MECHANISMS IN ENDOCRINOLOGY

Beyond the fixed setpoint of the hypothalamus–pituitary–thyroid axis

Eric Fliers[1], **Andries Kalsbeek**[1,2] and **Anita Boelen**[1]

[1]Department of Endocrinology and Metabolism, Academic Medical Center, University of Amsterdam, 1105 AZ Amsterdam, The Netherlands and [2]Hypothalamic Integration Mechanisms, Netherlands Institute for Neuroscience, Amsterdam, The Netherlands

Correspondence should be addressed to E Fliers

Email
e.fliers@amc.uva.nl

Abstract

The hypothalamus–pituitary–thyroid (HPT) axis represents a classical example of an endocrine feedback loop. This review discusses dynamic changes in HPT axis setpoint regulation, identifying their molecular and cellular determinants, and speculates about their functional role. Hypothalamic thyrotropin-releasing hormone neurons were identified as key components of thyroid hormone (TH) setpoint regulation already in the 1980s, and this was followed by the demonstration of a pivotal role for the thyroid hormone receptor beta in negative feedback of TH on the hypothalamic and pituitary level. Gradually, the concept emerged of the HPT axis setpoint as a fixed entity, aiming at a particular TH serum concentration. However, TH serum concentrations appear to be variable and highly responsive to physiological and pathophysiological environmental factors, including the availability or absence of food, inflammation and clock time. During food deprivation and inflammation, TH serum concentrations decrease without a concomitant rise in serum TSH, reflecting a deviation from negative feedback regulation in the HPT axis. Surprisingly, TH action in peripheral organs in these conditions cannot be simply predicted by decreased serum TH concentrations. Instead, diverse environmental stimuli have differential effects on local TH metabolism, e.g. in liver and muscle, occurring quite independently from decreased TH serum concentrations. The net effect of these differential local changes is probably a major determinant of TH action at the tissue level. In sum, hypothalamic HPT axis setpoint regulation as well as TH metabolism at the peripheral organ level is flexible and dynamic, and may adapt the organism in an optimal way to a range of environmental challenges.

Introduction

The tripeptide thyrotropin-releasing hormone (TRH) was the first hypothalamic hormone to be isolated and structurally characterised in the 1960s. Subsequent immunocytochemical studies in the rat hypothalamus revealed the presence of TRH neurons in a number of hypothalamic nuclei. A key role for TRH neurons in the paraventricular nucleus (PVN) of the hypothalamus in the neuroendocrine regulation of thyroid hormone (TH)

Invited Author's profile
Prof. E Fliers has been Head of the Department of Endocrinology and Metabolism at the Academic Medical Center in Amsterdam since 2007. Prof. E Fliers was one of the founders of the Netherlands Brain Bank. His current research interests include the hypothalamus–pituitary–thyroid axis, and the neuro-endocrine response to illness. He is also the current chair of the Dutch Endocrine Society.

was revealed in the 1980s when an inverse relationship of serum TH levels with *TRH* mRNA expression in the PVN was observed during experimentally induced hypo- and hyperthyroidism (1). TRH neurons in the medial and periventricular parvocellular subdivisions of the PVN project to the median eminence (ME), in line with observations in experimental hypothyroidism showing increased *TRH* mRNA only in these subdivisions of the PVN (2).

Together, these observations led to the concept of hypothalamus–pituitary–thyroid (HPT) axis setpoint regulation, reflected by highly constant intra-individual TH serum concentrations under basal conditions. The standard model of thyroid homeostasis postulates an intraindividual logarithmic relationship between serum free thyroxine (FT_4) levels and pituitary thyrotropin (TSH) release (for review see (3)). Twin studies showed that heritability accounts for $>60\%$ of the variation in serum

TSH and FT_4 (4), while later studies identified a number of genetic loci linked to the HPT axis setpoint (5). The concept of a fixed intra-individual TH serum concentration was reinforced in the clinical setting once serum TSH concentrations could reliably be measured. Elevated serum TSH became the key laboratory finding in patients with primary hypothyroidism, while the reverse (suppressed serum TSH) was true in primary hyperthyroidism. Moreover, serum TSH became the most important biochemical monitor in the treatment of patients with levothyroxine. However, already in the 1980s, it became clear that a variety of illnesses, including myocardial infarction, sepsis and surgical procedures, cause a decrease in serum triiodothyronine (T_3) levels and – in severe cases – T_4, without an elevation in TSH. Since then, additional examples of exogenous factors (schematically represented in Fig. 1) inducing deviations from fixed setpoint regulation have been uncovered. Examples of
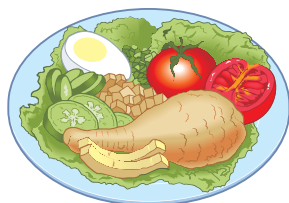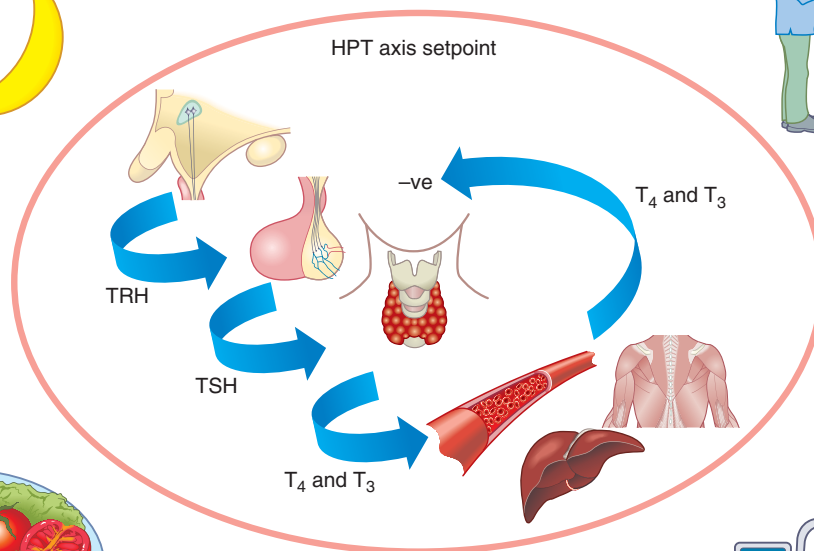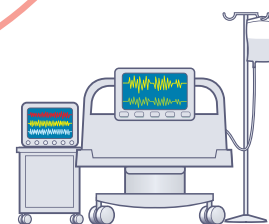

Figure 1

Exogenous determinants of the hypothalamus–pituitary–thyroid (HPT) axis setpoint. On the left side some examples are shown of physiological factors influencing the HPT axis setpoint, i.e. the day–night rhythm and the availability or absence of food. On the right side some pathophysiological factors are shown, i.e. acute inflammation and critical illness. Within the circle, the HPT axis setpoint is driven mainly by TRH neurons in the hypothalamic paraventricular nucleus (PVN).

physiological factors include the diurnal TSH rhythm with a clear nocturnal TSH surge (6), which is driven by the hypothalamic suprachiasmatic nucleus (SCN), and prolonged fasting, which induces a decrease both in serum T_3 (by 30%) and serum TSH (by 70%) in healthy men (7). By inference, feeding status and time-of-day effects should be considered in careful interpretation of serum TSH in a clinical setting. Examples of pathophysiological factors inducing low serum TH without an increase in serum TSH include acute inflammation and prolonged critical illness (8). This review discusses dynamic changes in HPT axis setpoint regulation, identifying their molecular and cellular determinants, and speculates about their functional role.

