill patients, diminished mean 24-h TSH concentrations were accompanied by decreased amplitude of the day-night rhythm, an almost 6-h delay shift in the acrophase, as well as absent TSH pulses in six, and a greatly diminished number in the other two patients (222).

The group of van den Berghe reported that nocturnal TSH profiles in patients in the ICU had low-normal mean TSH values, unchanged pulse frequency and diminished pulsatile secretion compared with reported literature data. In the patient group T4 levels ranged from low to normal values, T3 was decreased, but rT3 normal. GHRH infusion increased pulsatile TSH secretion, but basal TSH secretion and pulse frequency remained unchanged, as well as thyroid hormone levels (223). In another report by the same group, critically ill patients (mean Apache II score 14.1) received combined infusions of GH-releasing peptide-2 (GHRP2) and TRH for 5 d. This regimen increased (nocturnal) TSH secretion, and normalized serum T4 and T3 concentrations without changing rT3 (224). Interestingly, the secretion patterns of GH, prolactin and TSH were synchronized by GHRP-2, but not by GHRH or TRH (225). In another study combined infusions of GHRP-2, TRH and GnRH reactivated the GH, TSH and GnRH axes, normalized T4 and T3 levels, normalized IGF-I, IGFBP3 and acid-labile subunit (ALS) levels, and to a lesser degree increased testosterone and LH levels (226). Nevertheless, it has not been established whether this treatment is useful for the critical ill patient (220). In a postmortem study in 10 patients with varying degrees of nonthyroidal illness (NTI), a strong correlation was found between TRH gene expression in the PVN and serum TSH and T3 levels (227). In NTI hypothalamic T3 is also decreased (228). These observations point to suppression of hypothalamic TRH that is relatively unresponsive to the decrement in thyroid hormone feedback. The combined changes could contribute to the persistence of low serum T3 concentrations for the conservation of energy during serious illness.

In summary, the current available data indicates that critical illness leads to diminished TSH secretion, especially of the pulsatile component, with loss of the nocturnal increase and unchanged pulse frequency. In the very ill patient, T4 levels decrease and the magnitude of decrease is related to the chance of death (229). Although undernutrition, high glucocorticoids, and inflammatory cytokines likely play major roles, the precise pathophysiology

**Figure 11.**

The course of plasma TSH concentrations over 24 h in controls and NTI patients. Each curve represents the mean TSH values of all subjects in that group; statistical differences between groups are indicated for the 0800–2000 and 2000–0800 h intervals. Reproduced with the permission of The Endocrine Society from reference 221.

ical mechanisms are not fully known yet (230, 231). At present there is no consensus whether to treat NTI with thyroid hormones, either with T4 or T3. No clear positive clinical outcome has been demonstrated, but the size of studies was small. A major problem is that circulating hormone levels do not predict intracellular hormone content in the different organ systems (232).

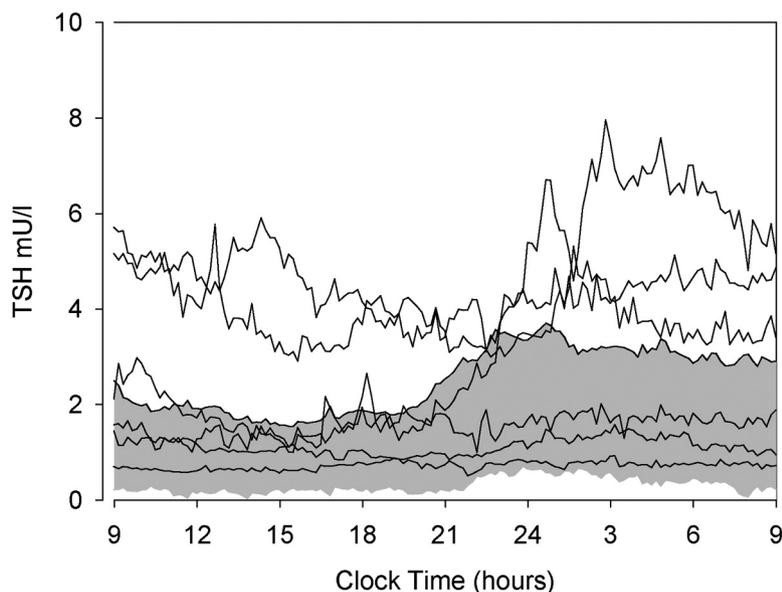
## VIII. Pituitary adenomas

### VIII a. Thyrotropinoma

Inappropriately elevated TSH values associated with central hyperthyroidism may be caused not only by T4 resistance, but also by thyrotropinoma (233–236). The first description of the TSH profile was in a 40 yr-old female patient (237). The patient was treated for supposed Graves' disease with radioactive iodine and became hypothyroid. The correct diagnosis was made when serum TSH failed to suppress to normal values during thyroxine replacement. Under a normal substitution dose of 200  $\mu$ g T4 per day, the serum TSH profile exhibited no diurnal variation. Pulsatility, determined with Cluster, was comparable in frequency with that of control subjects (7 pulses in the patient versus 9 in controls). There was no TSH response to TRH or dopamine, but octreotide decreased TSH to 30%. In 7 patients in another study of thyrotropinomas, 2 patients were sampled at 2-hr intervals for 24 h. One patient had no obvious diurnal rhythm, while it was preserved in the other patient. The response to TRH was normal in only one patient, and bromocriptine caused a

significant decrease in one patient. Octreotide administration in three patients caused a significant fall in TSH. Pulsatility was investigated in one patient over 8 h, and 3 pulses were detected with the Pulsar program (238). Another patient was investigated by Adriaanse. This patient underwent three 24-h sampling studies at 10-min intervals, one basal, one during octreotide treatment and one after the addition of bromocriptine. Mean TSH levels, but not TSH burst frequency, was increased compared with controls, while pulse amplitude was amplified. The nocturnal TSH surge was present at decreased magnitude, but increased during treatment (239). These studies suggest that pulsatility is preserved in TSH-secreting adenoma, and that diurnal variation may be still demonstrable.

