HOMEOSTASIS AND ALLOSTASIS OF THYROID FUNCTION

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HOMEOSTASIS AND ALLOSTASIS OF THYROID FUNCTION

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The homeostatic equilibrium point of the pituitary-thyroid axis is a dynamic attractor that is controlled by both central and peripheral factors. Rather than representing a fixed set point, the portal TRH concentration (purple) encodes an adaptive reference input that is governed by multiple nutritional, seasonal, inflammatory, environmental and emotional signals, thereby aligning energy expenditure with supply and both current and predicted demand. A previously overlooked part of this adjustment process is T3 (green) production in the thyroid gland, which is controlled by TSH concentration (red) and partly independent from T4 (blue) secretion. Image by Benedikt Dietrich, licensed under CC BY-SA 4.0.

The discovery of the negative feedback of thyroid hormones on pituitary thyroid-stimulating hormone (TSH) secretion, a classical endocrine feedback control system, has shaped diagnosis and treatment of thyroid disease for the last decades. Based on this concept, a unique diagnostic category of subclinical thyroid disorders was introduced, being defined exclusively by an abnormal TSH response in the presence of thyroid hormone concentrations within the reference range. Although this approach was able to deliver a conceptually straightforward disease definition problems surfaced in clinical practice as neither the diagnostic reference range nor the appropriate threshold for initiating substitution treatment are universally

agreed upon for subclinical thyroid disorders. The situation is further aggravated by the so-called syndrome T, which comprises a substantial but heterogeneous group of L-T4 treated patients with hypothyroidism with reduced quality of life despite "normal" TSH values.

A limited understanding of the physiological relationships between TSH and thyroid hormones may be a main reason for clinical difficulties in dealing with the causes of syndrome T and tailoring substitution therapy for hypothyroid patients with subclinical thyroid disorders.

Feedback regulation has recently been shown to be much more complex than previously assumed. The concept of homeostatic control has also been extended to include the lesser known but equally important allostatic thyroid regulation.The latter aims at adaptive homeostasis or stability through changing setpoints and modulating structural parameters of feedback control, as may be appropriate to adapt to a vast array of conditions spanning from fetal life, aging, pregnancy, exercise, starvation, obesity, psychiatric disorders to the severe non-thyroidal illness syndrome.

A better understanding of homeostatic and allostatic mechanisms, which govern the behaviour of pituitary-thyroid feedback control, is on the horizon. This promises to improve the diagnostic utility of laboratory methods, laying the foundation for personalised methods to optimise dosage and modality of substitution therapy. The emerging new world of thyroid physiology is reflected on the side of clinical medicine in a new, relational paradigm for diagnosis and treatment.

Considerable progress has been made in this respect in the following key areas:

- the significance of complementary information processing structures within the feedback loop, in particular ultrashort feedback of TSH on its own secretion and the action of a TSH-T3 shunt unburdening the thyroid from T4 synthesis in imminent thyroid failure,
- the unravelling of spatio-temporal dynamics of hormone concentrations ranging from ultradian to circannual rhythms and including hysteresis effects,
- the emergence of "non-canonical" mechanisms of thyroid hormone signalling beyond transcriptional control of gene expression,
- the physiological actions of thyronine metabolites, which have been previously regarded as biologically inactive, such as thyronamines and iodothyroacetates,
- the characterisation of distinct patterns in the adaptive processes to stress and strain and their conclusive explanation through reactions to type 1 and type 2 allostatic load.

This collective volume contains the contributions to the Research Topic "Homeostasis and Allostasis of Thyroid Function", which was originally published by the journal Frontiers in Endocrinology. Authored by an international team of experts from three continents ,the book provides a comprehensive overview on thyroid control from recent research in basic, computational and clinical thyroidology. Many aspects addressed here can be expected to stimulate future research. A more comprehensive view and better integration of in-vitro, in-silico and in-vivo investigations will be invaluable in paving the way to this new world of thyroidology.

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Table of Contents

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EDITORIAL
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Editorial: "Homeostasis and Allostasis of Thyroid Function"

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Editorial on the Research Topic

Homeostasis and Allostasis of Thyroid Function

CURRENT CHALLENGES IN THYROIDOLOGY

A basic understanding of thyroid control involving pituitary thyrotropin (TSH) has become a cornerstone for the contemporary diagnosis of thyroid disorders. However, long-held simplistic interpretations of the classical feedback concept fall short of the elusive goal of a universally applicable and reliable diagnostic test. Diagnostic ambiguities may arise from the dynamic nature of thyroid homeostasis. Concentrations of TSH and T3 are governed by circadian (1) and, additionally for TSH, ultradian rhythms (2). Plasticity of the hypothalamic–pituitary–thyroid axis in form of adaptive responses may promote misdiagnosis, especially in pregnancy and critical illness (3, 4). Diagnosis of subclinical dysfunction is also dependent on the mode of statistical analysis (5–9).

Consequently, the clinical care of thyroid patients faces major challenges, foremost ill-defined reference ranges for TSH and thyroid hormones (THs), and persistently poor quality of life in a substantial subset of treated hypothyroid patients (10). Divergent criteria by guidelines for defining thyroid disease and guiding therapeutic intervention have further added to the confusion. It remains unclear, if patients with subclinical hypothyroidism benefit from treatment and which are sensible targets of substitution therapy (11, 12).

By addressing predictive adaptation, the rather new theory of allostasis complements the established concept of homeostasis. In situations of strain and stress, allostasis ensures *stability through change* by modifying setpoints and parameters of feedback control (13–15). Despite being a basically beneficial reaction allostasis may also expose the organism to a new kind of strain referred to as allostatic load, which may result in even life-threatening diseases.

This research topic focusing on homeostasis and—still understudied—allostasis of thyroid function was initiated with the goal that deeper physiological insights in pituitary–thyroid feedback control may aid in solving the aforementioned problems. A series of articles summarizes the state of current scientific knowledge, and delivers new perspectives, as significant progress has been made in that regard.

THYROID HOMEOSTASIS—UNEXPECTED COMPLEXITIES IN A CLASSIC ENDOCRINE FEEDBACK LOOP

A review article by the editors (Hoermann et al.) provides an overview of homeostatic mechanisms in the light of recent research. The classical "short feedback" structure (*Astwood-Hoskins loop*) (16) is now complemented by additional motifs, an "ultrashort" autocrine loop, where TSH inhibits its own

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secretion, and a TSH-T3 shunt relaying stimulation from pituitary to intrathyroidal step-up deiodinases. Although documented for decades on a biochemical level (17, 18), the clinical importance of the TSH-T3 shunt has only recently been recognized (19–23).

Newly identified non-classical processing structures add to the complexity of the control system. They explain both pulsatile thyrotropin release and significant deviations from a log-linear relationship between FT4 and TSH concentrations [Hoermann et al.; (24–26)]. In onset hypothyroidism, rising TSH concentrations stimulate T3 formation (22), thereby maintaining thyroid signaling and unburdening the thyroid from T4 synthesis (Hoermann et al.).

A balancing concept for TSH, FT4, and FT3 is introduced under the term *relational stability* [Hoermann et al.; (22)]. Importantly, it is lacking in athyreotic patients and suspended when treatment with L-T4 reduces TSH concentration—an important argument against universal L-T4 substitution in subclinical hypothyroidism.

The novel clinical concepts feed back to theory. Berberich et al. describe an expanded physiology-based mathematical model of thyroid homeostasis that incorporates the rediscovered TSH-T3 shunt. This model extends a rich tradition of related "parametrically isomorphic" models (27–35), demonstrating that circadian variations of FT3 concentrations are well explained by TSH action and shedding a fresh light on the evolution of subclinical thyroid diseases (Berberich et al.).

Interpretation of thyroid function tests can be severely affected by homeostatic time constants resulting in hysteresis effects (36), as reviewed by Leow, extending implications to antithyroid treatment and LT4 substitution.

TECHNOLOGICAL ADVANCEMENTS AND NOVEL DIAGNOSTIC TOOLS

Although sensitive for primary hypothyroidism, TSH measurement has low specificity and is unable to detect dysfunctions of central origin. Isolated TSH measurements may be misleading in certain physiological (37) and allostatic conditions (38), including non-thyroidal illness (39).

In a short perspective article, we summarize methodological principles and clinical trial results (Dietrich et al.) for novel diagnostic approaches based on mathematical modeling, such as functional thyroid reserve capacity and step-up deiodinase activity. These calculated parameters deliver estimates for "hidden" structure parameters of thyroid homeostasis and provide early indicators of thyroid failure. Reconstructing the individual equilibrium point (the so-called *set point*) of thyroid homeostasis is facilitated by new tools and may prove useful as a personal target for L-T4 dosage titration (40, 41). Mathematical modeling can further improve interpretation of L-T4 absorption tests (42).

THE ENIGMATIC ROLE OF NON-CLASSICAL TH

The world of THs is composed of more than T4 and T3. Today, we know 27 metabolites derived from the thyronine skeleton, some of them being hormonally active [Hoermann et al.; (43)]. Thyronamines have received special attention, binding to trace amine-associated receptors (44) and acting as functional antagonists of iodothyronines (45, 46).

Glossmann et al. critically appraise suggested pharmacological uses of 3-monoiodothyronamine (3-T1AM), e.g., for therapy of stroke or in long-lasting space flights. Based on its pleiotropic effects they question if 3-T1AM can be a safe cryogenic drug. Some of the inconsistencies in reported serum concentrations may result from plasma protein binding, potential role of gut microbiota in the formation of thyronamines from iodothyronines or conversion of 3-T1AM to 3-iodothyroacetic acid (3-TA1), a possible major mediator of thyronaminergic signaling (47).

HYPOTHALAMUS–PITUITARY–THYROID AXIS—AN OPEN AND DYNAMIC SYSTEM

The traditional view of pituitary–thyroid feedback control holding T4 plasma concentration constant close to a fixed set point (48) has been challenged by variable concentrations of TSH and THs in certain physiological situations beyond thyroid disease (38, 49–55). Thyroid allostasis delivers a unified theory for a plethora of adaptive processes spanning from fetal life, pregnancy, starvation, exercise, obesity, aging, and general severe illness to psychiatric disorders. In strain and stress, type 1 and type 2 allostasis affect thyroid function in different ways, creating each distinctly recognizable patterns (Chatzitomaris et al.).

PROSPECTUS

Deeper insights in the physiology of thyroid function and its homeostatic control warrant a rethinking of diagnostic practice. The old paradigm employing TSH in the center of diagnostic work-up has to be replaced by a relational concept, where TSH is interlocked with FT4 and FT3, and multivariable distributions represent homeostatic equilibria (9, 30). This new approach allows for personalized interpretation of thyroid function and understands physiological influences as constituents of homeostatic/allostatic control modes (Hoermann et al.).

AUTHOR CONTRIBUTIONS

JD, JM, and RH wrote some of the papers in this Research Topic and participated as guest editors for manuscripts, where they were not coauthors themselves. All authors listed have made a substantial, direct, and intellectual contribution to this editorial and approved it for publication.