HPT axis regulation in the basal state

Hypothalamus

TRH neurons ▶ The hypothalamic tripeptide TRH was discovered in the 1960s and subsequently shown to regulate the synthesis, release and biological activity of TSH via the TRH receptor (TRHR). TRH-synthesising neurons are present in a number of hypothalamic nuclei, but only hypophysiotropic TRH neurons located in the PVN are involved in the central regulation of the HPT axis. In the rat hypothalamus, hypophysiotropic TRH neurons are found in the medial and periventricular subdivisions of the parvocellular PVN exclusively (for review see (2)). In the 1990s, the first studies on the distribution of TRH neurons in the human hypothalamus appeared (9, 10) showing that TRH-containing neurons and fibres are present in a number of hypothalamic nuclei, including the PVN, the SCN, which contains the circadian pacemaker of the brain acting as a biological clock, and the sexually dimorphic nucleus. The human PVN contains many spindle-shaped and spheric multipolar parvocellular TRH neurons, especially in its dorsocaudal portion, while only a small number of magnocellular neurons express TRH. Although the precise efferent projections of hypothalamic TRH-containing neurons in the human brain are unknown, dense TRH fibre networks, e.g. in the perifornical area, suggest an important role for nonhypophysiotropic TRH neurons in the human brain as demonstrated earlier in the rat. A key role for thyroid hormone receptor beta 2 (TRβ2) in TH negative feedback on TRH neurons in the PVN was demonstrated by studies carried out in TR isoform-specific knockout mice (11), although immunocytochemical studies showed that TRH neurons in the PVN may express all TR isoforms (12, 13).

Local TH metabolism ▶ A number of molecular determinants, including transporters and enzymes, are critical for local TH bioavailability. THs have to be transported into cells in order to be able to exert their effects. In the human hypothalamus, three types of TH transporters have been reported: the organic anion transporting polypeptide 1C1 (OATP1C1), which preferentially transports T_4, and the monocarboxylate transporter 8 (MCT8) and MCT10, facilitating both the uptake and efflux of T_3 and T_4 (14, 15, 16). Once transported into the cell, only T_3 binds to the TR in the nucleus, while the pro-hormone T_4 needs to be converted into the active hormone T_3 by the deiodinating enzymes (17). Deiodination of TH is catalysed by the selenoenzyme family of iodothyronine deiodinases, which consists of three deiodinases: type 1 (D1), type 2 (D2), and type 3 (D3). Both the inner (phenolic) ring and the outer (tyrosyl) ring of T_4 can be deiodinated, ultimately leading to the formation of the inactive 3,3′-di-iodothyronine (T_2). D1 is mainly expressed in liver, kidney, thyroid, and pituitary, and it can deiodinate both the inner- and the outer-ring of T_4. D2 and D3 are the major deiodinating enzymes in the central part of the HPT-axis. D2 is expressed in many areas of the brain, and also in the pituitary, brown adipose tissue (BAT), placenta and – although at remarkably low levels – in skeletal muscle. It represents the main T_3-producing enzyme in these tissues (18). D2 in the cortex and pituitary gland is negatively regulated by T_3 and T_4 at the pre- and post-transcriptional level respectively (19). D3 is a TH-inactivating enzyme, as it can only catalyse the inner-ring deiodination of T_4 and T_3. D3 is highly expressed in brain, especially during development, and in placenta (18). The interplay between tissue D2 and D3 determines the local availability of intracellular T_3 levels and, thereby, the level of T_3-regulated gene expression. Both D2 and D3 are expressed in the hypothalamus. D2 activity was reported in the rat hypothalamus, especially in the arcuate nucleus (ARC), already in the 1980s (20), and both D2 and D3 enzyme activities were reported in human pituitary and hypothalamic tissue samples obtained during autopsy (21). Moreover, D2 immunoreactivity is present in cells throughout the ependymal layer of the third ventricle, in the glial cells within the infundibular nucleus/ME region and in hypothalamic blood vessel walls. D3 expression showed a very different distribution, as D3 immunoreactivity was reported only in neurons in various hypothalamic nuclei, including the PVN, suggesting that D3 is expressed in T_3-responsive neurons to terminate T_3 action (for review see (22)).

Neurally mediated effects of intrahypothalamic T_3 on metabolism ▶ In addition to acting on hypophysiotropic TRH neurons in the PVN, thereby regulating the HPT axis, intrahypothalamic T_3 exerts metabolic effects in peripheral organs via neural routes, e.g. via sympathetic and parasympathetic outflow from the brain to BAT, liver, and heart (23). The first indication that metabolic effects of THs can be centrally mediated was obtained in mice heterozygous for a mutant *Trα1* with low affinity for T_3. These mice were hypermetabolic and showed a high BAT activity with increased thermogenesis and energy expenditure. The metabolic phenotype was blunted after a functional denervation of sympathetic signalling to BAT by housing them at thermoneutrality, suggesting that the CNS controlled the hypermetabolism of these mice through the autonomic nervous system (24). Then, Lopez *et al*. (25) showed that the activation of the thermogenic programme in the BAT through the sympathetic nervous system (SNS) depends on T_3-mediated activation of *de novo* lipogenesis by inhibiting AMPK in the ventromedial nucleus of the hypothalamus (VMH), establishing a role for T_3 in the VMH in the regulation of BAT. In addition to the VMH, T_3 was shown to act within the PVN to regulate hepatic glucose production and insulin sensitivity via sympathetic and parasympathetic outflow to the liver (26, 27). A third example of modulation by TH of neural outflow from the hypothalamus was recently provided in yet another hypothalamic neuron population, i.e. the parvalbuminergic neurons in the anterior hypothalamic area (AHA). These neurons require TR signalling for proper development and function and depend on THs to integrate temperature information with the regulation of cardiovascular parameters via modulation of central autonomic outflow (28). Finally, intrahypothalamic T_3 has stimulating effects on eating behaviour. Specifically, the T_3-mediated hyperphagia was shown to be mediated by activation of the mTOR pathway in the hypothalamic ARC, where mTOR co-localises with the TRα (29). These novel and topographically highly differential metabolic effects of intrahypothalamic T_3 are schematically represented in Fig. 2.

Functional connections ▶ Detailed studies in rodents have shed light on the numerous and complex neural inputs to hypophysiotropic TRH neurons. Together with humoral signals reaching the PVN via the circulation, TRH neurons can integrate metabolic and endocrine information obtained via neural projections, enabling them to adjust the activity of the HPT axis to the changing
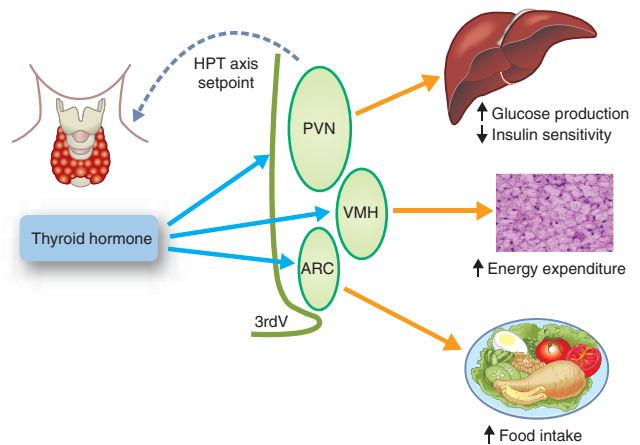

Figure 2

Thyroid hormone (TH) modulates energy metabolism via neural routes originating in hypothalamic nuclei. In the paraventricular nucleus (PVN), TH has a negative feedback action on hypophysiotropic TRH neurons, which are a major determinant of the HPT axis setpoint. In addition, TH modulates pre-autonomic neurons in the PVN, thereby modulating autonomic (both sympathetic and parasympathetic) outflow to the liver, in turn modulating endogenous glucose production and hepatic insulin sensitivity. In addition, TH affects neurons in the VMH, thereby stimulating energy expenditure in brown adipose tissue (BAT). Finally, TH acts on neurons in the arcuate nucleus (ARC) that modulate eating behaviour. 3rdV, third ventricle; yellow lines, neural pathways; blue lines, endocrine pathways.

environmental conditions. The ARC is an important hypothalamic nucleus sending efferent projections to TRH neurons in the PVN, thereby conveying information about the metabolic state of the organism. Within the ARC, two neuronal populations are particularly involved in the relay of metabolic information, i.e. the orexigenic neurons that produce neuropeptide Y (NPY) and agouti-related protein and the anorexigenic neurons that produce αMSH and CART. Similar innervation patterns of TRH neurons, with the exception of CART, have been reported in the human hypothalamus (for review see (30, 31)). In addition to the ARC, anatomical and physiological experiments have shown a role for the hypothalamic dorsomedial nucleus in the regulation of hypophysiotropic TRH neurons, but little information is available on the mechanisms involved. Finally, hypophysiotropic TRH neurons receive a dense catecholaminergic innervation from the brain stem, the majority of which is from

adrenergic neurons (32), and this input is probably involved in the response of TRH neurons to cold (33).