Our group studied 5 patients with TSH-secreting adenomas, all of whom underwent a 24-h 10-min blood sampling procedure. TSH dynamics were investigated with operator-independent deconvolution and diurnal rhythmicity was investigated by cosinor analysis, rather than comparing mean day and night values (Figure 12) (173, 240). Nonpulsatile (basal) TSH secretion was almost three-fold increased, but pulsatile secretion was statistically not greater than that found in controls (27.7 mU/L vs 16.3 mU/L). Interestingly, and comparable with studies of other hormone-secreting adenomas (e.g., acromegaly, Cushing's disease and prolactinoma), TSH burst frequency was increased in patients compared with controls (241–244). Parenthetically, operator-independent automated deconvolution analysis detected almost twice the number of TSH pulses in normal subjects compared with results obtained with the Detect, Pulsar or Desade pulse-detection programs. Other important novel results of this study were that the estimated serum half-life of TSH was unchanged in thyrotropinomas, and that the secretory burst-shape defined by its mode, i.e., the time to reach to maximal secretion rate was similar in patients and controls, suggesting an unchanged secretory process. The regularity of the pulse intervals was normal in patients. Finally, each patient had a significant diurnal rhythm, with normal amplitude, increased mean level, and delayed acrophase, as also described in the patient of the study by Adriaanse (239). In summary, TSH secretion in thyrotropinomas is characterized by increased basal secretion,

**Figure 12.**

Serum TSH concentration profiles of 6 patients with a thyrotropinoma. The shaded zone represents the 95% confidence interval (CI) of 18 healthy matched control subjects. Reproduced with permission of Pituitary from reference 240.

increased pulse frequency and preserved diurnal rhythm, due to a nocturnal surge. However, a limitation of most reports is the rather low mean TSH values studied. Therefore, data on TSH secretion in patients with higher levels are required to complete the description.

Approximate entropy (ApEn) in TSH-secreting adenoma is increased, indicating diminished pattern orderliness. Endocrine adenomas are characterized by autonomous hormone overproduction, with diminished feedback responses. A common feature of all hormone-secreting adenomas is increased ApEn, including GH-secreting adenoma, prolactinoma, ACTH-secreting adenoma, aldosterone- and cortisol-secreting adrenal adenoma and parathyroid adenoma (241, 245–247). Medical treatment of acromegaly with somatostatin analogs does not normalize ApEn, suggesting that the cause of increased ApEn is tumoral, rather than increased GH secretion per se (242). Such data are not available for medically-treated patients with a TSH-secreting adenoma. An unexpected finding is increased TSH ApEn in patients who harbor GH- and ACTH-secreting pituitary adenomas. The mechanism may be that hypothalamic somatostatin release, putatively induced by irregular GH or cortisol hypersecretion, is less well coordinated with respect to pituitary TSH release. ACTH ApEn is normalized in surgically treated patients with Cushing's disease, while curative surgery in acromegaly normalizes GH ApEn in 70% of patients. A possible explanation for the latter is the low

IGF-I feedback in the presence of mild GH deficiency after surgery alone (248). Indeed, GH deficiency occurring after pituitary irradiation is accompanied by increased GH ApEn (246, 249).

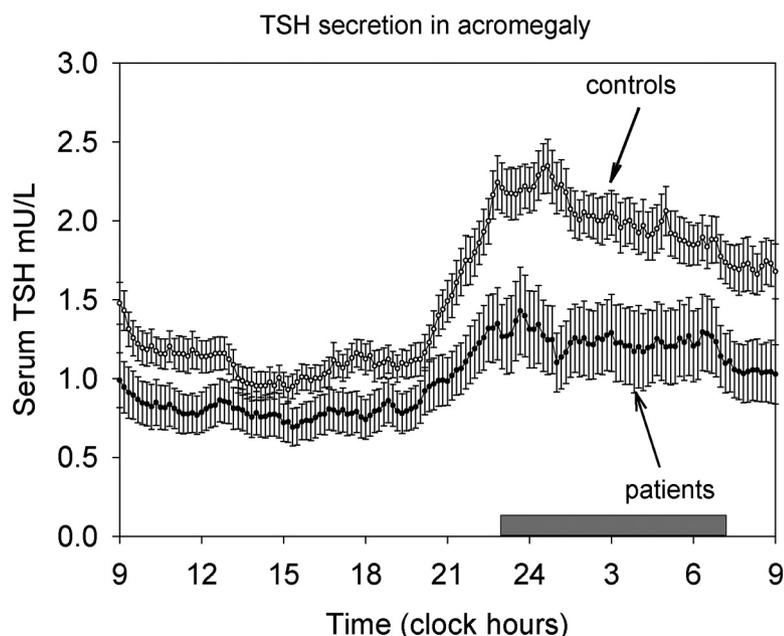
#### VIII b. Acromegaly

In acromegaly the TSH response to TRH administration is often blunted, although thyroid hormone levels are usually within the normal ranges (250–252). In experimental animals, GH modulates deiodinases in the liver and other organs, which may lead to (slightly) higher serum T3 and lower rT3 concentrations (23, 25, 253–255). Enhanced intracellular conversion of T4 into T3 in thyrotrope cells and/or folliculo-stellate cells of the pituitary gland in acromegaly may accentuate feedback inhibition of both the synthesis of  $\beta$ -subunit and secretion of intact TSH (23, 256). Similar inhibitory ef-

fects could be expected from GH-enhanced somatostatin and GH-diminished GHRH tonus (257). While hypothalamic somatostatin diminishes TSH release via SST2 and STT5 receptors, diminished GHRH release might also decrease the TSH-releasing effect of TRH. The latter consideration arises, because GHRH potentiates the TSH-releasing effect of TRH in healthy men and patients with acromegaly (258). Decreased leptin levels in active acromegaly could also contribute to reduced TSH by lowering TRH secretion (21, 34, 137, 259, 260).