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Homeostatic Control of the Thyroid–Pituitary Axis: Perspectives for Diagnosis and Treatment

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The long-held concept of a proportional negative feedback control between the thyroid and pituitary glands requires reconsideration in the light of more recent studies. Homeostatic equilibria depend on dynamic inter-relationships between thyroid hormones and pituitary thyrotropin (TSH). They display a high degree of individuality, thyroid-state-related hierarchy, and adaptive conditionality. Molecular mechanisms involve multiple feedback loops on several levels of organization, different time scales, and varying conditions of their optimum operation, including a proposed feedforward motif. This supports the concept of a dampened response and multistep regulation, making the interactions between TSH, FT4, and FT3 situational and mathematically more complex. As a homeostatically integrated parameter, TSH becomes neither normatively fixed nor a precise marker of euthyroidism. This is exemplified by the therapeutic situation with L-thyroxine (L-T4) where TSH levels defined for optimum health may not apply equivalently during treatment. In particular, an FT3–FT4 dissociation, discernible FT3–TSH disjoint, and conversion inefficiency have been recognized in L-T4-treated athyreotic patients. In addition to regulating T4 production, TSH appears to play an essential role in maintaining T3 homeostasis by directly controlling deiodinase activity. While still allowing for tissue-specific variation, this questions the currently assumed independence of the local T3 supply. Rather it integrates peripheral and central elements into an overarching control system. On L-T4 treatment, altered equilibria have been shown to give rise to lower circulating FT3 concentrations in the presence of normal serum TSH. While data on T3 in tissues are largely lacking in humans, rodent models suggest that the disequilibria may reflect widespread T3 deficiencies at the tissue level in various organs. As a consequence, the use of TSH, valuable though it is in many situations, should be scaled back to a supporting role that is more representative of its conditional interplay with peripheral thyroid hormones. This reopens the debate on the measurement of free thyroid hormones and encourages the identification of suitable biomarkers. Homeostatic principles conjoin all thyroid parameters into an adaptive context, demanding a more flexible interpretation in the accurate diagnosis and treatment of thyroid dysfunction.

Keywords: homeostasis, feedback regulation, TSH, thyroid hormones, deiodinase, set point

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DUAL ROLE OF HORMONES IN THYROID HOMEOSTASIS

The dynamic ability to maintain flexible homeostatic equilibria in response to environmental challenges is a hallmark of a healthy state of the organism. Thyroid hormones assume a dual role in homeostatic regulation, acting as controlling as well as controlled elements. They target a broad spectrum of metabolic effects but concomitantly are strongly regulated themselves. A basic understanding of thyroid control involving pituitary thyrotropin (TSH) has been readily exploited for the diagnosis of thyroid disorders (1–4). As a result, measurement of TSH, though an indirect indicator of thyroid homeostasis, has become central to contemporary thyroid function testing (4, 5). Our knowledge of the mechanisms involved in the regulation of thyroid hormones has greatly evolved in recent years. The underlying system is far more complex than previously thought (**Figure 1**). This requires a revision of long-held simplistic concepts and promotes a multifactorial concept of the feedback control between the thyroid and the pituitary gland (6–9). In this article, we review the role of thyroid homeostasis in the light of recent developments and discuss the resulting new perspectives for diagnosis and treatment of thyroid dysfunction.

HOMEOSTATIC ASPECTS OF THYROID FUNCTION CONTROL

From first principles, it is clinically important to understand clearly what distinguishes a controlling parameter from any other. A change in TSH concentration could be either merely adaptive to restore true euthyroidism or a failed attempt to maintain the euthyroid state. Corrective moves of the control parameter may therefore merely imply a change in the mechanism targeted. This depends on whether the correction sought for is successfully achieved or not. Any meaningful interpretation must respect those particularities in TSH, which do not apply to most other laboratory parameters.

The concept of a control loop feeding back information about the state of thyroid production to the pituitary gland was postulated as early as 1940 (11) and established experimentally before 1950 (12, 13). Models initially assumed an inverse linear correlation between TSH and T4 (14–17), but following more detailed analysis this was later changed to a log-linear relationship, which has remained the standard model ever since (18–22). As circulating thyroid hormones are bound to a large extent to transport proteins (TBG, transthyretin, and albumin) TSH has mostly been related to the unbound biologically active hormone, free T4 (FT4). **Table 1** summarizes various thyroid–pituitary

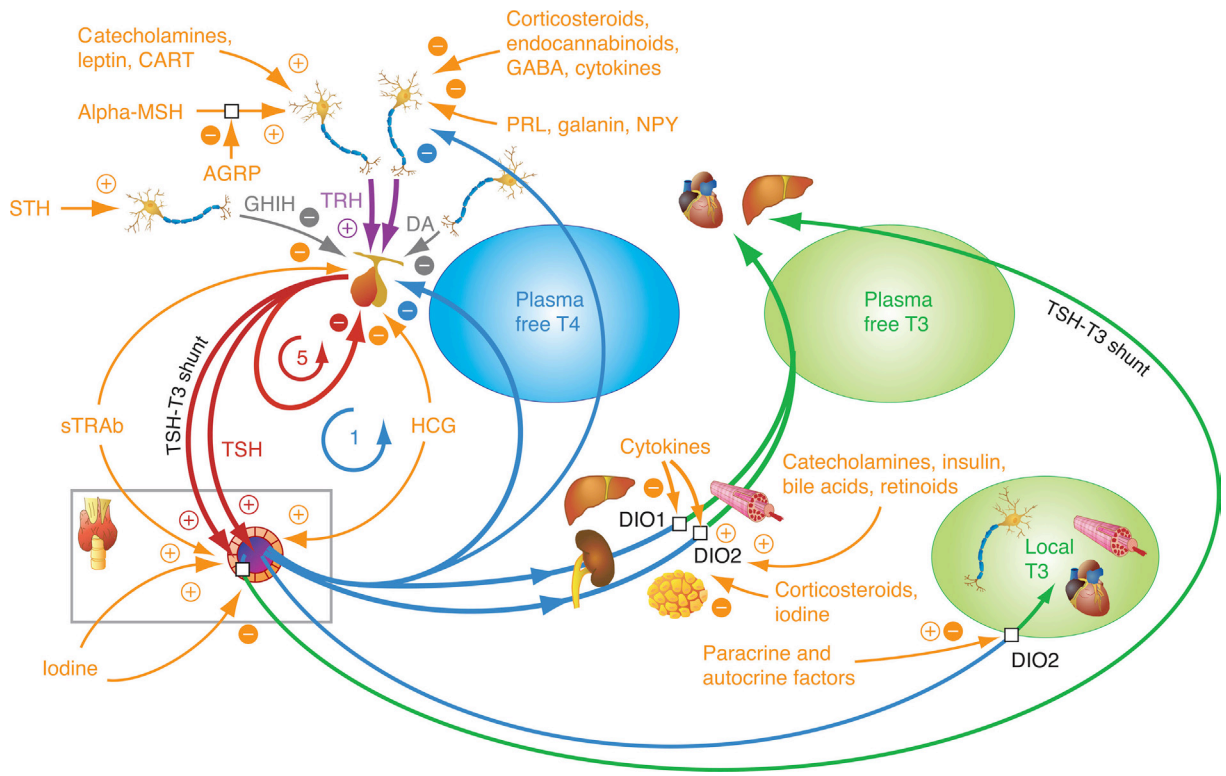

FIGURE 1 | Homeostatic integration of central, thyroidal, and peripheral influences. The integrated control involves several major control loops, a negative feedback control of thyroid hormones on pituitary TSH and hypothalamic TRH, positive stimulatory control of TRH on TSH, ultrashort feedback of TSH on its own secretion, and feedforward control of deiodinases by TSH. Other thyrotropic agonists than TSH, such as TSH receptor antibodies (TSH-R Ab) and human chorionic gonadotropin (hCG), play an important role in diseases, such as Graves' disease and pregnancy-related hyperthyroidism. A plethora of additional influences may fine-tune the responses at each level of organization. 1 refers to the classical Astwood–Hoskins loop, and 5 indicates ultrashort feedback loop of TSH on its own secretion, described in the text. Additional feedback loops (not shown here) control the binding of thyroid hormones to plasma proteins (8, 10).

TABLE 1 | Historical perspective on evolving models for the TSH-T4/FT4 relationship.

Author	Year of publication	Regression
Danziger and Elmergreen (14)	1956	Linear
Roston (23)	1959	Linear with basal secretion
Norwich and Reiter (15)	1965	Linear
DiStefano and Stear (36)	1968	Linear with basal secretion
DiStefano and Chang (16, 37)	1969 and 1971	Linear with basal secretion
Saratchandran et al. (24)	1976	Log-linear
Wilkin et al. (17)	1977	Restricted maximum secretion
Hatakeyama and Yagi (38)	1985	Power law and linear
Cohen (25)	1990	Exponential
Spencer et al. (19)	1990	Log-linear
Li et al. (26)	1995	Non-linear polynomial
Dietrich et al. (10, 27, 28, 40)	1997, 2002, and 2004	Michaelis–Menten kinetics, non-competitive inhibition, and first-order time constants
Sorribas and González (39)	1999	Power laws
Leow (18)	2007	Log-linear
Degon et al. (29)	2008	Non-linear
McLanahan et al. (30)	2008	Michaelis–Menten kinetics, non-competitive inhibition, and first-order time constants
Eisenberg et al. (31, 32)	2008 and 2010	Adopted from DiStefano
Benhadi et al. (21)	2010	Log-linear
Hoermann et al. (6, 9)	2010 and 2014	Erf (modulated log-linear) and polynomial
van Deventer et al. (22)	2011	Log-linear
Clark et al. (33)	2012	Polynomial
Midgley et al. (8)	2013	Segmented log-linear
Hadlow et al. (7)	2013	Polynomial
Jonklaas et al. (34)	2014	Segmented
Goede et al. (35, 41)	2014	Exponential (log-linear) and log-linear with Michael–Menten-type feedforward path

Models of DiStefano et al. and Eisenberg are based on the same platform. Likewise models of Dietrich et al., McLanahan et al., Midgley et al., and Hoermann et al. (9) are based on a common formalism. The model of Goede et al. (41) inherits from those of Leow (18) and Dietrich et al. (10, 27, 28, 40).

feedback models that have been proposed in the literature over the last decades (6–10, 14–19, 21–39). The feedforward path linking TSH levels to T4 output has been modeled as a simple linear relation in the majority of these models.

From the perspective of a sufficiently sensitive defensive response, however, linear or log-linear proportional relations between TSH and FT4 would not intuitively appear to be the most adequate solution. As in many technical systems, a dampened response could be more suited to maintain the controlled parameter at a given stable level with a minimal fluctuation. This consideration requires an examination of the system operating beyond the standard log-linear model.

A REASSESSMENT OF THYROID–PITUITARY FEEDBACK CONTROL

It would be ideal to follow individuals' responses during progression from the hypothyroid to the hyperthyroid state to study the changing pituitary response over the entire functional spectrum. Analyses that do not cover the full spectrum from the hypothyroid to the hyperthyroid extremes are problematic to interpret, because wide variations in the slopes of the logTSH–FT4 relationships have been reported (19, 21, 22, 42). Particularly, different weightings of the extreme, statistically most influential dysfunctional examples in the various patient panels impact heavily on the linear regression. Studies restricted to a narrower euthyroid panel have yielded TSH estimates when extrapolating the regression line to the hypothyroid state are much lower than those clinically observed in the hypothyroid patient (21, 22). Large cross-sectional studies have examined the TSH–FT4 relationship over the entire functional range but did not confirm a proportional and log-linear TSH–FT4 relationship, rather suggesting that the TSH response to changes in FT4 is curvilinear and damped in the middle part (6, 7, 9, 33) (**Figure 2**). Technically, using either a modulatory logistic function, a segmented approach, non-competitive inhibition, or polynomial approximation offers similar ways of examining the same underlying principles of a non-proportional adaptive response dependent on the actual thyroid hormone status (6–8, 33). The non-linearity of the logTSH–FT4 relationship has been independently confirmed by several groups and was replicated in a prospective study involving 1912 subjects (7–9, 33, 34). Thus, the TSH–FT4 relationship is not invariant but is impacted on by the thyroid status itself, which acts as a major determinant of the gradient relating TSH and FT4 (8). Accordingly, the thyroid state may be more vigorously defended, the greater is the deviation from a putative optimum state (6) (**Figure 2**). This behavior provides a far more flexible response than a simple log-linear template. It may conceivably arise from the integrated action of the multiple feedback loops operating at various levels of organization, as shown in **Figure 1**. The consequences of the non-proportional relationship for the clinical interpretation are discussed below.