Over the past decade, tanycytes have been recognised as important regulators of the HPT axis. These cells are specialised glial cells that line the ventrolateral wall and the floor of the third ventricle. Although there are several subtypes, they all have a small cell body located in the ependymal layer and a long process that may project to the ME, or the ARC, VMH, or DMH. The role of tanycytes in HPT axis regulation is increasingly recognised. These cells express TRs, as well as MCT8 and OATP1C1, and are capable of adapting their morphology according to the changes in circulating TH levels, perhaps regulating TRH release from hypophysiotropic terminals into the portal circulation. Furthermore, they express the TRH-degrading enzyme PPII, which is upregulated in hyperthyroidism. Finally, tanycytes are assumed to contribute to HPT axis feedback regulation by their expression of D2 and – under defined circumstances – D3 (for review see (31)).

Anterior pituitary

The anterior pituitary contains various types of adenohypophysial cells that are defined by the hormones secreted. Thyrotrophs secrete TSH and are preferentially located in the anteromedial and anterolateral portions of the pituitary. These cells express the TRHR, which is a member of the seven transmembrane-spanning, GTP-binding, G protein-coupled receptor family. Activation of this receptor by TRH stimulates both synthesis and release of TSH. Increased hormone production is thought to be regulated via activation of protein kinase C, while rapid release of stored TSH is regulated via activation of inositol 1,4,5-triphosphate (IP_3) and subsequent release of intracellular Ca^{2+}. TRH also stimulates the glycosylation of TSH, which is necessary for its full biological activity (34). TSH production and secretion are also regulated by circulating TH levels, as high TH levels inhibit TSH production and secretion while low TH levels activate TSH production. This so-called negative feedback regulation of TSH involves local D2-mediated conversion of T_4 into T_3, which is subsequently bound by TRβ2, finally resulting in the repression of the *TSHβ* gene (11). A crucial role of pituitary D2 in TSH regulation is supported by impaired TH feedback on TSH in D2-knockout mice (35).

Additional inhibitors of TSH secretion are the hypothalamic neuropeptide somatostatin, as well as dopamine and glucocorticoids. The latter impair the sensitivity of the pituitary to TRH. Pituitary peptides such as neuromedin B and PIT1, both expressed in thyrotrophs, are further

determinants of TSH secretion (36, 37, 38). Finally, IGSF1, a pituitary membrane glycoprotein, was recently identified as a novel player in TSH regulation. Loss-of-function mutations in the *IGSF1* gene result in congenital central hypothyroidism. Animal studies using *Igsf1*-knockout male mice exhibit diminished pituitary TRH-R expression, decreased pituitary and serum TSH levels, and decreased serum T_3 concentrations, in line with the clinical observations (39). The net result of these various peptidergic, enzymatic and neuroendocrine factors determines serum TSH concentration, which plays a critical role in the regulation of the thyroid gland by activating the TSHR on the follicular thyrocytes. The TSHR is also expressed by folliculo-stellate (FS) cells in the human anterior pituitary, suggesting that TSH secretion might be additionally regulated in a paracrine manner via FS cells (40).

Thyroid gland and peripheral organs

TH production by the thyroid gland is mainly regulated by TSH via binding to the TSHR on the follicular thyrocyte. Activation of the TSHR stimulates a variety of processes involved in TH synthesis, ultimately resulting in the release of T_4 (the prohormone) and T_3 (the active hormone) from thyroglobulin (41). In healthy individuals, 20% of daily T_3 production is secreted by the thyroid gland, whereas 80% is generated extrathyroidally by iodothyronine deiodinases (42). Once released, T_4 and T_3 circulate in the bloodstream bound to serum proteins including thyroid hormone-binding globulin, transthyretin, and albumin. Over 99% of serum THs is bound, leaving ∼1% of TH as freely available for uptake by target tissues. As mentioned earlier, TH are actively transported into cells in order to exert their effects, while the prohormone T_4 needs to be converted into the active hormone T_3 by deiodinating enzymes (17). It has been thought for many years that liver D1 is critical for release of T_3 into the circulation, but more recent studies have suggested that liver D1 is more important for TH clearance in the hyperthyroid state (43). Its expression is positively regulated by T_3 (44, 45).

In the last few years, polymorphisms of deiodinating enzymes have been reported (46). The consequences of these polymorphisms on the regulation of the HPT-axis are unknown at present, although subtle changes in serum TH concentrations occur in association with these polymorphisms. In the *DIO1* gene, two polymorphisms have been identified that affect serum T_3 and reverse T_3 (rT_3) concentrations in healthy subjects, i.e. D1-C785T

and D1-A1814G. The D1-785T variant is associated with higher rT_3 levels and a lower T_3/rT_3 ratio, suggesting that this substitution results in decreased D1 activity. By contrast, the D1-1814G substitution is associated with a higher T_3/rT_3 ratio, which indicates increased activity of D1. For the *DIO2* gene an association was reported in young subjects between the serum T_3/T_4 ratio and a polymorphism in a short open reading frame (*ORFa*) in the 5′-UTR of D2 (D2-ORFa-Gly3Asp) (47). Another polymorphism in the *DIO2* gene, D2-Thr92Ala, is not associated with serum TH or TSH levels, but with insulin resistance (48) and decreased bone turnover (49). The mechanism has remained enigmatic as cells transfected with D2-92A or D2-92Thr do not show altered D2 activity. As to the *DIO3* gene, one polymorphism has been identified (D3-T1546G), located in the 3′-UTR, but this variant does not affect serum TH levels in healthy subjects.

In target tissues, T_3 has to be bound by a TR to modulate gene transcription. The TR is a member of the nuclear receptor family, and the protein structure consists of different domains, i.e. the N-terminal activation function 1 (AF1) domain (A/B), the DNA-binding domain (C), the hinge region (D) and the C-terminal AF2 domain (E) (50). TRs are encoded by two genes: the *TRHA* and *TRHB* genes. Owing to alternative splicing and alternative promoter usage, the *TRHA*-gene may give rise to six isoforms: *TRα1*, *TRα2*, *TRΔ1* and *TRΔ2*, and *p46* and *p28* (51). The *TRHB* gene encodes the *TRβ1* and *TRβ2* isoform via alternative promoter usage (52). Only the *TRβ1*, *TRβ2*, and *TRα1* are bona fide TRs, having a ligand-binding domain and a DNA-binding domain which modulate gene-transcription (51). The function of the other isoforms is unknown, although *TRα2* and the short isoforms *TRΔα1* and *TRΔα2* are able to inhibit *TRα1* and *TRβ1*-mediated transcriptional activation (53). TRα binds T_3 with slightly higher affinity than TRβ1 (54). The DNA-binding domain of the receptor modulates gene transcription by binding to specific DNA sequences, known as thyroid hormone-response elements (TREs). TRs can bind to a TRE as monomers, as homodimers or as heterodimers with the retinoid X receptor, which is another member of the nuclear receptor superfamily that binds 9-*cis* retinoic acid. The heterodimer has the highest affinity and represents the major functional form of the receptor. *TRα1* and *TRβ1* show extensive sequence homology, specifically in domain C, D, and E. However, *TRα1* and *TRβ1* have isoform-specific roles in the mediation of T_3 action, which is supported by the fact that TRs are differentially expressed during embryonic development, in different tissues and even within the same organ (21, 55).