Investigations in patients with active acromegaly have revealed that diurnal TSH concentrations are diminished compared with values in matched healthy volunteers [Figure 13]. Diminished TSH secretion in acromegaly is associated with less basal and pulsatile secretion. The latter is caused by diminished TSH pulse amplitude, with no changes in pulse frequency or hormone half-life (261). Interestingly, the acute TSH response to TRH correlated positively with total TSH secretion, whereas the latter correlated negatively with log GH secretion.

In acromegalic patients, total T4 is typically normal, but T3 is (slightly) increased and rT3 decreased as a result of amplified D2 deiodinase activity in the liver under GH excess. In the face of diminished TSH secretion, one would expect decreased serum T4 concentrations. We did not find this, thus corroborating other studies in untreated patients with acromegaly (250, 252). Possible explana-

**Figure 13.**

Mean 24-h serum TSH-concentration time series in 21 patients with active acromegaly and 21 healthy matched controls. Blood samples were taken every 10 min for 24 h, starting at 0900 h. Lights were off between 2300 h until 0730 h. Reproduced with permission of The Endocrine Society from reference 261.

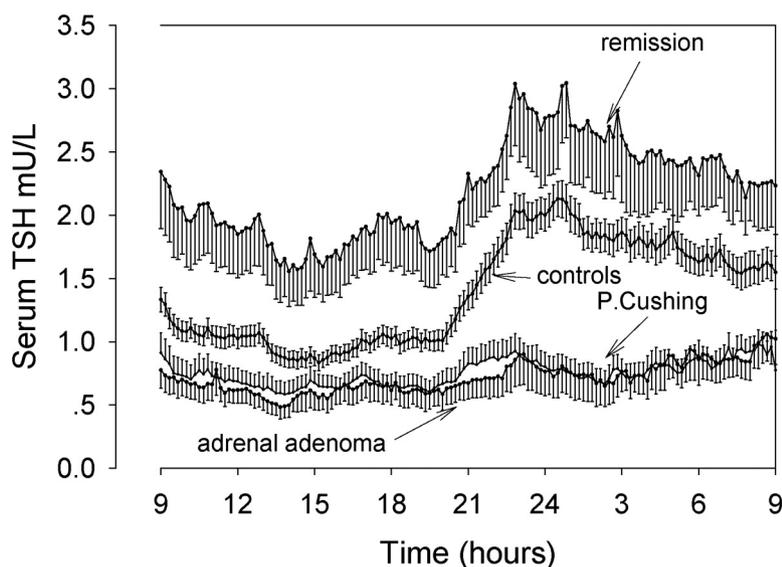
tions include: (I) increased biological activity of TSH by altered posttranslational processing of the oligosaccharide chains of the TSH molecule (175) and (II) enhanced sensitivity of the thyroid gland to TSH by factors such as elevated IGF-I or diminished inhibitory sympathetic neuronal input. The first possibility cannot be excluded without TSH bioassay and analysis of the carbohydrate moieties of the molecule. In favor of the second possibility are observations that the thyroid vasculature and follicular cells are stimulated by IGF-I and are richly innervated by the autonomic nervous system (262). Furthermore, there is a multisynaptic functional connection between the supra-chiasmatic nucleus and the thyroid gland in mammals, so that thyroid hormone secretion is regulated not only via TSH concentrations, but also by autonomic nervous-system modulation of glandular sensitivity to TSH (144). GH excess and low leptin levels diminish sympathetic activity, which may explain increased thyroid sensitivity to TSH. For instance, GH-overexpressing transgenic mice display reduced sympathetic outflow and reduced plasma and tissue norepinephrine concentrations, and patients with acromegaly exhibit reduced cardiac sympathetic activity (263, 264). Conversely, isolated GH deficiency may also be associated with abnormalities of 24-h TSH secretion pattern, but such studies are currently not available.

### VIII c. Cushing's disease

Previous reports have documented decreased efficacy of TRH in inducing TSH release in patients with hypercortisolism and in healthy subjects after glucocorticoid administration. Other reports have suggested a relationship between the degree of glucocorticoid excess and the TSH decrement (265–267). A single dose of glucocorticoids (1–2 mg dexamethasone) acutely suppresses pulsatile TSH production in healthy men (211). High-dose dexamethasone (16 mg/day) administration for 2.5 d decreased the mean 24-h concentration from 2.2 to 0.8 mU/L, and 24-h TSH secretion from 79 to 30 mU/day/m<sup>2</sup>. Although, T<sub>4</sub> and FT<sub>4</sub> levels remained unchanged, both T<sub>3</sub> and FT<sub>3</sub> levels fell. Finally, the TSH response to TRH decreased, pointing to a direct effect on the thyrotrope (265). Even a mild elevation of serum cortisol concentrations (about 32%) induced by timed cortisol infusions reduces pulsatile TSH

secretion by 50% (268).