It should be noted that these studies relied on immunometric FT4 assays, since tandem mass spectrometry (LC–MS/MS) is currently not practicable with large patient panels. However, none of the studies gave indication of a TSH–thyroid hormone mismatch. The inverse linear relation between thyroid hormones and logTSH was similarly broken with an immunoassay and LC–MS/MS in a clinically diverse sample contradicting an earlier report (22, 34). A subanalysis was conducted in a cohort of otherwise "healthy" out-patients without relevant comorbidity to ascertain FT3 or FT4 measurements and their relationships were not compromised by problematic conditions, such as pituitary dysfunction and the non-thyroid illness syndrome (6).

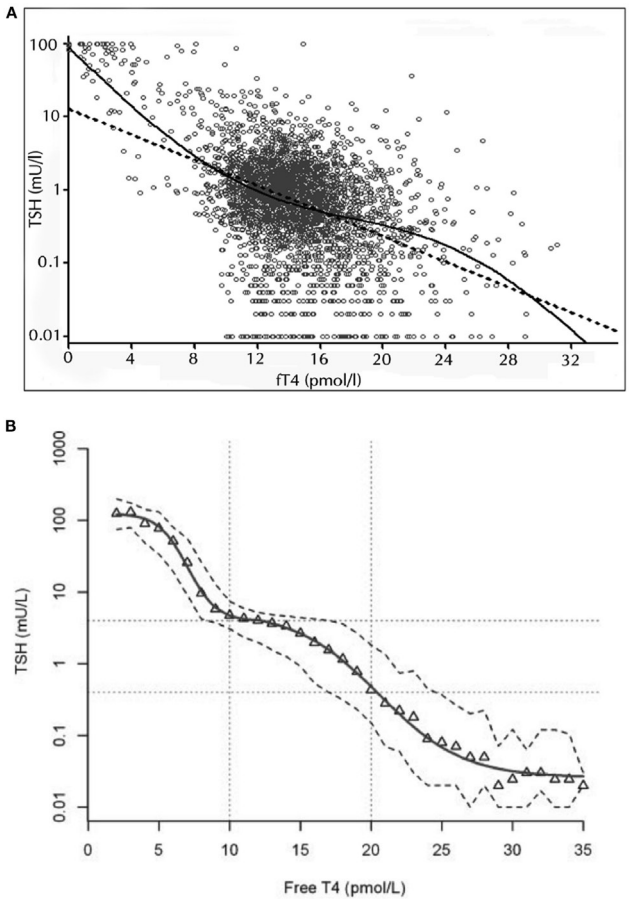

FIGURE 2 | Non-linear relationship between logTSH and free T4. The two studies by Hoermann et al. (6) **(A)** and Hadlow et al. (7) **(B)** show that the TSH–FT4 relationship may not follow a proportional log-linear model (dashed straight line), displaying a damped response in the euthyroid range and steeper gradients at the hypothyroid or hyperthyroid spectrum. The superiority of the non-linear modulatory logistic function shown over the standard log-linear model was statistically established by a strict curve-fitting template based on Akaike's information criterion (6). A multistep regulation of the FT4–TSH feedback control is discussed in the text. Adapted and reproduced with permission from Hoermann et al. (6) and Hadlow et al. (7).

Reliability of immunological methods and harmonization among various commercially available assays has been questioned (43, 44). In this respect, we have extensively evaluated the analytical performance of the immunoassays used in our inter-relational studies (45). In particular, we verified the reference range in the local population, demonstrated robustness of the relationships despite biological variation, and quantified other influences on the defining relationships, such as age and body mass index (9, 45). A clinically important and specific role of FT3 measurement was further supported by our pilot study in a large unselected predominantly euthyroid sample (46). FT3, in the range from 1 to 10 pmol/l, but not FT4 or TSH, showed a significant u-shaped relationship with the Hospital Anxiety and Depression Score (HADS) as well as the anxiety and depression subscales in a generalized linear-quadratic model (not a widely used, but unsuitable linear model) (46). Together these findings support suitability of FT3 for correlative studies when measured with the same instrument at a single institution but do not negate issues of insufficient validation and standardization with the methods discussed below.

MOLECULAR MECHANISMS INVOLVED IN THE FEEDBACK CONTROL

While this article focuses on homeostatic regulation and an in-depth review of the growing body of molecular details is beyond its scope, it should be briefly shown that key mechanisms are reconcilable with a non-proportional model. Both T3 and T4 following its conversion into T3 bind to specific intracellular TR receptors exerting a repressive action on various genes, including TSHβ and, to a lesser degree, α-subunit (47–52). Among the isoforms of TR expressed in various tissues, TRβ2 is active in the central nervous system, hypothalamus, and pituitary gland, with a reported sensitivity enhanced up to 10-fold to thyroid hormones, compared with TRβ1 (53, 54). Such a differential response should enable the central tissues to anticipate T3/T4 oversupply before it can affect less sensitive peripheral tissues. Similarly, deiodinases in central and peripheral tissues are also regulated differently, enhancing T3 conversion and providing a differentiated mechanism for oversensitively responding to changes in FT4 in the feedback loop (55–63). Specifically, type 2 deiodinase ubiquitination has

recently been shown to be instrumental in hypothalamic negative feedback regulation and is expressed in a non-uniform way among various tissues (64). Contrary to earlier assumptions, T3 and T4 do not diffuse freely across the plasma membrane but are actively transported by specialized transport proteins, such as MCT8, MCT10, and OATP1C1 (65). Intracellular trafficking involves intracellular binding substrates (IBSs) of thyroid hormones (e.g., CRYM) (66). These carriers appear to be necessary components of the feedback control, as simulated for IBS and demonstrated for MCT8 deficiency (27, 65). Additionally, in rodents their hypothalamic expression has been shown to be subject to regulation by T3 (67). Transmembrane transport control adds another layer of complexity to the system but is currently not well understood.

While negative thyroid hormone feedback mitigates thyroid hormone overproduction and hyperthyroidism, TRH is a potent defensive mechanism against undersupply, stimulating both pituitary TSH secretion and modulating its bioactivity (68–72). TSH stimulation of thyroid hormone production, in turn, is essential, because the TSH-independent basal capacity of the thyroid gland is limited and unable to maintain a euthyroid state. Tissue-specific glycosylation differentially regulates the hormone allowing for targeted signaling (73). Pars tuberalis-derived TSH has been shown to differ in its glycosylation pattern from the pars distalis-derived hormone, lacks the ability to stimulate the thyroid TSH receptor, and regulates deiodinase type 2 activity related to seasonality and behavior independent of thyroid hormone production (73). Long feedback control of TRH release by thyroid hormones involves both hypophysiotropic TRH neurons and tancytes, responding to humoral and neuronal inputs that can adjust the set point. The latter mechanism may integrate energy metabolism and thyroid function (74–76). This may play an important role in the pathogenesis of non-thyroidal illness (NTI) syndrome or thyroid allostasis in critical illness, tumors, uremia, and starvation (TACITUS) (74, 77–80).

Additionally, an ultrashort feedback loop involving the suppression of TSH by its own concentration has been proposed, mainly by one group (81–83). Our own studies including mathematical modeling of thyroid hormone homeostasis confirmed that this particular loop appears to be a relevant factor in influencing the TSH–FT4 relationship (8, 10).

The primary regulatory role of pituitary thyroid hormone feedback versus TRH stimulation has been studied using transgenic mice, but its relevance is still controversial (69, 70, 84, 85). In the pit-*D2* KO mouse, Fonseca et al. (86) demonstrated coordination between the hypothalamic and pituitary T3 pathways that involve type 2 deiodinase. The role of deiodinase in tancytes was increased in the absence of pituitary deiodinase in order to preserve euthyroid serum T3 levels (86). The selective loss of pituitary type deiodinase, while increasing basal TSH in the mouse, diminished TSH response to hypothyroidism (87). However, knock-out animals with various degrees of deficiencies in all types of deiodinase have suffered little as a consequence, being able to maintain sufficient homeostatic regulation (88–90). It appears that multiple adaptive layers exist to protect the basic functionality of the homeostatic feedback control from various challenges. Furthermore, a multitude of physiological and pathophysiological influences modulates the relationship between TSH and thyroid hormones at various sites of action, thereby influencing the location of the set point in health and disease (**Table 2**) (8, 9, 76, 91–111).

Complementing the idea of a multifaceted feedback control, we have recently proposed that a feedforward motif may also be operative, directly linking TSH with deiodinase activity and the control of corporeal conversion from T4 to T3 (123). While this study provides the first documentation for a TSH-deiodinase inter-relation in humans *in vivo*, the responsiveness of deiodinase type 1 and type 2 to TSH, presumably through a TSH receptor- and cAMP-dependent mechanism, has been well recognized (55, 124–130).

Like other glycoprotein hormones, TSH is secreted in a pulsatile manner. Faster oscillations with a mean pulse amplitude of 0.6 mIU/l and a rate of 5–20/24 h are superimposed on a circadian rhythm with maximum TSH levels shortly after midnight (**Figure 3**) (112, 113, 131). It is still debated whether fast TSH pulses emerge by pulsatile TRH input, which has been contradicted by Samuels et al. (114), through stochastic signals or via autocrine function of thyrotrophs, i.e. controlled oscillations emanating from ultrashort feedback (10, 40, 115). TSH pulsatility may be beneficial by preventing homologous desensitization of the thyrotropin receptor (132–134). This could partly explain why sialylated TSH has both prolonged half-life and reduced bioactivity (135, 136).

A direct TSH-deiodinase link may, at least partly, explain the T3 circadian rhythm accompanying that of TSH, while FT4 shows no such related circadian or seasonal rhythm (137–139). This response may be modulated by regulating TSH receptor density, as shown in rats with severe thyroid dysfunction (140).

Importantly, these mechanisms align the task of defending plasma FT3 with the central control system (123). Supply of T3 to peripheral tissues is therefore no longer to be seen exclusively as a locally and autonomously regulated process, rather as a part of an overarching, integrated, and central-peripheral control system that governs thyroid hormone signaling in both homeostatic and allostatic regulatory modes (123). This is particularly relevant for the treatment situation with levothyroxine, as discussed below (121).

Figure 1 presents a synopsis of central, thyroidal, and peripheral influences and their homeostatic integration. Taken together, the molecular mechanisms defining multiple feedback loops on several levels of organization, different time scales, and varying conditions of their optimum operation may explain the disproportional non-logarithmic behavior of the TSH–FT4 relationship (**Figure 2**) (6–9, 33, 34). They support a multistep regulation and functionally hierarchical model that has been proposed by our group (8). While we have focused on describing the essential principles, additional physiological contributors, such as ethnicity, gender, age, body mass, iodine intake, selenium supply, T4 treatment, genetic deiodinase polymorphisms, and many others, may all elaborate the complexity of the system. Thus, further fine-tuning of the adaptive responses occurs at both the central and peripheral levels (**Table 2**) (9, 74, 76, 91, 92, 95, 97–99, 118, 119, 141–144).