Additional levels of transcriptional regulation can be achieved by the potential of both isoforms to homo- or heterodimerise, by the type of TRE present on the promoters of T_3 target genes and by the interaction with various cellular proteins. These cellular proteins can also be expressed in a tissue-dependent and developmentally regulated manner (56).

Mutations in the TR give rise to a variety of clinical symptoms depending on the TR involved. Resistance to thyroid hormone (RTH) is a clinical syndrome wherein TH levels are increased without adequate suppression of TSH. The most common cause is heterozygous mutations in the *TRHB* gene, mostly affecting the ligand-binding domain and the hinge region. The mutant *TRβ* displays either reduced affinity for the ligand T_3 or disturbed interaction with cofactors necessary for T_3 action. RTH occurs to a similar extent in both sexes and has a world-wide distribution with an incidence of ~ 1 in 40 000 (57). Classic symptoms of RTH are goitre, tachycardia, developmental delay and failure to thrive, hearing loss, and bone age retardation (58), although the clinical picture is highly variable. Serum TH levels are increased in association with TSH within or just above the reference range due to nonresponsiveness of the pituitary and/or hypothalamus to regulate TSH production upon stimulation of the TRβ2. RTH patients display a hyperthyroid phenotype in tissues mainly expressing *TRα1*, such as the heart (tachycardia), while in tissues mainly expressing the *TRβ1* (liver and kidney) and *TRβ2* (hypothalamus, pituitary, cochlea, and retina) a hypothyroid phenotype is observed. Recently, mutations in the *THRA* gene have been reported, which are associated with growth and developmental retardation, skeletal dysplasia, and severe constipation. Of note, serum TH levels are only slightly abnormal. The clinical phenotype is a characteristic for hypothyroidism with regard to the skeleton, intestine and neural development, reflecting TRα-responsive tissues (59, 60).

HPT axis setpoint regulation: examples of physiological determinants

Clock time

One of the physiological determinants known to affect the HPT-axis is clock time: serum TSH is low during daytime, starts to increase in the early evening and peaks around the beginning of the sleep period. This phenomenon is known as the nocturnal TSH surge in humans (61, 62). The diurnal TSH rhythm is generated by the hypothalamic SCN, which is the biological clock of the brain, as

demonstrated by a number of experimental studies in rats (63). First, efferent fibres from the SCN contact TRH neurons in the PVN. Second, neuroanatomical studies using a retrograde transneuronal tracer revealed multi-synaptic neural connections between the hypothalamic SCN and the thyroid gland via sympathetic and para-sympathetic outflow. In addition, pre-autonomic neurons in the PVN, including TRH-immunoreactive neurons, were labelled after injection of the tracer into the thyroid gland (for review see (63)). Finally, a role for the SCN as the driver of the diurnal TSH rhythm in the circulation was confirmed by the observation that a thermic ablation of the SCN completely eliminates the diurnal peak in circulating TSH in rats (64). A recent study in healthy volunteers has confirmed that the 24-h TSH secretion is stable and robust, and not influenced by sex, BMI, or age (65). In spite of the clear diurnal variation in serum TSH levels, a diurnal rhythm in serum T_3 and T_4 concentrations is less obvious, illustrating that the diurnal TSH rhythm is not driven by negative feedback of serum TH on the level of the hypothalamus or pituitary. Finally, it should be noted that the physiologic meaning of the TSH rhythm is still elusive.

Feeding status

Feeding status is a major determinant of HPT-axis regulation. Fasting induces profound changes in TH metabolism characterised by decreased serum TH levels while serum TSH does not change or even decreases. The absence of a rise in serum TSH, which would be expected as a consequence of decreased negative feed-back regulation, suggests that the hypothalamus and/or pituitary is involved in the observed alterations, as they are reminiscent of central hypothyroidism. In line, animal experiments showed that the fasting-induced central hypothyroidism could be completely prevented by systemic leptin administration, i.e. by restoring the fasting-induced decrease in serum leptin concentrations (66). The primary target for leptin in this setting appeared to be the ARC, from which monosynaptic efferent connections to the PVN modulate the activity of hypophysiotropic TRH neurons. The observed down-regulation of the central component of the HPT axis is further characterised by an increase in D2 expression in the mediobasal hypothalamus, presumably increasing local T_3 concentrations, and a decrease in TRH expression in the PVN (67, 68) (see also (31)). Chan *et al*. showed that a period of 72-h fasting in healthy men induces a decrease in serum T_3 by 30%, and a

marked suppression of TSH secretion with a decrease in integrated area by over 70% as well as loss of the typical pulsatility characteristics observed in the fed state. Interestingly, administration of a replacement dose of leptin designed to maintain serum leptin at levels similar to those in the fed state largely prevented the starvation-induced changes in the HPT axis (7). In addition to these changes at the central level of the HPT axis during food deprivation, peripheral TH metabolism is also affected by fasting. For example, liver D3 activity increases in mice after 48 h of starvation, which may further decrease hepatic T_3 availability. Leptin administration selectively restores this starvation-induced D3 increase, independently of altered serum TH concentrations (69). The combination of central and peripheral alterations is likely to account for the fasting-induced decrease in serum TH levels. At present, it is unknown to what extent peripheral changes are mediated centrally. A recent study in mice, however, has shown that both the melanocortin receptors MC4R and NPY are required for the activation of hepatic pathways that metabolise T_4 during the fasting response (70), showing that starvation reduces TH availability both through central and peripheral circuits. The fasting-induced decrease in serum TH levels is assumed to be an important adaptive mechanism to conserve energy during times of food shortage (71).

HPT axis setpoint regulation: examples of pathophysiological determinants

It has been known for many years already that profound changes in TH metabolism occur during illness, the so-called nonthyroidal illness syndrome (NTIS) or the low-T_3 syndrome. NTIS is characterised by decreased serum T_3 and – in severe illness – serum T_4, as well as increased serum rT_3 concentrations. The expected increase in serum TSH is absent, reflecting a major change in negative feedback regulation (72). NTIS is a heterogeneous entity, and may occur in the setting of a great variety of illnesses (72). Recent studies have shown that TH action at the tissue level during illness is not a simple reflection of serum TH concentrations. Instead, NTIS has differential effects on local TH metabolism in various organs, which appear to occur quite independently from decreased serum T_3 and T_4 concentrations. The net effect of these differential changes is probably a major determinant of TH availability and, therefore, of TH action at the tissue level.

Acute inflammation

Acute inflammation is known to induce profound alterations in both circulating serum TH levels and tissue TH metabolism. Although the inflammation-induced alterations in local TH metabolism have not been studied extensively in humans, major surgery – an example of acute NTIS – induces a rapid inflammatory response characterised by activation of neutrophils and the release of a variety of proinflammatory cytokines (73, 74, 75). Simultaneously, significant alterations in serum T_3, T_4 and rT_3 concentrations and in T_3/rT_3 and T_3/T_4 ratios are observed, suggesting impaired TH conversion. Experimental studies in rodents have shown that administration of bacterial endotoxin (lipopolysaccharide (LPS)), which represents a model for severe and acute inflammation, results in down regulation of TRH expression in the PVN of the hypothalamus, probably via a local activation of D2 in tanycytes lining the third ventricle (76, 77). This may explain the absence of a TSH response to the decreased serum TH concentrations. LPS administration elicits a strong inflammatory response, characterised by the production of a variety of cytokines including tumor necrosis factor alpha, interleukin 1 (IL1), and IL6. For the induction of cytokines, the activation of inflammatory signalling pathways such as NFκB and activator protein 1 is mandatory (78). LPS administration also results in marked local changes in liver and muscle TH metabolism. For instance, hepatic D1 and D3 expression and activity decrease after LPS (79), presumably resulting in decreased liver TH concentrations, while D2 expression and activity increase in close correlation with liver IL1β. Inflammation-induced D2 expression was confirmed in macrophages and was absent in hepatocytes (Fig. 3). Moreover, D2 knockdown in macrophages attenuated LPS-induced granulocyte-macrophage colony-stimulating factor (GM-CSF) expression and affected phagocytosis in a negative way. Macrophages express *MCT10* and *TRα1*, while hepatocytes predominantly express the TRβ1. Thus, locally produced T_3, acting via the TRα, may be instrumental in the inflammatory response in the liver (Fig. 3). In line, LPS-treated TRα$^{0/0}$ mice showed a markedly decreased LPS-induced *Gm-csf* (*Csf2*) mRNA expression (80).