There are only a few studies elucidating TSH dynamics in pituitary-dependent hypercortisolism. One study of 3 patients showed decreased pulsatile TSH secretion (269). Another study investigated 16 patients with Cushing's disease, 11 patients with Cushing's syndrome caused by unilateral or bilateral adrenal adenoma, 7 patients in long-term remission after curative pituitary surgery and 27 healthy control subjects (270). Mean serum TSH concentration profiles of patients with hypercortisolism and controls are displayed in [Figure 14], showing diminished TSH concentrations in patients with pituitary-dependent hypercortisolism and those with cortisol-secreting adrenal adenoma and recovery of TSH concentrations in the patients in remission after pituitary surgery. Pulse frequency, TSH secretion-burst shape (waveform), and estimated TSH half-lives were similar in all groups. The regularity of interburst intervals was also unchanged, as demonstrated by Weibull distribution estimates. Pulsatile (and thereby total) TSH secretion declined to a similar degree in both forms of hypercortisolism (i.e., pituitary-dependent or primary adrenal adenoma), which was mediated by a diminished pulse mass. In contrast, total TSH secretion rose in patients in remission due to amplified basal (nonpulsatile) secretion especially during daytime. T<sub>3</sub> levels were de-

**Figure 14.**

Twenty-four hour serum TSH-concentration time series in 16 patients with pituitary-dependent hypercortisolism (M. Cushing), 11 patients with primary adrenal hypercortisolism, 7 patients in remission after transsphenoidal surgery and 27 healthy controls. 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. Reproduced with permission of European Society of Endocrinology from reference 270.

increased in all patient groups, except surgically cured individuals. The severity of cortisol excess correlated negatively with TSH secretion, a result which resembles the relation between GH excess and TSH secretion in acromegaly (261). 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 serum free T4 concentrations levels remained within normal limits, as reported in other studies (266, 267, 269). Possible explanations for the relatively normal thyroxine concentrations during hypercortisolemia are that T4 to T3 conversion is blocked by glucocorticoids and that the biological activity of TSH is increased by altered posttranslational processing of its oligosaccharide chains (175).

Several *in vitro* studies have suggested a direct inhibitory effect of glucocorticoids on pituitary TSH secretion (267, 271). Subsequent studies have identified a role for annexin1 (lipocortin1), a protein produced by pituitary folliculo-stellate cells, which acts as a paracrine mediator of acute glucocorticoid effects on TSH, ACTH, prolactin and luteinizing hormone (LH) secretion (LH) (272–275). Suppression of TSH secretion by glucocorticoids in the human, either in acute experiments in volunteers or in patients suffering from chronic glucocorticosteroid excess, are consistent with *in vitro* data, particularly because a direct pituitary-inhibitory effect would not necessarily

change the pulse frequency or half-life of TSH. However, other hormonal regulatory systems are involved in TSH secretion in hypercortisolism. Somatostatin directly inhibits TSH secretion by activation of the SST2 and SST5 receptor-subtypes expressed on the thyrotrope (276). Animal studies have demonstrated that glucocorticoids rapidly increase hypothalamic somatostatin mRNA and somatostatin release (277–279). There is also experimental evidence that glucocorticoids repress TRH release by the paraventricular nucleus, which contains glucocorticoid receptor-immunoreactive TRH neurons (280–282). Furthermore, glucocorticoid administration induces long-lasting inhibition of TRH secretion in rats (283, 284). Lastly, glucocorticoids inhibit TRH mRNA expression in the PVN of the human hypothalamus (285). Precisely how these men-

tioned mechanisms operate collectively in the human under physiological and pathophysiological states requires clarification.

#### VIII d. Prolactinoma

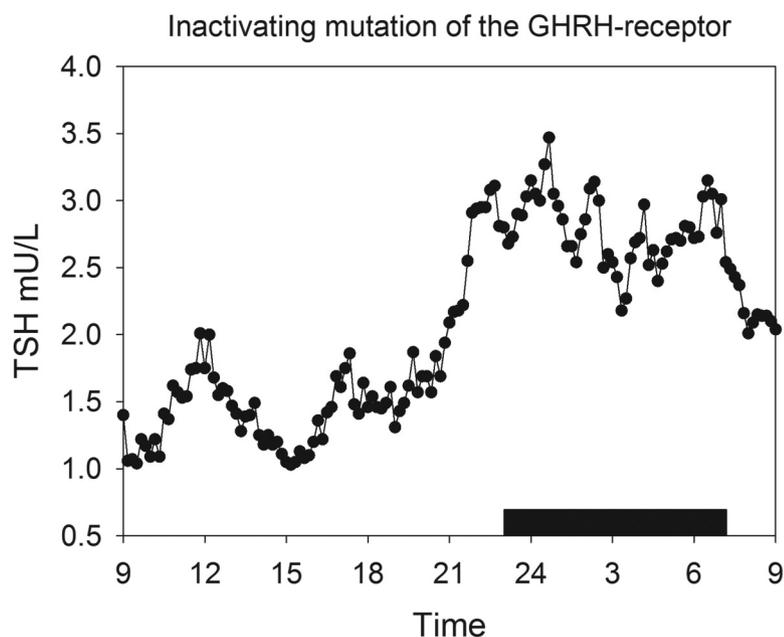
In patients with a prolactinoma the increase of TSH to TRH administration is similar to that found in normal subjects (286, 287), although one study reported an amplified response in patients (288). Administration of antidopaminergic drugs, e.g., sulpiride and domperidone resulted in a larger TSH increase in patients than in controls, suggesting a heightened endogenous dopamine outflow in patients (286, 287, 289). Nevertheless, serum T4 and T3 concentrations are unchanged in prolactinoma patients. There is only one study on TSH profiles in patients with prolactinoma available (290). In this study with hourly samples, patients displayed a clear diurnal variation, with higher serum concentrations during the nocturnal phase than during the day. No data on pulse frequency and other relevant rhythm parameters are currently available.