TABLE 2 | Physiological and pathophysiological influences that may modulate the relationship between TSH and thyroid hormones.

Factor	Main site of action	Predominant mechanism	Main effect	Reference
Age	Pituitary and hypothalamic	Altered sensitivity of thyroid hormone feedback	Diminished TSH response with increasing age	(9, 91–95)
BMI	Pituitary, hypothalamic, and adipose tissue	Central modulators (e.g., leptin) and hyperdeiodination	Hyperthyrotropinemia	(9, 96–99)
Time of day	Pituitary and deiodinases	Circadian TRH rhythm and ultrashort TSH feedback	Circadian rhythms of TSH and FT3 and pulsatile TSH release	(40, 100, 112–115)
Pregnancy	Thyroid gland	TSH receptor stimulation by placental factors (hCG)	Stimulation of thyroid hormone secretion and TSH suppression	(101, 109, 110)
Non-thyroidal illness	Multiple	Set point alteration	Low-T3/T4 and inappropriate TSH response	(116, 117)
Genetic polymorphism	Pituitary	Set point variation	TSH variation	(118, 119)
Epigenetics	Pituitary	Long-term set point alteration	Resetting the system	(120)
Thyroid state	Pituitary and hypothalamic	Variable TSH response depending on distance from putative optimum	Exaggerated response or dampening effect	(6, 8)
TSH quantity	Pituitary	Ultrashort feedback loop	TSH suppression	(8, 82)
TSH quality	Pars tuberalis and pars distalis	Tissue-specific glycosylation of TSH	TSH bioactivity	(72, 73)
TSH agonists or antagonists (TSH-R Ab and hCG)	Thyroid gland	TSH receptor stimulation or blockade	Thyroid hormone stimulation/inhibition and TSH suppression/stimulation	(68, 101, 109, 111)
TRH	Pituitary	TSH production and TSH glycosylation	TSH stimulation and bioactivity	(69, 70, 72)
Neuromodulators (dopamine and somatostatin)	Pituitary	Set point modulation	TSH	(76)
Leptin	Central and hypothalamus	TRH stimulation	TSH increase	(74)
Cytokines (interleukin-6)	Pituitary	TSH inhibition	TSH decrease	(106)
Cortisol and glucocorticoids	Pituitary	TSH inhibition	TSH suppression	(104)
Deiodinase type 2	Central, hypothalamus, and pituitary	T4–T3 conversion	Sensitive feedback regulation by T4	(60, 75, 86)
Deiodinase type 1	Peripheral tissues	T4–T3 conversion	T3 generation	(57)
MCT8 and MCT10	Hypothalamus and pituitary	T3-dependent mRNA expression and thyroid hormone transport	Intra- versus extracellular thyroid hormone gradient	(65, 67)
CRYM	All cells	Intracellular binding substrate (IBS)	Intracellular thyroid hormone trafficking	(66)
Thyroid hormone receptor (TR) β2	Pituitary and hypothylamus	T3 binding	Receptor occupancy	(48)
TR costimulator cosuppressor (RXR)	Pituitary and hypothylamus	T3 binding	Receptor occupancy	(54, 71)
Iodine supply and iodine deficiency	Thyroid gland and autonomously functioning thyroid nodule(s)	Thyroid volume-related TSH response and TSH receptor or G protein mutations	TSH increase/decrease	(107, 111)
L-T4 treatment	Pituitary	Altered thyroid hormone feedback and set point	TSH–FT3 disjoint and FT3–FT4 dissociation	(121, 122)
Other thyroid-related compounds or drugs	Multiple sites	Thyroid inhibitors, thyroid mimetica, and endocrine disruptors	Changes in TSH, FT3, and FT4 and inhibition of conversion or T3 actions	(102, 103)

EMERGING ROLE OF NON-CLASSICAL THYROID HORMONES

Some less recognized non-classical thyroid hormones, such as reverse triiodothyronine (rT3), 3,5-diiodothyronine (T2), iodothyroacetates, and thyronamines, have recently been revisited and found to play an active physiological role (**Figure 4**) (145, 146). rT3 (3,3′5′-T3) is a T3 isomer that is deiodinated in the 3′ position. It is upregulated in fetal life and NTI and interferes by blocking characteristics on thyroid signaling (147). 3,5-T2 exerts agonistic effects at nuclear thyroid hormone receptors, although its concentrations parallel those of rT3 in critical illness (148–150). Elevated 3,5-T2 concentrations in the non-thyroid illness syndrome could, at least in part, explain why patients displaying the low-T3 syndrome may not benefit from substitution therapy with L-thyroxine (L-T4) or L-triiodoythyronine (L-T3) (151).

Iodothyroacetates are smaller, deaminated variants of thyroid hormones and have similar effects to those of iodothyronines (152, 156). However, their plasma half lives and affinity to receptors and transporters differ from the latter (157, 158). Due to its smaller molecule size triiodothyroacetate (TRIAC) is used for the treatment of resistance to thyroid hormone (RTH), but this effect does not seem to be beneficial for all mutant variants (157, 158).

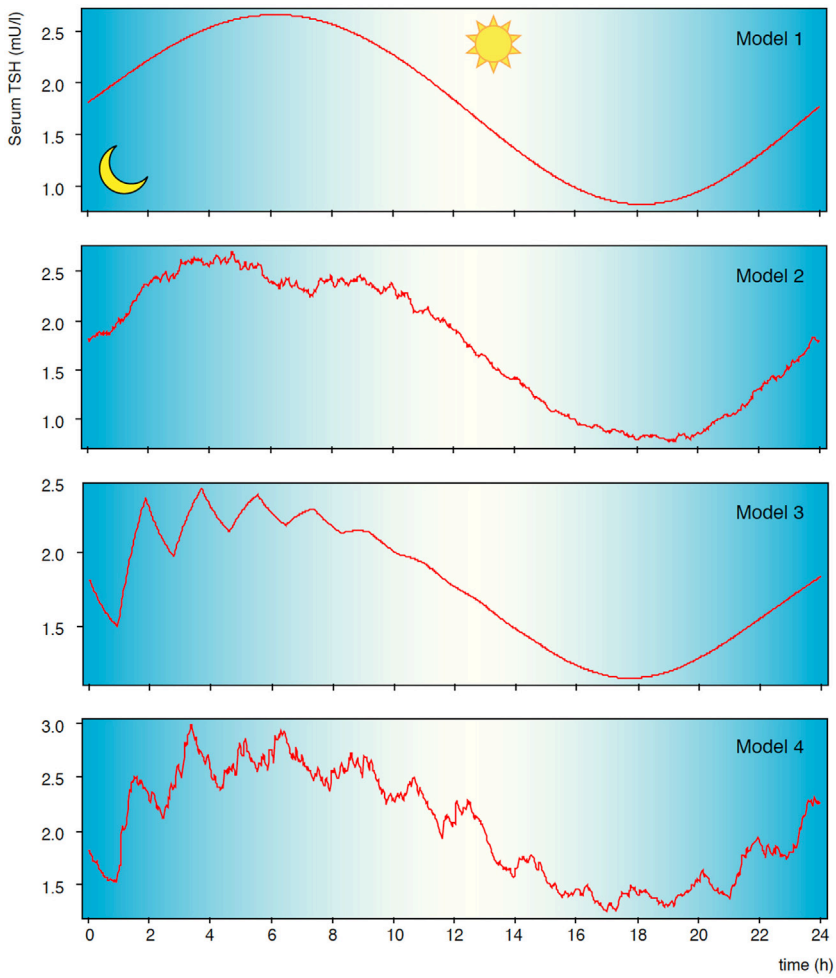

FIGURE 3 | Pulsatility of TSH secretion. Secretion of thyrotropin is subject to circadian and ultradian variation. Shown are results of computer simulations with circadian input only (model 1), additional stochastic afferences (model 2), additional ultrashort feedback of TSH secretion (model 3), and combined stochastic input and ultrashort feedback (model 4). Statistical properties and fractal geometry of model 4 is identical to that of natural time series, while the simpler models differ (10).

Although thyronamines originate from follicular thyroid tissue and are structurally similar to iodothyronines, their biological effects are different. In many respects, their effects are antagonistic effects to those of the classical thyroid hormones (153, 159, 160). Classical and non-classical thyroid hormones can be interconverted by enzymes in certain body compartments (**Figure 4**) (153–155). While the effect of non-classical thyroid hormones on the overall behavior of thyroid homeostasis is still to be elucidated in more detail, some molecules including 3,5-T2, TRIAC, and TETRAC have thyromimetic effects at TR-β receptors, thereby exerting TSH-suppressive actions (156, 157, 161–163). This suggests a role of non-classical thyroid hormones as important modulators of the overall control system in supporting feedback loops controlling release and conversion of thyrotropin and the classical thyroid hormones. The resulting complexity of the homeostatic system is reflected in the non-proportional relationship between FT4 and TSH concentrations (**Figure 2**).

CONSEQUENCES FOR THYROID FUNCTION TESTING

Accordingly, the novel insights into thyroid–pituitary hypothalamic regulation of thyroid hormones described above have important consequences for thyroid function testing. The initial discovery that pituitary TSH responds inversely to the underlying thyroid hormone concentration has greatly influenced current clinically applied thyroid testing (4). Its exaggerated response allows much greater sensitivity to subtle changes in the thyroid hormone status. The first TSH-based thyroid test strategies emerged in the 1980s (164). Whilst the vast majority of studies concentrated on TSH testing, there were few attempts at physiologically based modeling (**Table 1**) (10, 26, 101, 165, 166). The consensus of TSH as a more sensitive diagnostic test than FT4 measurement has been summarized repeatedly in laboratory-focused procedures on TSH measurement and clinical guidelines on its practical use (2, 4, 167). Technically, routinely employed