Chronic inflammation, sepsis, and critical illness

Chronic inflammation, sepsis, and critical illness are all associated with profound decreases in serum TH levels. The magnitude of the decrease in serum T_3 is related to the
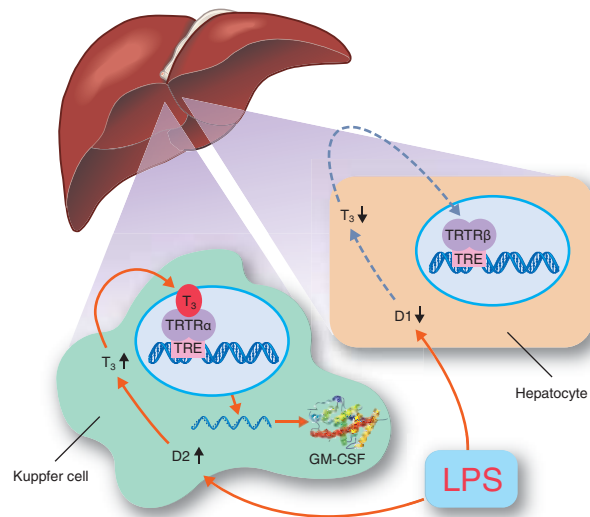

Figure 3

Differential intra-hepatic effects of acute inflammation on thyroid hormone (TH) metabolism. After administration of LPS, which serves as an experimental model for acute inflammation, the activity of type 1 deiodinase (D1) in the hepatocyte decreases. By inference, intracellular T_3 concentrations decrease, resulting in less T_3-dependent gene expression via thyroid hormone receptor beta (TRβ). By contrast, the expression of D2 in Kuppfer cells is stimulated by LPS. This will result in a higher intracellular T_3 concentration and, thereby, induction of T_3-dependent gene expression via TRα, including *GM-CSF*.

severity of illness; serum T_3 may become very low or even undetectable in critical illness. In severe cases, serum T_4 decreases as well and is inversely correlated with mortality: when serum T_4 falls below 50 nmol/l the risk of death increases to 50%, and with serum T_4 below 25 nmol/l mortality increases even further to 80% (for reviews see (8, 72)). A systematic review of studies in patients with sepsis and/or septic shock confirmed a correlation between decreased thyroid function at baseline and worse outcome (81). Careful analysis of the secretory TSH profile in patients with critical illness showed a loss of the nocturnal TSH surge as well as a loss of the pulsatile fraction, with a dramatically suppressed pulse amplitude in the prolonged phase of illness (82). Thus, although a simple TSH measurement can be within the reference range, the lack of TSH pulse amplitude correlates positively with the low serum T_3. Together, these observations point to altered setpoint regulation at the level of hypophysiotropic TRH neurons in the PVN. Indeed, *TRH* mRNA expression in the PVN was reduced in the hypothalamus of patients who had died after prolonged illness, and correlated positively

(instead of negatively) with serum T_3 (83). The latter observations were confirmed in a rabbit model for critical illness (for review see (8)). As the infusion of exogenous TRH together with the growth hormone (GH) secretagogue GH-releasing peptide 2 in critically ill patients restored not only pulsatile TSH and GH secretion but also circulating T_3 and T_4 levels, the suppression of the HPT axis in critical illness seems primarily of hypothalamic origin. In addition to changes in HPT axis setpoint regulation, the net result of which is decreased serum TH concentrations, there are marked changes in peripheral tissue TH uptake, metabolism and signalling. Only few studies have addressed TH tissue concentrations in this setting (84). Other examples of studies in ICU patients at the tissue level have shown that the decrease in serum T_3 is associated with changes in deiodinase expression in liver and muscle (85). Two recent review articles have addressed this issue extensively (8, 72).

Although NTIS may represent an adaptive response during acute inflammation, NTIS might turn disadvantageous during prolonged critical illness, necessitating mechanical ventilation, dialysis and inotropic support. There are many studies to suggest that the neuroendocrine response to illness can be seen as a dynamic process, with distinct features in the acute and chronic phase of critical illness (86), but only very few studies have addressed the changes in local TH metabolism in patients with prolonged critical illness. These studies were mostly based on samples obtained from critically ill patients shortly after death. Liver T_3 and T_4 concentrations were reported to be low in samples of NTIS patients as compared with healthy controls, indicating that the liver may be deficient in THs during prolonged critical illness (87). In agreement with this are the decreased liver T_3 levels observed in a rabbit model of prolonged critical illness (88). Prolonged critically ill patients develop a neuroendocrine dysfunction with suppressed hypothalamic TRH expression. The mechanism behind the suppression at the central level of the HPT axis in these patients is unknown at present. It is important to note that prolonged critically ill patients may theoretically benefit from correction of the TH changes, but this challenging hypothesis has not been tested to date.

Conclusion

Under basal conditions, the HPT axis is regulated by negative TH feedback at the hypothalamic and pituitary level, resulting in stable circulating FT_4 concentrations. However, a number of environmental challenges induce complex interactions of novel players, including D2

in hypothalamic tanycytes, which result in a net TH setpoint change. For example, during food deprivation and inflammation, TH serum concentrations decrease without a concomitant rise in serum TSH. Surprisingly, TH action at the tissue level in these conditions is not a simple reflection of decreased TH serum concentrations. Instead, there appear to be differential effects on local TH metabolism in liver and muscle, which occur quite independently from TH serum concentrations. In sum, hypothalamic HPT axis setpoint regulation as well as TH metabolism at the peripheral organ level appear to be dynamic, and may help to adapt the organism to a range of environmental challenges.

References

1 Segerson TP, Kauer J, Wolfe HC, Mobtaker H, Wu P, Jackson IM & Lechan RM. Thyroid hormone regulates TRH biosynthesis in the paraventricular nucleus of the rat hypothalamus. *Science* 1987 **238** 78–80. (doi:10.1126/science.3116669)

2 Lechan RM & Fekete C. The TRH neuron: a hypothalamic integrator of energy metabolism. *Progress in Brain Research* 2006 **153** 209–235.

3 Dietrich JW, Landgrafe G & Fotiadou EH. TSH and thyrotropic agonists: key actors in thyroid homeostasis. *Journal of Thyroid Research* 2012 **2012** 351864. (doi:10.1155/2012/351864)

4 Hansen PS, Brix TH, Sørensen TI, Kyvik KO & Hegedüs L. Major genetic influence on the regulation of the pituitary–thyroid axis: a study of healthy Danish twins. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 1181–1187. (doi:10.1210/jc.2003-031641)

5 Panicker V, Wilson SG, Spector TD, Brown SJ, Kato BS, Reed PW, Falchi M, Richards JB, Surdulescu GL, Lim EM *et al.* Genetic loci linked to pituitary–thyroid axis set points: a genome-wide scan of a large twin cohort. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 3519–3523. (doi:10.1210/jc.2007-2650)

6 Roelfsema F & Veldhuis JD. Thyrotropin secretion patterns in health and disease. *Endocrine Reviews* 2013 **34** 619–657. (doi:10.1210/er.2012-1076)

7 Chan JL, Heist K, Depaoli AM, Veldhuis JD & Mantzoros CS. The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short-term starvation in healthy men. *Journal of Clinical Investigation* 2003 **111** 1409–1421. (doi:10.1172/JCI200317490)

8 Mebis L & Van den Berghe G. Thyroid axis function and dysfunction in critical illness. *Best Practice & Research. Clinical Endocrinology & Metabolism* 2011 **25** 745–757. (doi:10.1016/j.beem.2011.03.002)

9 Fliers E, Noppen NW, Wiersinga WM, Visser TJ & Swaab DF. Distribution of thyrotropin-releasing hormone (TRH)-containing cells

and fibers in the human hypothalamus. *Journal of Comparative Neurology* 1994 **350** 311–323. (doi:10.1002/cne.903500213)

10 Guldenaar SE, Veldkamp B, Bakker O, Wiersinga WM, Swaab DF & Fliers E. Thyrotropin-releasing hormone gene expression in the human hypothalamus. *Brain Research* 1996 **743** 93–101. (doi:10.1016/S0006-8993(96)01024-4)

11 Abel ED, Ahima RS, Boers ME, Elmquist JK & Wondisford FE. Critical role for thyroid hormone receptor β2 in the regulation of paraventricular thyrotropin-releasing hormone neurons. *Journal of Clinical Investigation* 2001 **107** 1017–1023. (doi:10.1172/JCI10858)

12 Lechan RM, Qi Y, Jackson IM & Mahdavi V. Identification of thyroid hormone receptor isoforms in thyrotropin-releasing hormone neurons of the hypothalamic paraventricular nucleus. *Endocrinology* 1994 **135** 92–100.