As summarized in Table 4, pituitary adenomas alter several key measures of HPT dynamics, including the nocturnal TSH surge and TSH pulsatility. Somatostatin may mediate some of the pattern changes, but nonexclusively.

**Table 4.** Thyroid hormone and TSH levels in pituitary hormone secreting adenomas

	T4(fT4)	T3(fT3)	TSH	Nocturnal surge	Rhythm study	References
Thyrotropinoma	↑	↑	↑ =	↓	yes	173,237,240
Acromegaly	=	↑ (mild)	↓	↓	yes	250,252,261
Cushing's disease	↓	↓	↓	↓	yes	266,267,269,270
Prolactinoma	=	=	=	=	yes	288,290

↑ increased, ↓ decreased, = no change

**Figure 15.**

Serum TSH concentration profile of a male patient with an inactivating mutation of the GHRH-receptor. Details on the defect and the impact on GH and PRL secretion were described earlier (Reference 292). Unpublished figure.

## IX. Other Endocrine Disorders

### IX a. Growth hormone deficiency and treatment

The causes of GH deficiency are diverse and include genetic abnormalities of the development of the pituitary gland, organic disease of the pituitary gland, e.g., pituitary adenoma, sarcoidosis, hypophysitis, hypothalamic tumors, such as craniopharyngioma, irradiation of the brain, trauma and inactivating mutations of the GHRH receptor. There are only a few studies on the 24-h TSH secretion profile available, of which two in children. Villarís investigated 4 GH-deficient prepubertal patients with a 20-min blood sampling scheme. Cluster analysis revealed higher TSH levels in patients, both during the day and night, increased pulse amplitude and area and interpulse levels, but unchanged pulse frequency. In addition, the diurnal TSH variation and T4 and T3 levels were not different from those in controls (291). We have investigated the serum TSH concentration profile in a patient

with an inactivating mutation of the GHRH-receptor (292). The profile obtained showed normal pulsatility and a clear nocturnal surge, all within normal limits (Figure 15). In contrast, in a much larger study comprising 52 children with pituitary diseases, of whom 44 were GH-deficient, the nocturnal TSH increase was diminished (mean increase 22% versus 124% in normal children). There was no correlation between the TSH increase after TRH administration and the maximal nocturnal TSH concentration unlike in normal children (178, 293). The heterogeneity of etiologies of GH deficiency may explicate some of the foregoing differences.

Replacement therapy with GH may affect the HPT-axis, although reports are not unanimous. GH treatment in 15 patients with an isolated form of GH deficiency, caused by an inactivating mutation of the

GHRH receptor had increased serum T3, but fT4, rT3 and TSH levels remained unchanged (294). GH treatment in GH-deficient patients with coexisting T4-substituted hypothyroidism decreased T4 and rT3 levels and increased T3 levels, without TSH changes, suggesting increased peripheral conversion of T4 to T3 (295). These investigators also investigated the 24-h TSH profile by measuring hourly samples (296). They found decreased TSH levels across the 24 h in 10 patients and loss of the weak diurnal rhythm, accompanied by an increase of serum T3 and fT3, and a decrease of rT3 levels (296). In another study in 49 patients with GH deficiency of whom the majority had multiple deficiencies and 70% received T4 replacement, GH treatment reduced FT4 levels, but only 2 patients developed overt hypothyroidism, and in the replacement group the dose had to be increased in only one patient (297). In contrast, the largest study of the effect of GH replacement on the HPT axis in 243 adult GH-deficient

patients described the emergence of biochemical hypothyroidism in 34 of 84 euthyroid patients (298). Sixteen percent of the T4-substituted patients required an increase in dose. Two studies in children suggest that the absence of the nocturnal TSH increase predisposes to the development of hypothyroidism during GH replacement (293, 299). In one small study in children T4 levels decreased and T3 levels increased, but remained within normal limits (300). In a larger study in 75 children, 8 became hypothyroid, but their basal levels were not predictive of GH-induced hypothyroidism (301) nor did children with organic pituitary disease differ from those suffering from isolated GH-deficiency (302). In summary, these reports suggest that the development of hypothyroidism during GH substitution is related to a compromised HPT axis of which early failure may be the absent or diminished nocturnal TSH increase.

#### **IX b. Addison's disease**

Early investigations in 3 patients with Addison's disease showed that withholding hydrocortisone substitution for 2 d elevates TSH levels (303). Inhibition of cortisol secretion by metyrapone administration to healthy subjects resulted in a mean TSH increase from 1.6 to 3.1 mU/L (265). In more extensive studies, metyrapone administration to healthy subjects, which reduced serum cortisol by 39% between 0800–1345 h and by 47% between 0200 and 0745 h, but not otherwise, selectively increased daytime TSH by 35%. The inverse relationship with cortisol suggests that the physiological cortisol increase during waking is responsible for the normal daytime TSH decrease (304). Hangaard and colleagues studied daytime TSH levels from 8.00 to 19.00 h during different glucocorticoid replacement doses in 12 patients. Hydrocortisone reduced the mean TSH concentration, but pulse frequency and pulse amplitude were unchanged, suggesting an effect on basal TSH secretion. They also noted an increase in T3 levels (305). In another study the effect of hydrocortisone was also studied in 7 patients. The authors devised different infusion protocols, one which imitated the normal serum cortisol pattern, another a day-night reversed cortisol pattern, one continuous administration and the last a saline control infusion. During all these administration schemes, TSH secretion remained pulsatile, and the mean 24-h TSH levels were similar in the 4 groups. However, day-time TSH was only lower and nighttime TSH higher during the imitation of the normal serum cortisol pattern, thus restoring the diurnal variation. This study therefore strongly suggests that physiological cortisol levels present during the morning hours can inhibit TSH secretion during that period, while this effect is minimized during the night (306).