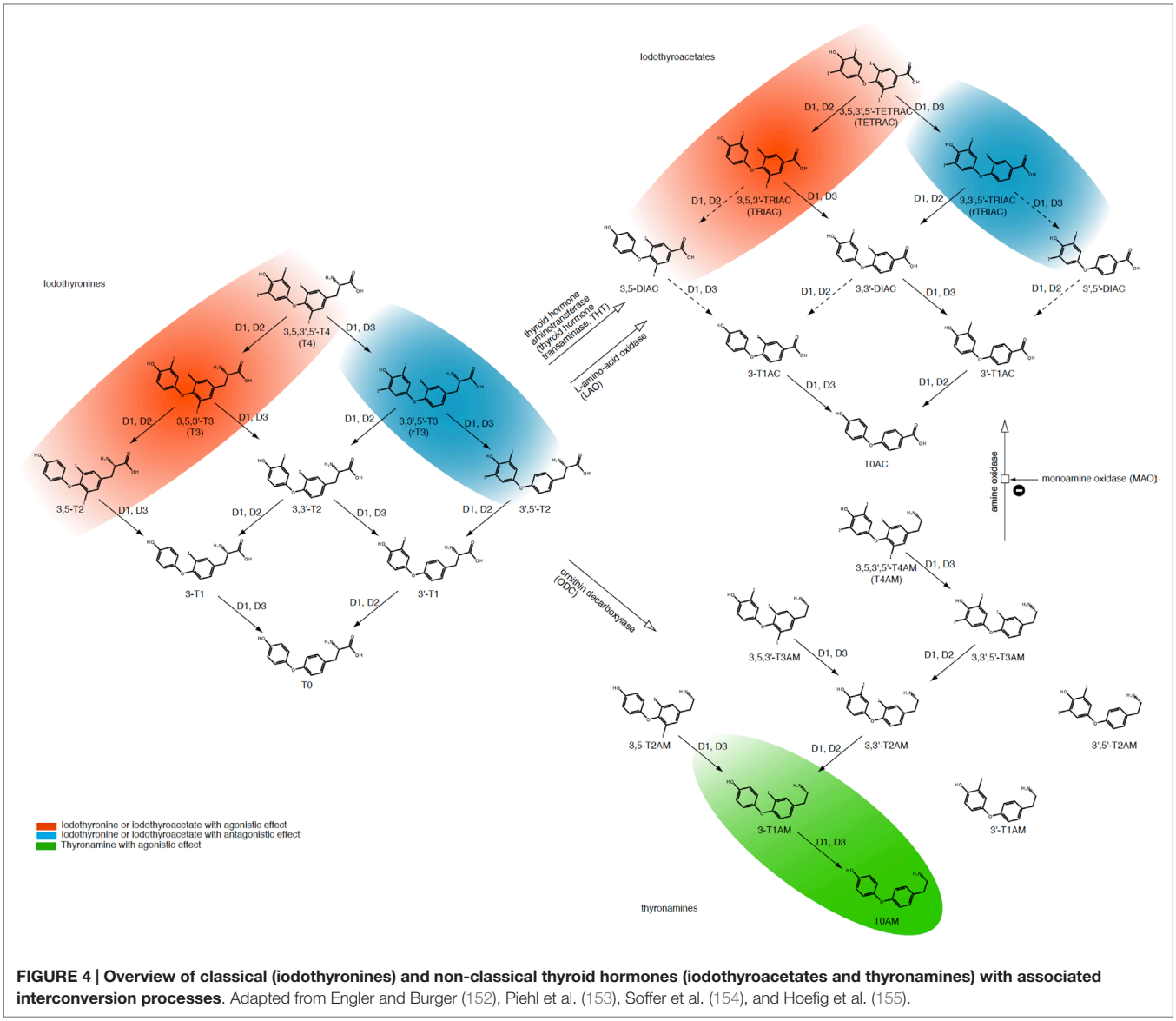

FIGURE 4 | Overview of classical (iodothyronines) and non-classical thyroid hormones (iodothyroacetates and thyronamines) with associated interconversion processes. Adapted from Engler and Burger (152), Piehl et al. (153), Soffer et al. (154), and Hoefig et al. (155).

TSH assays are now in their third generation with each advance significantly enhancing functional assay sensitivity and the ability to clearly separate suppressed TSH levels observed in overt hyperthyroidism from levels at the lower reference limits seen in euthyroid subjects (168). Clinicians have embraced the availability of such a sensitive and cost-effective instrument. TSH is also employed in numerous prognostic studies which define it as a statistical marker of future outcomes (169–175).

This has important consequences as to how TSH has become viewed by the thyroid community as a simple and efficient diagnostic parameter. The ease of measurement was translated into simplicity of interpretation, ignoring the fact that TSH is both an indirect measure reflective of thyroid hormone homeostasis and a controlling element. Thereby, this concept obscured the intricate relationship of the TSH response with the underlying change in the hormonal milieu. By separating TSH from its physiological roots and primary role as a controlling element (**Figure 1**), not only did it become a statistical parameter in its own right, but also it thereby gained the role of the dominant thyroid function test. Consequently, definitions of hypothyroidism or hyperthyroidism were adjusted, introducing new laboratory-based and TSH-derived disease entities of subclinical hypothyroidism and hyperthyroidism, which are defined by an abnormal TSH level while FT3 and FT4 still dwell within their reference ranges (176–178). This was a major conceptual shift, as a disease had now become exclusively defined by measuring a single laboratory value, and, as a result, thyroid disease prevalence was thereby linked to the performance of a single test (179).

HOMEOSTASIS AND THE REFERENCE RANGE OF TSH

While acknowledging strategic advantages of TSH measurement, such as ease of use, suitability for first-line screening, detection

of subtle functional abnormalities, and association with various health outcomes including mortality, there are considerable risks of distorting its integrated physiological importance. The misconception is highlighted by the ongoing controversy surrounding the reference limits of TSH, particularly its upper limit defining subclinical hypothyroidism (167, 180, 181). Proposed amendments to the range, taking into account additional factors such as hidden autoimmunity, ethnicity, gender, and age, offer minor corrections but still fall short of a satisfactory solution. The issues may be more fundamental in nature (182). Even logarithmic transformation of TSH does not totally succeed in restoring a normal distribution. Some authors have attributed this failure to the presence of hidden pathologies, such as autoimmune disorders, others disagreeing with that conclusion (183, 184). We have adopted an alternative statistical approach to the conventional method of establishing the reference interval (45). This involves extrapolation from a normally distributed, robust middle part of the range to the respective boundaries and is suitable for verifying proposed reference ranges by third parties, such as laboratories and manufacturers, using their own retrospective sample of the target population.

However, this does not overcome the problems of diagnostic interpretation using TSH. Unlike many other laboratory parameters, TSH values are personalized measures exhibiting a high degree of individuality. The ratio of the interindividual to the intraindividual variation may serve as an estimate of "individuality," being much higher for TSH than most laboratory parameters, for example, 2 in an earlier report and 2.9 in a recent study (45, 185). Accordingly, the same TSH value could be "normal" for one individual but pathological for another. This also holds for patients with subclinical dysfunction, in whom the relationship between FT4 and TSH shows elements both of normality and abnormality (101). Apart from the statistical requirement that a TSH value in the subclinical range must change by 30% to be confidently classified as change rather than variation or fluctuation, the true nature of TSH referencing is bivariate in relation to an appropriate individual TSH level when combined with a certain FT4 level (101, 186–189). Pulsatility of TSH release adds to the intraindividual variation in TSH levels, which is higher than that of circulating FT4 concentrations (45, 190). Circadian and ultradian rhythms of TSH levels reduce diagnostic accuracy unless reference intervals are adapted or blood sampling is restricted to morning time (100).

Furthermore, the observed rather flat TSH–FT4 relationship within the euthyroid range of the population makes a particular TSH measurement more ambiguous in its prediction of the underlying thyroid state than it does when related by a steeper gradient (**Figure 2**). This questions reliance solely on TSH measurements whenever precise estimates of thyroid function are warranted, but to consider all three thyroid parameters TSH, FT4, and FT3 and their inter-relationships. However, only a few published studies have followed this approach, establishing as a proof of concept truly multivariate reference ranges for thyroid parameters, instead of a combination of two statistically independent univariate reference intervals (191–194). A model that respects the relationship between TSH and thyroid hormones raises the concept of an individually and conditionally determined set point. This is the intersecting point on the overlaid characteristic curves for thyroidal T4 production and pituitary TSH secretion (35, 41, 101). It is important to appreciate that the homeostatic relationship of TSH and FT4 defines the reference range of TSH in a "kite-shaped" graphical configuration, as opposed to the rectangular area obtained by plotting the two univariate parameters (101). A mathematical algorithm has recently been proposed to reconstruct the set point in an individual independently of a population-based reference range (35, 195). The clinical potential of this novel approach awaits further trials. It might prove useful in assessing the appropriateness of a TSH value in a given patient, thus legitimizing a personalized TSH target for thyroid hormone replacement therapy.

While relatively stable in thyroid health, the set point is, however, not fixed but acts as an important physiological integrator and modulator for the homeostatic and allostatic regulation of thyroid hormones (**Figure 1**) (9, 116). This demands a careful diagnostic interpretation taking into account additional information about the clinical condition and various historical influences that may temporarily or permanently impact on the location of the set point at various hierarchical levels (**Table 2**). In extremis, the notion of a non-fixed TSH set point is typified in the NTI syndrome and other constellations of thyroid allostasis where TSH measurement fails as a diagnostic test for that reason (117). The persistence of a significant homeostatic deviation for a prolonged period of time, may, in turn, irrevocably alter the position of the set point, which then assumes a "normality" that is now vigorously defended anew (120). This potential plasticity of thyroid homeostasis is part of a broader concept of epigenetic influences where the bidirectional interchange between heredity and the environment plays a defining role.

In conclusion, the conventional reference system and reliability of TSH measurement as a clinically adequate measure of euthyroidism is compromised by its indirect influence dependent on its fundamental relationship with the underlying thyroid hormones. This, however, is neither proportional (log-linear), as previously thought, nor is it unconditional, but rather complex, hierarchical, and highly individual. Consequently, subclinically hypothyroid patients therefore comprise a heterogeneous population of truly dysfunctional and truly euthyroid subjects. Hence, current definitions of subclinical hypothyroidism or hyperthyroidism cannot serve as a satisfactory and consistent aid to an accurate disease classification in itself. Emerging integrated and personalized diagnostic concepts need to be evaluated and appropriate new markers of tissue euthyroidism must be developed.

HOMEOSTATIC CONSIDERATIONS IN T4-TREATED PATIENTS

The rationale for using TSH as an important treatment target is that the patient's own pituitary gland is generally assumed to be a good determinant for establishing the dose adequacy of L-T4 treatment, even though differences between the T3 utilization of various tissues may exist (4, 5). Thereby, the TSH value derived from optimum health is deemed an appropriate level to aim at for treatment in most patients, excluding systemic NTI and pituitary

disorders where the TSH response is compromised. However, when put to the test, we and others found this assumption to be invalid (9). On the contrary, the interlocking inter-relationships between FT3, FT4, and TSH were not invariably fixed but conditionally and homeostatically determined. In L-T4-treated patients, we showed a significant upward or downward shift and change in the gradients of the FT4 or FT3 regression line with logTSH, compared to untreated controls (9). The phenomenon reveals a disjoint in the relationship between TSH and FT3 (122). While earlier studies already hinted that TSH normalization may not suffice to guarantee a normal serum T3, the more detailed inter-relationships have only recently been analyzed (9, 122, 196–199). From a homeostatic point of view, evidence suggests that the stability of serum T3 is maintained over a wide variation in the endogenous thyroid hormone production in healthy subjects but is lost in the L-T4-treated athyreotic patient (**Figure 5**) (121, 123). This observation raises questions regarding T3 adequacy in treated hypothyroidism. The lower FT3 levels frequently documented in athyreotic L-T4-treated patients, as compared to untreated controls, have received scant attention, being widely dismissed as easily compensated at the tissue level in humans. However, feedback regulation seems clearly compromised in athyreotic patients on L-T4 treatment resulting in T3 instability and homeostatic equilibria that differ significantly from those in healthy subjects (123) (**Figure 5**). In this respect, the presence of the thyroid gland itself and the size of the remnant thyroid tissue after thyroid surgery have recently been shown to play an important role in stabilizing serum FT3, presumably through TSH stimulated intrathyroidal T3 conversion (123, 200). This may explain why athyreotic patients are particularly vulnerable, with approximately 15% living in a chronically low-T3 state below reference, even if they are able

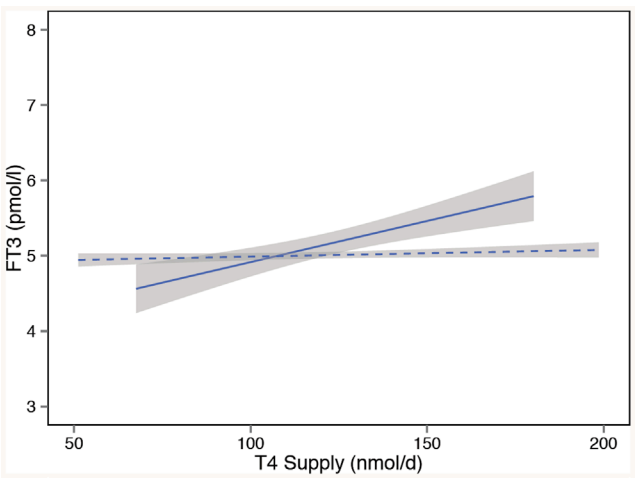

FIGURE 5 | Loss of T3 stability in L-T4-treated athyreotic patients with thyroid carcinomas. In controls (dashed line), serum T3 remains stable over a wide variation in the endogenous thyroid hormone production. In contrast, in L-T4-treated patients (solid line), compensatory T3 regulation is broken, and serum T3 unstably varies with the exogenous T4 supply. Adapted and reproduced with permission from Hoermann et al. (123).