13 Alkemade A, Vuijst CL, Unmehopa UA, Bakker O, Vennstrom B, Wiersinga WM, Swaab DF & Fliers E. Thyroid hormone receptor expression in the human hypothalamus and anterior pituitary. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 904–912. (doi:10.1210/jc.2004-0474)

14 Heuer H & Visser TJ. Minireview: Pathophysiological importance of thyroid hormone transporters. *Endocrinology* 2009 **150** 1078–1083. (doi:10.1210/en.2008-1518)

15 Alkemade A, Friesema EC, Kuiper GG, Wiersinga WM, Swaab DF, Visser TJ & Fliers E. Novel neuroanatomical pathways for thyroid hormone action in the human anterior pituitary. *European Journal of Endocrinology* 2006 **154** 491–500. (doi:10.1530/eje.1.02111)

16 Alkemade A, Friesema EC, Kalsbeek A, Swaab DF, Visser TJ & Fliers E. Expression of thyroid hormone transporters in the human hypothalamus. *Journal of Clinical Endocrinology and Metabolism* 2011 **96** E967–E971. (doi:10.1210/jc.2010-2750)

17 Yen PM, Ando S, Feng X, Liu Y, Maruvada P & Xia X. Thyroid hormone action at the cellular, genomic and target gene levels. *Molecular and Cellular Endocrinology* 2006 **246** 121–127. (doi:10.1016/j.mce.2005.11.030)

18 Gereben B, Zeold A, Dentice M, Salvatore D & Bianco AC. Activation and inactivation of thyroid hormone by deiodinases: local action with general consequences. *Cellular and Molecular Life Sciences* 2008 **65** 570–590. (doi:10.1007/s00018-007-7396-0)

19 Burmeister LA, Pachucki J & St Germain DL. Thyroid hormones inhibit type 2 iodothyronine deiodinase in the rat cerebral cortex by both pre- and posttranslational mechanisms. *Endocrinology* 1997 **138** 5231–5237.

20 Riskind PN, Kolodny JM & Larsen PR. The regional hypothalamic distribution of type II 5′-monodeiodinase in euthyroid and hypothyroid rats. *Brain Research* 1987 **420** 194–198. (doi:10.1016/0006-8993(87)90260-5)

21 Alkemade A, Friesema EC, Unmehopa UA, Fabriek BO, Kuiper GG, Leonard JL, Wiersinga WM, Swaab DF, Visser TJ & Fliers E. Neuroanatomical pathways for thyroid hormone feedback in the human hypothalamus. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 4322–4334. (doi:10.1210/jc.2004-2567)

22 Fliers E, Alkemade A, Wiersinga WM & Swaab DF. Hypothalamic thyroid hormone feedback in health and disease. *Progress in Brain Research* 2006 **153** 189–207.

23 Fliers E, Klieverik LP & Kalsbeek A. Novel neural pathways for metabolic effects of thyroid hormone. *Trends in Endocrinology and Metabolism* 2010 **21** 230–236. (doi:10.1016/j.tem.2009.11.008)

24 Sjogren M, Alkemade A, Mittag J, Nordstrom K, Katz A, Rozell B, Westerblad H, Arner A & Vennstrom B. Hypermetabolism in mice caused by the central action of an unliganded thyroid hormone receptor α1. *EMBO Journal* 2007 **26** 4535–4545. (doi:10.1038/sj.emboj.7601882)

25 Lopez M, Varela L, Vazquez MJ, Rodriguez-Cuenca S, Gonzalez CR, Velagapudi VR, Morgan DA, Schoenmakers E, Agassandian K, Lage R *et al*. Hypothalamic AMPK and fatty acid metabolism mediate thyroid regulation of energy balance. *Nature Medicine* 2010 **16** 1001–1008. (doi:10.1038/nm.2207)

26 Klieverik LP, Sauerwein HP, Ackermans MT, Boelen A, Kalsbeek A & Fliers E. Effects of thyrotoxicosis and selective hepatic autonomic denervation on hepatic glucose metabolism in rats. *American Journal of Physiology. Endocrinology and Metabolism* 2008 **294** E513–E520. (doi:10.1152/ajpendo.00659.2007)

27 Klieverik LP, Janssen SF, van Riel A, Foppen E, Bisschop PH, Serlie MJ, Boelen A, Ackermans MT, Sauerwein HP, Fliers E *et al*. Thyroid hormone modulates glucose production via a sympathetic pathway from the hypothalamic paraventricular nucleus to the liver. *PNAS* 2009 **106** 5966–5971. (doi:10.1073/pnas.0805355106)

28 Mittag J, Lyons DJ, Sällström J, Vujovic M, Dudazy-Gralla S, Warner A, Wallis K, Alkemade A, Nordström K, Monyer H *et al*. Thyroid hormone is required for hypothalamic neurons regulating cardiovascular functions. *Journal of Clinical Investigation* 2013 **123** 509–516. (doi:10.1172/JCI65252)

29 Varela L, Martínez-Sánchez N, Gallego R, Vázquez MJ, Roa J, Gándara M, Schoenmakers E, Nogueiras R, Chatterjee K, Tena-Sempere M *et al*. Hypothalamic mTOR pathway mediates thyroid hormone-induced hyperphagia in hyperthyroidism. *Journal of Pathology* 2012 **227** 209–222. (doi:10.1002/path.3984)

30 Fliers E, Unmehopa UA & Alkemade A. Functional neuroanatomy of thyroid hormone feedback in the human hypothalamus and pituitary gland. *Molecular and Cellular Endocrinology* 2006 **251** 1–8. (doi:10.1016/j.mce.2006.03.042)

31 Fekete C & Lechan RM. Central regulation of hypothalamic–pituitary–thyroid axis under physiological and pathophysiological conditions. *Endocrine Reviews* 2013 **35** 159–194. (doi:10.1210/er.2013-1087)

32 Fuzesi T, Wittmann G, Lechan RM, Liposits Z & Fekete C. Noradrenergic innervation of hypophysiotropic thyrotropin-releasing hormone-synthesizing neurons in rats. *Brain Research* 2009 **1294** 38–44. (doi:10.1016/j.brainres.2009.07.094)

33 Perello M, Stuart RC & Nillni EA. The role of intracerebroventricular administration of leptin in the stimulation of prothyrotropin releasing hormone neurons in the hypothalamic paraventricular nucleus. *Endocrinology* 2006 **147** 3296–3306. (doi:10.1210/en.2005-1533)

34 Chiamolera MI & Wondisford FE. Thyrotropin-releasing hormone and the thyroid hormone feedback mechanism. *Endocrinology* 2009 **150** 1091–1096. (doi:10.1210/en.2008-1795)

35 Schneider MJ, Fiering SN, Pallud SE, Parlow AF, St Germain DL & Galton VA. Targeted disruption of the type 2 selenodeiodinase gene (DIO2) results in a phenotype of pituitary resistance to T_4. *Molecular Endocrinology* 2001 **15** 2137–2148. (doi:10.1210/mend.15.12.0740)