#### **IX c. Sheehan's syndrome**

Sheehan's syndrome is a condition that affects women who have major blood loss during or after childbirth, leading to ischemic necrosis of the pituitary gland. Early symptoms are absence of lactation, amenorrhea and fatigue. Other anterior pituitary deficiencies may also occur. Nine patients with untreated Sheehan's syndrome had diminished FT4 levels and increased TSH levels. The paradoxical increase of TSH was attributed to diminished TSH bioactivity, caused by increased sialylation (176). In another study, afternoon TSH concentrations were compared with nocturnal levels. TSH levels were increased in both periods however without nocturnal increase (307). In an extension of this study the 24-h profile was investigated. Mean TSH concentrations, quantitated by Cluster, were increased almost 3-fold in patients, due to amplified basal (nonpulsatile) TSH levels, with normal pulse frequency, amplitude, duration and interpulse interval. Concomitantly, the diurnal TSH variation was absent (308). At present the role of GH and/or cortisol deficiency in altered TSH dynamics is uncertain, because specific pituitary function tests were not reported.

#### **IX d. Diabetes mellitus**

Patients with severe ketoacidosis manifest all the biochemical characteristics of the low T3 syndrome, i.e., diminished T3 levels, low normal T4 levels, increased rT3 levels and unchanged morning TSH (309). Five days after normalization of ketoacidosis and glucose levels, normal thyroid hormone levels are restored, but the response of TSH to TRH remains subnormal (310). Comparable changes are described in patients with uncontrolled type II diabetes, suggesting that ketoacidosis per se is not responsible for the low T3 syndrome (310). The nocturnal TSH increase was investigated in patients with and without C-peptide secretion by Coiro (311). During the uncontrolled state both groups had diminished T3 levels and an absent nocturnal TSH increase. After correction of the hyperglycemic state T3 levels normalized in both groups but the nocturnal TSH increase was restored only in patients with some endogenous insulin secretion, possibly pointing to a more favorable metabolic state. Finally one study compared patients with insulin-dependent and non-insulin dependent patients during hyperglycemia without ketoacidosis. All patients had the low T3 syndrome and absence of the nocturnal TSH surge. Five to six weeks after treatment all parameters were normalized, including the nocturnal TSH surge (312), suggesting that complete normalization of the HPT-axis requires several weeks and not days. There is only one study available in which the whole 24-h profile was studied in young male well-controlled young diabetes patients, albeit with hourly samples (170).

The TSH profile showed a clear circadian variation of TSH levels, with a statistically significant nocturnal increase. Collectively, these reports indicate that TSH secretion in well controlled patients is normal, although no details on secretion characteristics such as pulse amplitude, basal secretion and pulse frequency are currently available. Patients with ill-controlled diabetes mellitus develop the low T3 syndrome.

## **X. TSH secretion in psychiatric diseases**

### **X a. Endogenous depression**

Several investigations have shown that the TSH response to TRH is diminished in depressed patients (313–316). The first study exploring diurnal TSH variation in depressed patients was done by Weeke in 1978, by comparing TSH levels sampled at 2 pm and midnight. The nocturnal increase in TSH concentration correlated negatively with the severity of clinical symptoms. TSH changes also paralleled serum free T3 levels (317). Two years later this group published results of a complete 24-h TSH profile in 4 patients. Three patients still had a nocturnal TSH increase, although of decreased magnitude (318). In another study, 32 patients were sampled at 4-h intervals. They had decreased mean, maximum and minimum TSH values as well as attenuated amplitude of the day-night rhythm, compared with 32 matched controls. After clinical remission, all values increased (319). Three other studies reported partly conflicting results. One study in 13 patients found an absent nocturnal TSH increase and diminished TSH values in unipolar depressed patients, while bipolar patients had higher TSH values with an intact nocturnal increase (320). The second study in 12 patients also reported a diminished nocturnal rise in bipolar depressed patients (321), while the last study in nine depressed patients did not detect an abnormal TSH profile (322).

Sleep has a profound influence on the nocturnal TSH rise by suppressing values during this period. Sleep deprivation in healthy volunteers, in contrast, causes sustained TSH secretion during the night (83, 84). Several groups reported a transient improvement of clinical symptoms in patients after TRH injection (323, 324), although not confirmed by others (325).

Interestingly, sleep deprivation in depression may also lead to a short-lived improvement of clinical symptoms (326). These observations motivated studies in which the effect of sleep deprivation on the TSH-thyroid axis was investigated. Compared with healthy controls, patients with bipolar depression had lower TSH concentrations, no sustained nocturnal secretion and a blunted TSH response to TRH (327). Another study in which the bioac-