to normalize TSH (122, 198). Three remarkable phenomena have been observed in L-T4-treated patients, (1) a dissociation between FT3 and FT4, (2) a disjoint between TSH and FT3, and (3) an L-T4-related conversion inefficiency (121, 123). Hence, L-T4 dose escalation may not invariably remedy T3 deficiency but could actually hinder its attainment (121, 201). In addition to substrate inhibition and an inhibitory action of reverse T3 on the enzyme activity of deiodinase type 2, experimental studies in the rat elaborate on molecular details involving ubiquitination that may explain a lack of effect of increasing T4 dose in this condition (64). In the rodent, FT3 concentrations in the circulation remained low after escalation of the L-T4 dose (64). More importantly, irrespective of local variations by tissue or type of deiodinase involved, tissue hypothyroidism persisted in all organs examined including brain, liver, and skeletal muscle despite a normal TSH (64). The recent findings are in agreement with earlier studies in the rat (64, 202, 203). T3 supply is locally controlled by several mechanisms, such as active thyroid hormone uptake, tissue-specific expression and activity of two distinct types of deiodinases converting T3 from T4, and thyroid hormone inactivation by deiodination or degradation by sulfatation, deamination, or glucoronidation (57–66). The regulation varies by tissue as the brain predominantly expresses type 2 deiodinase, whereas type 1 deiodinase is abundant in other tissues of the body, and by thyroid state, as T3 excess upregulates type 1 deiodinase but downregulates type 2 deiodinase, which is upregulated in hypothyroidism (57–64). Type 3 deiodinase produces T3 in an inactive form, reverse T3. Although T3 utilization may be locally adjusted to meet the specific demands of each organ, tissue supply is not autonomously independent, but subject to the overarching central control, as discussed above. While corresponding data on tissue T3 in humans are widely lacking and the physiological proportions of T3 derived by conversion versus thyroidal secretion may differ in humans and rodents, the animal models indicate widespread tissue hypothyroidism of target organs in the presence of low serum T3 and normal TSH. This suggests that the disequilibria recognized between circulating FT3 concentrations and both FT4 and TSH in patients on L-T4 may remain intracellularly uncompensated and truly reflective of tissue deficiencies. However, the long-term consequences of the altered ratios are presently unknown. Interestingly, a strong TSH–FT3 relationship was a marker of familial longevity in a recent study confirming the prognostically important role of the equilibria measured in the circulation (204).

The novel implications of homeostatic regulation require further careful study and clinical follow-up. In humans, quality of life may be reduced in a substantial portion of hypothyroid patients taking levothyroxine, even though normal TSH levels suggest restoration of euthyroidism (205). Importantly, the interpretation of TSH values is not uniform among different pathophysiological conditions. A given TSH value in an athyreotic patient on L-T4 has a diagnostic implication entirely different from the same value in an untreated euthyroid subject. While concentrating on treatment-related aspects of thyroid homeostasis, we have not specifically addressed the treatment of hypothyroidism, which has been covered by several recent

specialized articles (205–211). Though optimum treatment targets and modalities invite a fuller debate and further research, it is, however, increasingly clear that L-T4 treatment in its current form, which lacks approximately 10% naturally secreted T3 component, is at base an unphysiological treatment modality where the resulting homeostatic responses operate differently from normality. The diagnostic situation cannot therefore be judged by the same TSH-based criteria defining optimum health (9, 122).

Hence, we suggest that the use of TSH, valuable though it is in many situations, should be scaled back to a supporting role that is more appropriate to its conditional interplay with peripheral thyroid hormones. We emphasize that measurement and consideration of FT3 and conversion efficiency is equally important, particularly in known situations where TSH and FT3 dissociate. This reopens the debate on measurement of free thyroid hormones and encourages the identification of suitable biomarkers. While TSH assays are traceable to a single WHO standard, FT4 and especially FT3 methods are in urgent need of equivalent standardization and harmonization if they are to play a clinically acceptable role in an integrated concept (212). It remains general good clinical practice to only interpret laboratory tests in conjunction with a clinical assessment of the patient history and symptoms and to obtain appropriate confirmation or follow-up before commencing treatment. While TSH may be suitable for screening of asymptomatic conditions, the integrated interpretation of TSH, FT4, and FT3 and their conditional equilibria should benefit decision making, particular on dose adequacy of replacement therapy. Possible adverse effects of the homeostatic disequilibria that arise under the current standard treatment of L-T4 replacement also warrant careful study, and new treatment strategies should aim at maintaining more physiological equilibria.

SUMMARY AND FUTURE OUTLOOK

The concept of thyroid homeostasis offers new perspectives to optimize the interpretation of thyroid function tests and minimize the diagnostic misuse of an isolated and inappropriate statistical interpretation of TSH. The latter approach has wrongly assumed a level of diagnostic certainty that is inherently lacking in this indirect, conditional, and highly individual measure of thyroid function. We have described a new integrative concept, in which TSH becomes a context-sensitive conditional variable but is neither a precise marker of euthyroidism nor optimal for the fine-tuning of thyroid control. TSH levels defined for optimum health may not apply in many L-T4-treated patients. Because of a discernible disjoint between FT3 and TSH concentrations in athyreotic patients, this can result in an inability of T4 monotherapy to adequately address their therapeutic needs. Unlike in the healthy subject with adequate correction, FT3 levels now become unstably dependent on exogenous T4 supply. Furthermore, the T4-related conversion inefficiency may outweigh the benefits of escalating the L-T4 dose in some patients. Homeostatic principles question the isolated interpretation and disease-defining diagnostic value of TSH measurements, hence promoting both a more personalized approach and consideration of diagnosis in a more conditional adaptive context.

These perspectives raise a variety of issues that warrant further exploration and require carefully designed clinical studies before advancing to broader clinical application. The questions relate to multivariate reference limits, personalized set point reconstructions, and the additional value of FT3 for defining thyroid status and assessing dose adequacy in thyroid hormone replacement. There also may be clinical consequences and long-term risks of an unphysiological FT3–FT4 ratio, FT3–TSH disjoint, and impaired deiodinase activity on L-T4 replacement, supporting a possible role of combined treatment with T3 and T4 in selected patients with poor conversion efficiency.

SEARCH STRATEGY

References for this review were identified through the authors' personal files and searches of PubMed for articles published from January, 1971, to March, 2015, by the use of broader terms, such as thyroid homeostasis, feedback regulation, feedback control, reference interval, TSH, triiodothyronine, deiodinase, set point, and synonyms or combinations of the terms. Historically, relevant articles published between 1918 and 1971 were obtained through searches in the authors' personal files, Google Scholar, and other Online Archives' Collections. Articles published in English, French, and German were considered. Articles resulting from these searches and relevant references cited in those articles were reviewed. A more narrow search for homeostasis was done using the following terms: "pituitary AND thyroid AND (feedback OR homeostasis)" and "thyroid AND (simulation OR modeling)." Combined search strategy delivered more than 2000 articles to identify homeostatic models and mechanisms related to thyroid–pituitary feedback. To narrow down the listed references from the vast literature found, individual articles deemed highly original and most relevant were included, and broader concepts were covered by comprehensive review articles whenever possible.

AUTHOR CONTRIBUTIONS

The authors jointly collaborated in conception of this review article, literature research, interpreting and condensing the results, and drafting of the manuscript. RH, JM, and RL provided **Figures 2** and **5**, and JD and RH created **Figures 1** and **4** and **Table 1**.

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Relational Stability in the Expression of Normality, Variation, and Control of Thyroid Function

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Thyroid hormone concentrations only become sufficient to maintain a euthyroid state through appropriate stimulation by pituitary thyroid-stimulating hormone (TSH). In such a dynamic system under constant high pressure, guarding against overstimulation becomes vital. Therefore, several defensive mechanisms protect against accidental overstimulation, such as plasma protein binding, conversion of T4 into the more active T3, active transmembrane transport, counter-regulatory activities of reverse T3 and thyronamines, and negative hypothalamic–pituitary–thyroid feedback control of TSH. TSH has gained a dominant but misguided role in interpreting thyroid function testing in assuming that its exceptional sensitivity thereby translates into superior diagnostic performance. However, TSH-dependent thyroid disease classification is heavily influenced by statistical analytic techniques such as uni- or multivariate-defined normality. This demands a separation of its conjoint roles as a sensitive screening test and accurate diagnostic tool. Homeostatic equilibria (set points) in healthy subjects are less variable and do not follow a pattern of random variation, rather indicating signs of early and progressive homeostatic control across the euthyroid range. In the event of imminent thyroid failure with a reduced FT4 output per unit TSH, conversion efficiency increases in order to maintain FT3 stability. In such situations, T3 stability takes priority over set point maintenance. This suggests a concept of relational stability. These findings have important implications for both TSH reference limits and treatment targets for patients on levothyroxine. The use of archival markers is proposed to facilitate the homeostatic interpretation of all parameters.

Keywords: TSH, thyroid hormones, TSH feedback control, thyroid homeostasis, set point

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INTRODUCTION

Maintenance of body composition and its internal milieu both over long periods of time and during varying imposed external conditions is a primary goal for attaining human health. This is expressed as biological normality, which is more narrowly defined as a perceived optimal state for an individual rather than through a reference range for a population (1–3). In contrast, the statistical definition of normality is inferred by the Gaussian distribution of the variation or measurement error frequently observed in a population (4). Biological variation is not expressed by an error term, but has an important evolutionary role (5).

The human body is essentially a highly complex, interactive biological system where normality and variation are tightly controlled by various mechanisms (6, 7). Typically, the hypothalamus–pituitary–thyroid–axis is closely concerned with energy metabolism and multiple specific functions within the organism. Gaining control over energy expenditure independently of environmental short-term supply offers major systemic advantages (8). It is therefore unsurprising that the thyroid, within the broader biological complex, embodies these basic biological necessities, showing also associations with longevity in older populations (9–13). By nature, such systems tend to operate far away from their unstimulated resting point, and being metastable require considerable energy and systemic pressure in order to avoid collapse and to maintain a desired equilibrium point. Subtle variations in responding to challenges either by an individual or among populations provide stabilization against adventitious reactions that could otherwise cause wide fluctuations and turbulences in the control outputs (14).

In this short review, we will examine the expression of normality, variation, and control of thyroid function in humans and draw some conclusions on their practical use for diagnosis and treatment.