36 Shupnik MA. Thyroid hormone suppression of pituitary hormone gene expression. *Reviews in Endocrine & Metabolic Disorders* 2000 **1** 35–42. (doi:10.1023/A:1010008318961)

37 Steel JH, Van Noorden S, Ballesta J, Gibson SJ, Ghatei MA, Burrin J, Leonhardt U, Domin J, Bloom SR & Polak JM. Localization of 7B2, neuromedin B, and neuromedin U in specific cell types of rat, mouse, and human pituitary, in rat hypothalamus, and in 30 human pituitary and extrapituitary tumors. *Endocrinology* 1988 **122** 270–282. (doi:10.1210/endo-122-1-270)

38 Ortiga-Carvalho TM, Curty FH, Nascimento-Saba CC, Moura EG, Polak J & Pazos-Moura CC. Pituitary neuromedin B content in experimental fasting and diabetes mellitus and correlation with thyrotropin secretion. *Metabolism* 1997 **46** 149–153. (doi:10.1016/S0026-0495(97)90293-6)

39 Sun Y, Bak B, Schoenmakers N, van Trotsenburg AS, Oostdijk W, Voshol P, Cambridge E, White JK, le Tissier P, Gharavy SN *et al*. Loss-of-function mutations in IGSF1 cause an X-linked syndrome of central hypothyroidism and testicular enlargement. *Nature Genetics* 2012 **44** 1375–1381. (doi:10.1038/ng.2453)

40 Prummel MF, Brokken LJ, Meduri G, Misrahi M, Bakker O & Wiersinga WM. Expression of the thyroid-stimulating hormone receptor in the folliculo-stellate cells of the human anterior pituitary. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 4347–4353. (doi:10.1210/jcem.85.11.6991)

41 Scanlon MF, Toft AD. Regulation of thyrotropin secretion. In: *The Thyroid*, 8th Ed, pp 234–253. Eds LE Braverman & RD Utiger. Philadelphia, PA, USA: Lippincott 2005.

42 Maia AL, Kim BW, Huang SA, Harney JW & Larsen PR. Type 2 iodothyronine deiodinase is the major source of plasma T3 in euthyroid humans. *Journal of Clinical Investigation* 2005 **115** 2524–2533. (doi:10.1172/JCI25083)

43 Schneider MJ, Fiering SN, Thai B, Wu SY, St Germain E, Parlow AF, St Germain DL & Galton VA. Targeted disruption of the type 1 selenodeiodinase gene (Dio1) results in marked changes in thyroid hormone economy in mice. *Endocrinology* 2006 **147** 580–589. (doi:10.1210/en.2005-0739)

44 Jakobs TC, Schmutzler C, Meissner J & Kohrle J. The promoter of the human type I 5′-deiodinase gene – mapping of the transcription start site and identification of a DR+4 thyroid-hormone-responsive element. *European Journal of Biochemistry* 1997 **247** 288–297. (doi:10.1111/j.1432-1033.1997.00288.x)

45 Toyoda N, Zavacki AM, Maia AL, Harney JW & Larsen PR. A novel retinoid X receptor-independent thyroid hormone response element is present in the human type 1 deiodinase gene. *Molecular and Cellular Biology* 1995 **15** 5100–5112.

46 Peeters RP, van der Deure WM & Visser TJ. Genetic variation in thyroid hormone pathway genes; polymorphisms in the TSH receptor and the iodothyronine deiodinases. *European Journal of Endocrinology* 2006 **155** 655–662. (doi:10.1530/eje.1.02279)

47 Peeters RP, van den Beld AW, Attalki H, Toor H, de Rijke YB, Kuiper GG, Lamberts SW, Janssen JA, Uitterlinden AG & Visser TJ. A new polymorphism in the type II deiodinase gene is associated with circulating thyroid hormone parameters. *American Journal of Physiology. Endocrinology and Metabolism* 2005 **289** E75–E81. (doi:10.1152/ajpendo.00571.2004)

48 Canani LH, Capp C, Dora JM, Meyer EL, Wagner MS, Harney JW, Larsen PR, Gross JL, Bianco AC & Maia AL. The type 2 deiodinase A/G (Thr92Ala) polymorphism is associated with decreased enzyme velocity and increased insulin resistance in patients with type 2 diabetes mellitus. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 3472–3478. (doi:10.1210/jc.2004-1977)

49 Heemstra KA, Hoftijzer H, van der Deure WM, Peeters RP, Hamdy NA, Pereira A, Corssmit EP, Romijn JA, Visser TJ & Smit JW. The type 2 deiodinase Thr92Ala polymorphism is associated with increased bone turnover and decreased femoral neck bone mineral density. *Journal of Bone and Mineral Research* 2010 **25** 1385–1391. (doi:10.1002/jbmr.27)

50 Aranda A & Pascual A. Nuclear hormone receptors and gene expression. *Physiological Reviews* 2001 **81** 1269–1304.

51 Bassett JH, Harvey CB & Williams GR. Mechanisms of thyroid hormone receptor-specific nuclear and extra nuclear actions. *Molecular and Cellular Endocrinology* 2003 **213** 1–11. (doi:10.1016/j.mce.2003.10.033)

52 Wood WM, Dowding JM, Haugen BR, Bright TM, Gordon DF & Ridgway EC. Structural and functional characterization of the genomic locus encoding the murine β2 thyroid hormone receptor. *Molecular Endocrinology* 1994 **8** 1605–1617.

53 Liu RT, Suzuki S, Miyamoto T, Takeda T, Ozata M & DeGroot LJ. The dominant negative effect of thyroid hormone receptor splicing variant α 2 does not require binding to a thyroid response element. *Molecular Endocrinology* 1995 **9** 86–95.

54 Yen PM. Physiological and molecular basis of thyroid hormone action. *Physiological Reviews* 2001 **81** 1097–1142.

55 Stoykov I, Zandieh-Doulabi B, Moorman AF, Christoffels V, Wiersinga WM & Bakker O. Expression pattern and ontogenesis of thyroid hormone receptor isoforms in the mouse heart. *Journal of Endocrinology* 2006 **189** 231–245. (doi:10.1677/joe.1.06282)

56 Cheng SY, Leonard JL & Davis PJ. Molecular aspects of thyroid hormone actions. *Endocrine Reviews* 2010 **31** 139–170. (doi:10.1210/er.2009-0007)

57 Refetoff S & Dumitrescu AM. Syndromes of reduced sensitivity to thyroid hormone: genetic defects in hormone receptors, cell transporters and deiodination. *Best Practice & Research. Clinical Endocrinology & Metabolism* 2007 **21** 277–305. (doi:10.1016/j.beem.2007.03.005)

58 Ferrara AM, Onigata K, Ercan O, Woodhead H, Weiss RE & Refetoff S. Homozygous thyroid hormone receptor β-gene mutations in resistance to thyroid hormone: three new cases and review of the literature. *Journal of Clinical Endocrinology and Metabolism* 2012 **97** 1328–1336. (doi:10.1210/jc.2011-2642)

59 Bochukova E, Schoenmakers N, Agostini M, Schoenmakers E, Rajanayagam O, Keogh JM, Henning E, Reinemund J, Gevers E, Sarri M et al. A mutation in the thyroid hormone receptor α gene. *New England Journal of Medicine* 2012 **366** 243–249. (doi:10.1056/NEJMoa1110296)

60 van Mullem A, van Heerebeek R, Chrysis D, Visser E, Medici M, Andrikoula M, Tsatsoulis A, Peeters R & Visser TJ. Clinical phenotype and mutant TRα1. *New England Journal of Medicine* 2012 **366** 1451–1453. (doi:10.1056/NEJMc1113940)

61 Brabant G, Prank K, Ranft U, Schuermeyer T, Wagner TO, Hauser H, Kummer B, Feistner H, Hesch RD & von zur Mühlen A. Physiological regulation of circadian and pulsatile thyrotropin secretion in normal man and woman. *Journal of Clinical Endocrinology and Metabolism* 1990 **70** 403–409. (doi:10.1210/jcem-70-2-403)

62 Allan JS & Czeisler CA. Persistence of the circadian thyrotropin rhythm under constant conditions and after light-induced shifts of circadian phase. *Journal of Clinical Endocrinology and Metabolism* 1994 **79** 508–512.