tivity of TSH was also measured reported higher TSH values and sustained secretion during sleep deprivation in responders, i.e., patients who had a temporary improvement of clinical symptoms (328), while serum TSH patterns in nonresponding patients and controls were similar. The authors concluded that depressed patients have mild compensated thyroid resistance to thyrotropin. Responding patients compensate this state with increased TSH secretion, while nonresponders secrete TSH with enhanced bioactivity. In recent study basal thyroid hormones and TSH concentrations were investigated in 113 unipolar, nonhospitalized and medication-free patients and compared with a carefully matched control population (329). Thyroid hormone levels in patients and controls were similar, but TSH slightly, but significantly elevated in patients. Finally, a study in which only part of the diurnal TSH profile was investigated, reported similar TSH values in the morning in patients and controls, but no nocturnal increase in patients, as well as diminished T4 and T3, and increased rT3, along with biochemical signs suggesting liver hypothyroidism (314). In short, reported findings in major depression are conflicting, possibly due to analysis of different patient groups, stages of psychiatric disease, outpatient or hospitalized subjects, nonoptimal blood sampling and small study groups. Altogether, available studies generally point to a diminished nocturnal TSH surge. A limitation is that none has employed intensive blood sampling, so that no information on pulsatility and secretory regularity is available. In addition, the mechanistic basis for TSH suppression is not established. One study evaluated TSH production indirectly in major depression. The investigators found a decrease in TRH gene expression in the hypothalamic paraventricular nucleus in 5 patients with major depression (330). Another possibility for diminished nocturnal TSH secretion in depression is accentuated inhibition of TSH by relatively elevated cortisol levels during the evening and first part of the night (321, 331). Conversely, some patients with (primary) hypothyroidism are depressed. Detection is important inasmuch as coexistent hypothyroidism may worsen major (endogenous) depression.

### **X b. Schizophrenia**

TSH and thyroid hormone secretion is less intensively studied in schizophrenia than in endogenous depression. Only one study is available on the diurnal variation of TSH measured by cosinor analysis on eight 3-h samples obtained in 90 treatment-free patients, 25 patients on neuroleptic treatment and 34 controls. The TSH mesor and amplitude were diminished and the acrophase somewhat advanced (earlier in time) shift. Treatment had no effect on the circadian parameters (332). There have been reports

on the beneficial effect of TRH on the clinical symptoms in uncontrolled studies (333), but these results could not be confirmed in double-blind placebo-controlled crossover studies (334–337).

### **X c. Nonspecified major psychiatric disease**

Hyperthyroxinemia can be present at the time of clinical admission of patients with psychiatric disorders. One study recorded 95 patients (3%) with elevated T4 and normal TSH in group of 3093 first-time admissions (338). The same study recorded elevated TSH in 5.9% of the admissions, especially in patients suffering from drug abuse. During the hospital stay and treatment of psychiatric patient with different etiologies, elevated TSH and T4 and T3 levels decrease (339, 340). Finally, one study found raised T4 levels in acutely ill schizophrenic patients, but TSH, T3 and rT3 levels were normal. Clinical signs were correlated with T4 levels, and during treatment levels fell and the decrease correlated significantly with clinical improvement. Patients in chronic remission or with stable residual disease did not show thyroid-axis dysfunction (341).

### **X d. Posttraumatic stress disorder**

Posttraumatic stress disorder (PTSD) is a severe anxiety disorder that can develop after exposure to threat of death to oneself or to someone else, or to one's own or someone else's physical, sexual, or psychological integrity, overwhelming the individual's ability to cope. Diagnostic symptoms for PTSD include re-experiencing the original trauma(s) through flashbacks or nightmares, avoidance of stimuli associated with the trauma, and increased arousal—such as difficulty falling or staying asleep, anger, and hypervigilance.

Most neuroendocrine investigations examined the hypothalamo-corticotrope-adrenal axis (342, 343). There are also several, although rather limited, studies on TSH-thyroid hormones in combat veterans, survivors of earthquakes, in childhood sexually abused women, military survival training, and refugees from the former German Democratic Republic (DDR). Combat veterans exhibited a moderate increase in total T3, accompanied by increased free T3 (344–346). Single TSH and FT4 levels did not differ from values in control subjects, and no detailed 24-h blood sampling profile studies were performed in any study on PTSD. Soldiers ( $n = 21$ ) who were exposed to survival training, which included captivity and intense interrogations, had lower total and free T3 with a tendency of increased TSH. No data were obtained on rT3. The whole group (72 subjects) experienced a drop in all thyroid hormones and TSH at the end of the training. The authors suggested that TSH alterations during the inter-

rogations reflected a combination of sleep and food deficits resulting in mild central hypothyroidism, although they did not exclude hypercortisolism as a possible mechanism (347). Unfortunately, no follow-up data are available. Interestingly, a comparable decrease of the thyroid hormones and TSH was described in 84 refugees of the former DDR, within 6 wk of their arrival in free Berlin (348). These subjects did not suffer from obvious clinical hypothyroidism, but appropriate investigations for confirming organ-level (e.g., liver and heart) hypothyroidism were not performed. Another study reported TSH levels in adolescents (mean age 14 yr), victims of an earthquake in 1988 in Armenia. A cohort from a city which was almost completely destroyed with many deaths had a slightly higher TSH than a control cohort from a city distant from the epicenter and which had sustained minor damage (349). One report on the effects of childhood sexual abuse comprised 63 patients and 42 healthy controls. Only total T3 was higher in patients, but not FT4 and total T4, free T3, TBG and TSH, not unlike findings found in combat veterans (350). Whereas PTSD studies are partly conflicting, subjects suffering from combat or sexual abuse PTSD tend to have elevated total T3 and frequently elevated free T3, suggesting peripheral changes in the metabolic fate of thyroxine, while other groups exhibit (mild) central hypothyroidism.

### **X e. Attention deficit hyperactivity disorder**

Attention deficit hyperactivity disorder (ADHD) is a developmental brain disorder, characterized by the coexistence of attentional problems and hyperactivity with signs and symptoms starting before the age of seven years. The cause of the syndrome is unknown, but there is a strong genetic background. Interestingly, about 40%–50% of the young patients suffering from thyroid hormone resistance also have ADHD (351). Serum T4 and T3, but not TSH correlated with symptoms. Specifically, thyroid hormone levels in ADHD patients are generally normal in some reports (352, 353), albeit correlated with symptoms (354). An exception is a report of abnormal thyroid hormone parameters with normal TSH (355). Exogenous T4 in therapeutic doses does not induce ADHD, since patients who received early treatment for congenital hypothyroidism display introversion and clumsiness rather than social negativity and inattention (356). In view of the sparse findings, there is certainly a need for detailed studies of the TSH rhythm in ADHD patients.