BACKGROUND

The human thyroid gland produces and then releases into the circulation, a large amount of thyroxine (T4) and a lesser proportion of triiodothyronine (T3). Hormone concentrations in the circulation depend on both specific and unspecific binding to proteins, such as TBG, transthyretin, and albumin. Only very small concentrations exist as the respective unbound free forms. The free molecules are biologically active, free T3 (FT3) significantly more so than free T4 (FT4). The two hormones are interrelated by conversion of T4 into T3 by enzymatic 5′ monodeiodination (15, 16). Most of T4 circulates throughout the body, whereas T3 is predominantly found within cells. T3 transport in and out of the cell is potentiated *via* specific transport mechanisms and not by passive diffusion (17, 18). Within the cell, T3 is eventually transported to the nucleus where it binds to thyroid hormone receptors to exert its mostly genomic actions (19–21). Non-classical actions include binding of thyroid hormones to membrane receptors (22). The basal unstimulated glandular output of thyroid hormones is relatively low (23). It potentiates the euthyroid state only upon stimulation by pituitary thyroid-stimulating hormone (TSH). This puts the system of T4 and T3 production under constant high pressure. Consequently, such a high pressure system requires defensive mechanisms to protect the cells from the dangers of being flooded and overwhelmed by an over-supply of thyroid hormones. This includes binding of thyroid hormones to plasma proteins, prior activation of the pro-hormone T4 (T4–T3 conversion), gate control at cell entry (active transport across the cell membrane), and production of locally expressed counter-regulatory derivatives such as reverse T3 and thyronamines that exert short loop inhibitory control (17, 24–26). There is additionally a systemic negative feedback control at the pituitary and hypothalamic level that both controls thyroid hormone production and sets an appropriate internal reference point for pituitary TSH secretion (27).

Modern assays allow readily available measurements of all three thyroid parameters, TSH, FT4, and FT3 (28–30). This does not apply to TRH whose circulatory concentrations are too low for reliable detection and result from multiple sources including hypothalamus, spinal cord, and gastrointestinal tract. At the present time, there are 27,184 publications on TSH in PubMed. However, most studies have focused exclusively on TSH, or examined the parameters in isolation, downplaying the interrelationships with the other thyroid hormones. TSH has assumed a dominant and statistically independent role as the result of its exceptional sensitivity, compared to thyroid hormones, thus divorcing it from its physiological roots as an indirect controlling element (27, 29, 31, 32). This, in turn, has fostered a widely held belief that its exceptional sensitivity in response translates into superior diagnostic performance, thereby making the additional consideration of thyroid hormones largely redundant (33). We have examined the validity of this tenet below.

NORMALITY OF THYROID PARAMETERS

Statistical normality applies to FT4 and FT3 in a sufficiently large and healthy population sample, thus making it easy to define appropriate 95% confidence limits or reference intervals for a population. For TSH, which is not normally distributed, a logarithmic transformation was used as an accepted statistical procedure (34). This method has had limited success and the remaining skewness of the logarithmically transformed TSH was explained by the putative presence of clinically hidden pathologies such as thyroid autoimmunity that are highly prevalent in the population (35–38). Further investigations showed considerable unexpected variation in the upper limit of the reference range (39–41). This hiatus in defining the TSH reference range has been widely discussed and various influences, among others, methodology, geography, ethnicity, and age have been suggested to explain the discrepancies (34–54). However, the disagreement has not been resolved. This may indicate an important underlying problem.

By its physiological role as discussed above, TSH is interlocked with FT4, being the main driving force behind the rise in concentration of the latter hormone to its normal euthyroid level. In homeostatic equilibrium, the two values deliver the so-called set point (55). This represents the intersecting point between the characteristic curves for thyroidal FT4 production and the pituitary response of TSH feedback control. The set point is less variable in an euthyroid individual, and, importantly, intraindividual variability of TSH is only about half as wide as its interindividual variability (55–59). TSH differs thereby from many other laboratory parameters where intra-subject and between-subject variation are nearly equal. A two-dimensional or three-dimensional distribution of TSH and FT4/FT3 in the euthyroid range describes clusters of set points appropriate for healthy individuals (60–64). Conceptionally, this questions the use of the presently employed isolated univariate reference ranges for single parameters and promotes a composite expression of multivariate normality

in the collective. Between-subject variation should therefore best be addressed by paired measurements of TSH and FT4 in healthy individuals. Using a large sample from a prospective study, we have derived bivariate and trivariate reference limits for TSH and thyroid hormones (64). We have further examined their diagnostic performance, compared to a univariate TSH reference range (64). This study revealed frequent discrepancies in the placement of results between composite multivariate reference limits and a combination of the univariate single reference intervals. Method-associated reclassification from thyroid dysfunction to euthyroidism was as high as 26% by using the bivariate limit or 42% for the trivariate limit, respectively (64). These recent findings agree with the few previous studies applying the concept of multivariate normality to clinical data (60, 63), extending the earlier findings to evaluating diagnostic performance (64). This demonstrates that statistical analytic techniques heavily influence current TSH-reliant thyroid disease classification. Hence, joint application of the dual roles of TSH as a sensitive screening test and an accurate diagnostic tool becomes highly questionable and consequently the roles must be separated from each other. The current classification of the disease entities of subclinical hypothyroidism or hyperthyroidism, which is solely based on abnormal TSH values when thyroid hormones concentrations remain within their respective reference ranges, seems no longer tenable (31, 32). New markers for clinical endpoints or tissue-based definitions of thyroid function are therefore urgently needed. Over-reliance on TSH as a gold standard has long impeded the advancement of the field, since the first doubts were raised and disagreements emerged on the setting of the reference intervals (34–54). While sole reliance on TSH must therefore be scaled back, good clinical practice taking into account the full history and symptoms displayed by a patient has to be re-instituted as a primary tool (65, 66).

RELATIONAL STABILITY BETWEEN THYROID PARAMETERS

Although current definitions of subclinical thyroid dysfunction follow a narrow TSH-based distinction, recent studies demonstrated that the cardiovascular and mortality risk increases within the "normal" thyroid function range (67–69). This suggests that transition into thyroid disease occurs more gradually, and risks may not be reliably assessed by univariate TSH normality. The situation is further complicated by issues with TSH reference limits discussed above. A combined view of all thyroid parameters and their interrelationships may provide a more comprehensive picture as a moderately raised TSH could either indicate a failed attempt at restoration of euthyroidism or signal successful homeostatic adaptation. The expression of adaptive homeostatic equilibria between TSH and thyroid hormones may provide an early defense line against the abrupt onset of thyroid failure following less severe or temporary disruptions. Clinical studies support this view showing no adverse outcomes for mortality, cardiovascular events, fracture risk, or cognitive impairment in association with mildly elevated TSH levels (5–10 mIU/l) (70). In elderly patients with subclinical hypothyroidism, in contrast, life expectancy was found to be compromised with lower, not higher TSH values (9–13).

We hypothesized that early adaption under system stress should be testable by studying the expression of homeostatic equilibria across the spectrum of euthyroid subjects (71). The concept was termed relational stability, describing adaptive interrelations between the parameters rather than univariate expression of normality of a single component maintaining system stability. We found an inverse correlation between TSH-standardized T4 production and T4–T3 conversion across the euthyroid reference range (71). This contradicts the assumption that variation between thyroid hormones and TSH may be randomly defined in euthyroid subjects by genetic variation in the formation of set points (72–74), rather indicating early homeostatic control across the euthyroid spectrum (71). The euthyroid reference range for FT3 becomes dependent on a progressive alteration in the controlling interplay between TSH, FT4, and FT3 across the range. The concept extends to the diseased state, e.g., in patients with autoimmune thyroiditis where the observed pattern of control was similar, albeit shifted at a lower level (71).

Hence, maintaining stable FT3 positions takes priority over set point fixation in expressing the TSH–FT4–FT3 relationship, as the system seeks early compensation from the very onset of thyroid capacity stress, progressively increasing global deiodinase activity as thyroidal production declines (71). This T3-stabilizing behavior may emanate from the expression of TSH feedforward control on deiodinase activity (27). Phase-shifted coupling of the circadian rhythms of FT3 and TSH reflects this example of physiological control (75). *In vitro* and *in vivo* studies on athyreotic patients under levothyroxine (LT4) treatment further suggested a direct role of TSH in integrating cooperative elements of central and peripheral control (27, 76–80). Recent pathophysiological support from studies in the rat or genetically modified animals suggests that defending appropriate FT3 concentrations has high priority (81–83). As reviewed elsewhere (84, 85), invasive procedures carried out in the animals, not ethically possible in humans, extend many of our findings to tissue equilibria.

The newly proposed concept of relational stability assigns maintenance of T3 stability equal physiological relevance to central set point control. Where conflicts between the two regulatory elements may arise, T3 stability takes priority over set point maintenance. Importantly, this indicates that the set point is not only dramatically adjusted in extreme conditions such as the non-thyroidal illness syndrome, as has long been recognized (86, 87), but may be modified as part of an early response of the system when challenged by minor disturbances.

Understanding the progressive variation in control responses has important implications for clinical decision-making. First, it brings a homeostatic perspective to the controversial debate on the validity of TSH reference limits. Lowering the conventional reference range for TSH to 2 mIU/l has been proposed by some authors, based on imposing a statistical normal distribution, so as to sensitively define subclinical hypothyroidism (40, 45). Others see no need to redefine the upper reference limit (39, 40). If the non-random statistical

pattern is explained by subtle heterogeneity in the expression of control in euthyroid individuals, the 2 mIU/l limit may not necessarily reflect the beginning of a diseased state, rather indicating a compensatory response to early capacity stress. This favors a more conservative approach for treatment decisions in patients with subclinical hypothyroidism (32, 70). However, given considerable individual variation, such findings of modest elevations in TSH should not countervail appropriate treatment if clinical presentation warrants it. Second, some unexpected outcomes may arise from balancing effects between the feedback and feedforward regulation. LT4 administration for instance may impair feedforward regulation by reducing stimulatory TSH levels more than anticipated from the added supply of exogenous T4, resulting in decreased FT3 concentrations in patients with autoimmune thyroiditis (88). This encourages further clinical study of calculated homeostatic parameters and interrelational measures (89).

INTERRELATIONAL MEASURES AND EMERGING NEW CONCEPTS OF THYROID HOMEOSTASIS

While TSH alone can play a role as a sensitive screening test in asymptomatic subjects, it cannot also simultaneously assume the second role of a reliable diagnostic tool (gold standard) for defining true euthyroidism. The clinical interpretation of TSH should therefore be appropriately scaled back. TSH reference intervals for euthyroidism are uncertainly defined. From a homeostatic perspective, all three parameters TSH, FT4, and FT3 must be viewed together (27, 88).

To develop this concept further, we propose archival markers should be laid down for the individual subject. This may serve as a homeostatic reference point if any disturbance or thyroid disease arises in the future. Such markers will be readily available prior to surgery and may deliver personal targets to guide dose titration. In situations where they are unavailable or not applicable (e.g., after surgery for toxic adenoma or toxic multinodular goiter), reconstructing the set point from multiple TSH–FT4 pairs may offer an alternative option (90–92). As a strength, the latter approach provides individual markers with less variation, compared to univariate or multivariate reference ranges, but it has the disadvantage of failing to deliver FT3 targets and being sensitive to variations in deiodinase activity.

Potential treatment-related adjustments are not considered by this method, and the path of set points from hypothyroidism to euthyroidism can usually only be mapped in individuals under open loop conditions, i.e., under LT4 therapy in most cases. This becomes clinically relevant, because the set point has been demonstrated to differ in the same patient from health to disease, e.g., before and after thyroidectomy (27, 88, 93, 94). Hence, the equilibria in individuals appropriate for their healthy state do not remain unaltered and cannot act as equivalent targets for their diseased state. Consequently, log-linearity of the TSH–FT4 relationship cannot be safely assumed over the entire functional range (27, 95–99). While linearity roughly holds true in the open loop situation and hypothyroid state, the relationship becomes progressively damped toward the euthyroid range (88, 95, 99–101). Within the euthyroid range, factors other than TSH dominate the expression of control (27). Thereby, this fine-tunes the response, adjusting it to the conditional and situational needs. While this has been convincingly shown for populations in cross-sectional studies, longitudinal data are more difficult to interpret, because most patients followed are treated, and LT4-treatment has in itself a dose-related influence on the relationships (27, 88, 101).