63 Kalsbeek A & Fliers E. Daily regulation of hormone profiles. *Handbook of Experimental Pharmacology* 2013 **217** 185–226.

64 Kalsbeek A, Fliers E, Franke AN, Wortel J & Buijs RM. Functional connections between the suprachiasmatic nucleus and the thyroid gland as revealed by lesioning and viral tracing techniques in the rat. *Endocrinology* 2000 **141** 3832–3841. (doi:10.1210/endo.141.10.7709)

65 Roelfsema F, Pijl H, Kok P, Endert E, Fliers E, Biermasz NR, Pereira AM & Veldhuis JD. Thyrotropin secretion in healthy subjects is robust and independent of age and gender, and only weakly dependent on body mass index. *Journal of Clinical Endocrinology and Metabolism* 2013 **99** 570–578. (doi:10.1210/jc.2013-2858)

66 Legradi G, Emerson CH, Ahima RS, Flier JS & Lechan RM. Leptin prevents fasting-induced suppression of prothyrotropin-releasing hormone messenger ribonucleic acid in neurons of the hypothalamic paraventricular nucleus. *Endocrinology* 1997 **138** 2569–2576.

67 Diano S, Naftolin F, Goglia F & Horvath TL. Fasting-induced increase in type II iodothyronine deiodinase activity and messenger ribonucleic acid levels is not reversed by thyroxine in the rat hypothalamus. *Endocrinology* 1998 **139** 2879–2884.

68 Rondeel JM, Heide R, de Greef WJ, van Toor H, van Haasteren GA, Klootwijk W & Visser TJ. Effect of starvation and subsequent refeeding on thyroid function and release of hypothalamic thyrotropin-releasing hormone. *Neuroendocrinology* 1992 **56** 348–353. (doi:10.1159/000126248)

69 Boelen A, van Beeren M, Vos X, Surovtseva O, Belegri E, Saaltink DJ, Vreugdenhil E, Kalsbeek A, Kwakkel J & Fliers E. Leptin administration restores the fasting-induced increase of hepatic type 3 deiodinase expression in mice. *Thyroid* 2012 **22** 192–199. (doi:10.1089/thy.2011.0289)

70 Vella KR, Ramadoss P, Lam FS, Harris JC, Ye FD, Same PD, O'Neill NF, Maratos-Flier E & Hollenberg AN. NPY and MC4R signaling regulate thyroid hormone levels during fasting through both central and peripheral pathways. *Cell Metabolism* 2011 **14** 780–790. (doi:10.1016/j.cmet.2011.10.009)

71 Boelen A, Wiersinga WM & Fliers E. Fasting-induced changes in the hypothalamus–pituitary–thyroid axis. *Thyroid* 2008 **18** 123–129. (doi:10.1089/thy.2007.0253)

72 Boelen A, Kwakkel J & Fliers E. Beyond low plasma T3: local thyroid hormone metabolism during inflammation and infection. *Endocrine Reviews* 2011 **32** 670–693. (doi:10.1210/er.2011-0007)

73 Boelen A, Platvoet-ter Schiphorst MC & Wiersinga WM. Association between serum interleukin-6 and serum 3,5,3′-triiodothyronine in

nonthyroidal illness. *Journal of Clinical Endocrinology and Metabolism* 1993 **77** 1695–1699.

74 Hashimoto H, Igarashi N, Yachie A, Miyawaki T & Sato T. The relationship between serum levels of interleukin-6 and thyroid hormone in children with acute respiratory infection. *Journal of Clinical Endocrinology and Metabolism* 1994 **78** 288–291.

75 Raja SG & Berg GA. Outcomes of off-pump coronary artery bypass surgery: current best available evidence. *Indian Heart Journal* 2007 **59** 15–27.

76 Boelen A, Kwakkel J, Thijssen-Timmer DC, Alkemade A, Fliers E & Wiersinga WM. Simultaneous changes in central and peripheral components of the hypothalamus–pituitary–thyroid axis in lipopolysaccharide-induced acute illness in mice. *Journal of Endocrinology* 2004 **182** 315–323. (doi:10.1677/joe.0.1820315)

77 Fekete C, Gereben B, Doleschall M, Harney JW, Dora JM, Bianco AC, Sarkar S, Liposits Z, Rand W, Emerson C *et al*. Lipopolysaccharide induces type 2 iodothyronine deiodinase in the mediobasal hypothalamus: implications for the nonthyroidal illness syndrome. *Endocrinology* 2004 **145** 1649–1655. (doi:10.1210/en.2003-1439)

78 Palsson-McDermott EM & O'Neill LA. Signal transduction by the lipopolysaccharide receptor, Toll-like receptor-4. *Immunology* 2004 **113** 153–162. (doi:10.1111/j.1365-2567.2004.01976.x)

79 Boelen A, Kwakkel J, Alkemade A, Renckens R, Kaptein E, Kuiper G, Wiersinga WM & Visser TJ. Induction of type 3 deiodinase activity in inflammatory cells of mice with chronic local inflammation. *Endocrinology* 2005 **146** 5128–5134. (doi:10.1210/en.2005-0608)

80 Kwakkel J, Surovtseva OV, de Vries EM, Stap J, Fliers E & Boelen A. A novel role for the thyroid hormone-activating enzyme type 2 deiodinase in the inflammatory response of macrophages. *Endocrinology* 2014 **155** 2725–2734. (doi:10.1210/en.2013-2066)

81 Angelousi AG, Karageorgopoulos DE, Kapaskelis AM & Falagas ME. Association between thyroid function tests at baseline and the outcome of patients with sepsis or septic shock: a systematic review. *European Journal of Endocrinology* 2011 **164** 147–155. (doi:10.1530/EJE-10-0695)

82 Van den Berghe G, De Zegher F, Veldhuis JD, Wouters P, Gouwy S, Stockman W, Weekers F, Schetz M, Lauwers P, Bouillon R *et al*. Thyrotrophin and prolactin release in prolonged critical illness: dynamics of spontaneous secretion and effects of growth hormone-secretagogues. *Clinical Endocrinology* 1997 **47** 599–612. (doi:10.1046/j.1365-2265.1997.3371118.x)

83 Fliers E, Guldenaar SEF, Wiersinga WM & Swaab DF. Decreased hypothalamic thyrotropin-releasing hormone gene expression in patients with nonthyroidal illness. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 4032–4036.

84 Peeters RP, van der Geyten S, Wouters PJ, Darras VM, van Toor H, Kaptein E, Visser TJ & Van den Berghe G. Tissue thyroid hormone levels in critical illness. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 6498–6507. (doi:10.1210/jc.2005-1013)

85 Peeters RP, Wouters PJ, Kaptein E, Van Toor H, Visser TJ & Van den Berghe G. Reduced activation and increased inactivation of thyroid hormone in tissues of critically ill patients. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 3202–3211. (doi:10.1210/jc.2002-022013)

86 Van den Berghe G. Novel insights into the neuroendocrinology of critical illness. *European Journal of Endocrinology* 2000 **143** 1–13. (doi:10.1530/eje.0.1430001)

87 Arem R, Wiener GJ, Kaplan SG, Kim HS, Reichlin S & Kaplan MM. Reduced tissue thyroid hormone levels in fatal illness. *Metabolism* 1993 **42** 1102–1108. (doi:10.1016/0026-0495(93)90266-Q)

88 Weekers F, Van Herck E, Coopmans W, Michalaki M, Bowers CY, Veldhuis JD & Van den Berghe G. A novel *in vivo* rabbit model of hypercatabolic critical illness reveals a biphasic neuroendocrine stress response. *Endocrinology* 2002 **143** 764–774. (doi:10.1210/endo.143.3.8664)