## **XI. Neurological disorders**

The rationale for investigating thyroid function in some neurological diseases is possible involvement of the thal-

amus and hypothalamus, e.g., narcolepsy, Huntington's disease and Parkinson's disease.

#### XI a. Narcolepsy

Narcolepsy is a neurological disorder characterized by excessive daytime sleepiness, hypnagogic hallucinations, cataplexy, and sleep paralysis (357). Disruption of hypocretin (orexin) neurotransmission underlies the disease in animals and humans (358). Hypocretins are produced by a small group of neurons in the lateral hypothalamus projecting widely throughout the central nervous system (CNS), including to the paraventricular nucleus, which synthesizes TRH (359–361). Conversely, hypocretin neural circuits are modulated by the activity of hypothalamic TRH neurons in animals (362). Unfortunately, few clinical studies have investigated the HPT axis in narcolepsy. In one study in 7 patients and controls 10-min blood samples were collected and TSH measured (363). Mean TSH concentrations were diminished, although still in the normal range. The decrement was caused by reduced pulsatile and basal secretion. Total T4 and T3, however, did not differ between the groups. The cause for the decreased TSH-T4 relation is not known [Figure 15].

#### XI b. Huntington's disease

Huntington's disease is a progressive autosomal dominant neurodegenerative disorder caused by CAG repeat expansion in exon 1 of the *huntingtin* gene (HTT), resulting in a long polyglutamine tract in the N-terminus of the encoded protein (364). The disease is characterized by motor disturbances, cognitive decline and behavioral problems. Other hallmarks are progressive weight loss, muscle wasting and abnormalities in glucose homeostasis (365). Neuronal degeneration is most prominent in the cerebral cortex and striatum, but neuronal death is also found in the tuberal nucleus of the hypothalamus. The last hypothalamic abnormality could potentially modify major endocrine axes (366, 367). In addition, loss of hypothalamic dopamine D2 receptors was demonstrated by *in vivo* studies in early stage patients (368). These findings justify a full exploration of the hypothalamo-pituitary axes. There are relatively few studies on the HPT axis in this disease, but in general no abnormal levels of T4, T3 and TSH are observed in single specimens (369–371). However, TSH and fT3 correlated with disease severity in one study (371). In a study of 24-h TSH secretion the mean 24-h level and total secretion rate estimated by deconvolution (AutoDeconv) were about 20% higher in patients ( $n = 9$ ) than in controls ( $n = 9$ ), but these differences were not statistically significant (372). Interestingly, the nocturnal TSH surge appeared larger in patients than in controls, but this was not tested separately. In line with this finding were the in-

creased levels of T4 and T3 in patients, albeit still in the normal range. Verifying mild central activation of the HPT-axis will require analyses in additional patients with more advanced Huntington's disease.

#### XI c. Parkinson's disease

Parkinson's disease is another degenerative disorder of the CNS, characterized by death of dopamine-generating cells in the substantia nigra. Early signs and symptoms are movement-related, including tremor, rigidity, slowness of movement and difficulty walking. Sleep disruption, emotional, cognitive and behavioral problems may arise later, with dementia occurring in late stages of the disease. Several investigations, but not all, have shown an increased prevalence of (primary) hypothyroidism, often subclinical (373–377). Two recent independent studies reported loss of hypocretin-secreting and melanin-concentrating hormone (MCH) neurons in the lateral hypothalamus of Parkinson patients (378, 379). In the same area, Lewi bodies, the pathological hallmark of Parkinson's disease, were found. The hypocretin and MCH neurons project widely throughout the CNS and play a key role in the regulation of the sleep-wake cycle, body-energy homeostasis and autonomic function (380, 381). Nevertheless, tissue and CSF levels of hypocretin were still measurable, unlike in patients with narcolepsy. A possible decrease of TRH drive, and thereby of TSH, was tested by intensive 24-h blood sampling in 9 patients in the early stage of the disease (382). Basal, pulsatile and total TSH secretion rates did not differ. Pulse frequency and mean pulse mass, quantitated by AutoDeconv were comparable in patients and controls. The nocturnal surge seemed to be diminished, but the latter was not quantitated separately. Free T4 levels were higher in patients than in controls (16.7 vs 13.9 pmol/L), and T3 levels were comparable. Summarizing, available data do not point to gross abnormalities of the HPT-axis, but an increased incidence of primary hypothyroidism and a diminished nocturnal TSH surge are not excluded. These studies require confirmation in a larger cohort of patients with more advanced stages of the disease and suggest a role for monitoring thyroid function during follow-up.

### XIII. Conclusions and perspectives

Basal, pulsatile and entropic features of TSH secretion are regulated by feedforward and feedback signals acting on the hypothalamic-thyrotrope unit in healthy volunteers, obese premenopausal women, patients with pituitary disorders and primary hypothyroidism. Inhibitory effects of dopamine, somatostatin and glucocorticoids operate in healthy subjects. There is also a negative impact of calorie deprivation and nonthyroidal illness. While T4

and T3 both feedback negatively on TSH secretion, comparable to cortisol on ACTH, the long half-lives of total T3 and T4 have precluded precise analyses of feedback kinetics and endogenous dose-responsive inhibition. Dynamics of the HPT axis remain largely unexplored in common clinical and social conditions, such as overweight, chronic stress, puberty, diabetes mellitus and aging. Such potentially important, but expensive and time-consuming, investigations should ultimately include other neuroendocrine systems in order to dissect multihormone interactive physiology.

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