Athyreotic patients show particularly wide variations in their biochemical responses to LT4 treatment (102). This provokes alterations in the interlocking relationships and governing equilibria (27). While FT3 is uncorrelated with TSH in healthy subjects indicating T3 stability, it becomes TSH-related and unstable in LT4-treated patients (**Figure 1**). This flexible behavior precludes both the isolated interpretation of TSH levels and challenges the validity of the clinical application of fixed set points. Rather the balanced interrelationship between FT3 and TSH must be considered as a key component of control and should be interpreted more dynamically. FT3 as the primary target of stability cannot thus left be out of the equation. Neither can the importance of its absolute position be ignored nor can its equally important indirect influence on the TSH–FT4 relationship be neglected. However, equilibrium and optimum reference points may dissociate on LT4. Ingestion of a T4 load unaccompanied by the production of a physiologically appropriate T3-fraction may drive the system attempting regulatory balance into non-optimum positions (101). While LT4 dose escalation or the use of liquid formulations effectively control elevated TSH levels in various conditions including gastric problems, intestinal malabsorption, or drug interference (103, 104), this situation is different, as low FT3 concentrations persist in these patients despite suppressed

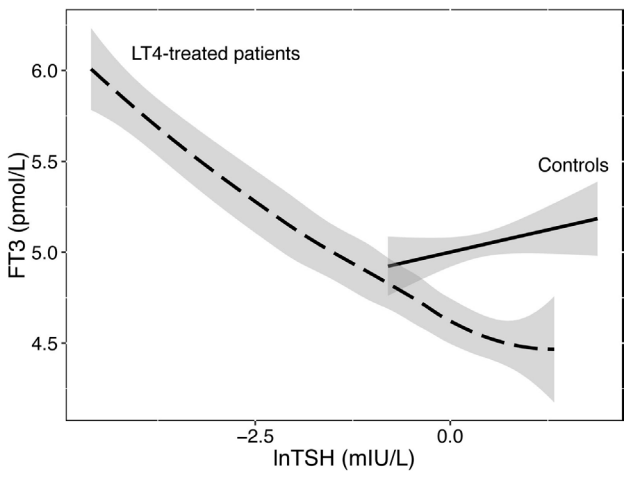

FIGURE 1 | Relationship between FT3 and TSH in healthy controls and LT4-treated patients. While stable and uncorrelated with logarithmic TSH levels in controls, serum FT3 concentrations were unstably associated with TSH in LT4-treated patients. Data are from a published trial (88). The regression lines shown and their 95% confidence interval (shaded area) were fitted by a linear model in 207 controls or locally weighted scatterplot smoothing in 353 patients on LT4.

TSH levels and elevated FT4 concentrations (102). Apparently, a minority of patients on LT4 still fail to concomitantly raise their serum FT3 concentrations as the T4 excess itself impairs T3–T4 conversion (102, 105). The clinical relevance was recently confirmed as euthyroid TSH targets could not adequately raise resting energy expenditure (REE) adjusted for lean body mass in LT4-treated women, compared to healthy controls, only FT3 levels being positively correlated with REE (106). A recent study using currently available evidence-based treatment options suggests hypothyroid patients can expect their quality of life to improve, but not a full recovery to a level characteristic of the healthy population (107). Recognizing and targeting homeostatic equilibria may improve future treatment strategies for hypothyroid patients, which remains an important goal.

CONCLUSION

Thyroid-stimulating hormone gains unique properties from its embedment into a biological system and its primary role as a controlling element. Important differences thus arise exceeding the statistical concept of univariate normality conventionally applied to univariate reference ranges. Biological variation, homeostatic interrelations, equilibria, and set points between TSH, FT4, and FT3 become relevant for the observed expression of multivariate normality and relational stability in thyroid health. FT3 becomes a primary target for system stability whose absolute position and indirect influence on the TSH–FT4 relationship must be recognized. System instability may produce non-optimal equilibria under LT4 monotherapy in some patients. The triple roles of TSH as a sensitive screening test, an accurate diagnostic tool, and a therapeutic target require separation. A conjoint view of all thyroid parameters leads to the proposition of archival thyroid markers as future reference points.

AUTHOR CONTRIBUTIONS

RH, JM, and JD drafted the manuscript; RL contributed some revisions. All the authors read, amended, and approved the final manuscript.

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REVIEW
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A Review of the Phenomenon of Hysteresis in the Hypothalamus–Pituitary–Thyroid Axis

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The existence of a phase of prolonged suppression of TSH despite normalization of serum thyroid hormones over a variable period of time during the recovery of thyrotoxicosis has been documented in literature. Conversely, a temporary elevation of TSH despite attainment of euthyroid levels of serum thyroid hormones following extreme hypothyroidism has also been observed. This rate-independent lag time in TSH recovery is an evidence of a "persistent memory" of the history of dysthyroid states the hypothalamus–pituitary–thyroid (HPT) axis has encountered after the thyroid hormone perturbations have faded out, a phenomenon termed "hysteresis." Notwithstanding its perplexing nature, hysteresis impacts upon the interpretation of thyroid function tests with sufficient regularity that clinicians risk misdiagnosing and implementing erroneous treatment out of ignorance of this aspect of thyrotropic biology. Mathematical modeling of this phenomenon is complicated but may allow the euthyroid set point to be predicted from thyroid function data exhibiting strong hysteresis effects. Such model predictions are potentially useful for clinical management. Although the molecular mechanisms mediating hysteresis remain elusive, epigenetics, such as histone modifications, are probably involved. However, attempts to reverse the process to hasten the resolution of the hysteretic process may not necessarily translate into improved physiology or optimal health benefits. This is not unexpected from teleological considerations, since hysteresis probably represents an adaptive endocrinological response with survival advantages evolutionarily conserved among vertebrates with a HPT system.

Keywords: memory effect, lagging TSH recovery, hysteresis, epigenetic regulation, histone modification and chromatin structure

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INTRODUCTION

Given the exquisite potency of thyroid hormones on the body, the hypothalamus–pituitary–thyroid (HPT) axis is under extremely delicate homeostatic control to ensure that the circulating thyroid hormone levels are finely adjusted to physiological concentrations critical for normal cellular, tissue, and organ development, function as well as the overall survival of the organism (1–3). In human beings, the normal population range of serum-free thyroxine (FT4) lie approximately between 10

and 20 pmol/L, free triiodothyronine (FT3) between 4.0 and 8.0 pmol/L, and that of serum thyrotropin (TSH) between 0.5 and 5.0 mIU/L (4, 5). Within any given individual, there is clear evidence that the normal ranges of the above hormones are much narrower than the population ranges (6–8) and appear to oscillate around a relatively stable and unique mean operating level of FT4 and TSH called the euthyroid set point that defines the individual's optimal and physiological state of health (9, 10). The HPT axis is naturally regulated by a negative feedback loop in order to keep FT3 and FT4 from swinging off the normal limits. In this system, excessive FT4 (when deiodinated to FT3 intracellularly) and FT3 suppresses the expression of TRH and TSH. Conversely, when FT4 and FT3 are deficient, their lack of inhibition on the hypothalamus and pituitary leads to pronounced elevations of TRH and TSH. Thus, in states of thyroid hormone deficiency when FT3 and FT4 are falling away from the set point and have gone below their lower normal limits, serum TSH will increase and rise beyond the upper limit. During thyroid hormone excess when FT3 and FT4 are rising and have exceeded their upper limits, serum TSH will decline and even become suppressed below the lower limit of normal.

This inverse log-linear pattern of variation between TSH and FT3/FT4 is well known to physiology and medical students as well as doctors and endocrinologists (11, 12). However, a strange observation has been noticed by clinicians treating patients with thyroid hormone disorders. This pertains to elevated serum TSH for a variable period despite restoration of euthyroid levels of FT3/FT4, following treatment of severe hypothyroidism (13, 14). Similarly, it has been noted for decades that serum TSH can become drastically suppressed sometimes for weeks or even months, following the recovery of severe thyrotoxicosis (15, 16). Such a phenomenon of persistent elevation or suppression of serum TSH in the face of normalized FT3/FT4 after recovery of hypo- and hyperthyroidism is termed as "hysteresis" and first described as such in a formal treatise in 2007 (12). Clinicians have been perplexed by this and have also wondered if this implies a residual thyroid dysfunction that deserves treatment to hasten the recovery of serum TSH to the normal range. The following review is devoted to the discussion of hysteresis of the HPT axis and its clinical implications.

BRIEF HISTORICAL PERSPECTIVES OF HYSTERESIS

Hysteresis is a Greek term that means "shortcoming" and "to be late." It was originally proposed by the late Scottish engineer and physicist, Sir James Alfred Ewing, to refer to the phenomenon observed in systems exhibiting a memory effect such that the response to an input is delayed by a lag time (17). Hysteresis has since been identified in many fields, including physics, economics, and biology. In the area of physiology, hysteresis is encountered in pulmonary mechanics, parathyroid homeostasis, and even urodynamics (18–20). Although the phenomenon of persistent TSH suppression and elevation with consequent lagging of thyrotroph recovery following severe thyrotoxicosis and hypothyroidism had been observed for many years, the first formal description of hysteresis involving the HPT axis was enunciated in 2007 (12).

CLINICAL SCENARIOS

Thyroid function test (TFT) data have revealed that mild departure of FT4 and TSH away from their respective normal ranges often led to the recovery of FT4 and TSH to their expected baseline levels in a coupled fashion fairly rapidly. But when thyroid status swings all the way to the extremes far beyond the limits of the normal ranges, TSH inevitably remained either suppressed or amplified for a variable period of time before finally settling down to the baseline values for any given FT4 or FT3 level. The following illustrates some common examples of TFT disturbances encountered in usual clinical settings.

Patient 1

The first clinical vignette involves a 35-year-old woman with a history of acute lymphoblastic leukemia as a young child cured with high dose chemoradiation followed by allogeneic bone marrow transplant. She was diagnosed with stage 1 papillary thyroid carcinoma from fine needle aspiration biopsy of a solid 2 cm × 2 cm nodule involving her right thyroid lobe. Pre-surgery TFT revealed a biochemically euthyroid status with serum-free T4 (FT4) level of 14 pmol/L and serum thyrotropin (TSH) level of 2.8 mU/L. Total thyroidectomy was performed, and she was allowed to become hypothyroid before undergoing high dose radioiodine remnant ablation using 100 mCi of I-131. She was then put on TSH-suppressive doses of L-thyroxine (L-T4) till she achieved an FT4 of 21 pmol/L and TSH of 0.09 mU/L. About 2 years later, she was withdrawn from L-T4 for a stimulated thyroglobulin and whole body iodine scanning assessment followed by TSH-suppressive doses of L-T4. The anonymized **Table 1** shows her TFT data over time.

This can also be illustrated in the form of a graph of TSH vs. FT4 (**Figure 1**), which revealed the presence of two distinct clockwise hysteresis loops.

TABLE 1 | Change in thyroid function tests of "Patient 1" over time.

Day	1 (pre-op euthyroid set point values)	14 (total thyroid resection done)	60 (I-131 remnant ablation today)	88 (L-T4 started 2 weeks ago and titrated)	110	290	462	1200	1235	1330
FT4 (pmol/L)	14	8	3	11	16	19	21	4	15	20
TSH (mU/L)	2.8	20.4	66.8	36.3	7.56	0.32	0.09	42.6	24.1	0.15
L-T4 (μg/day)	0	0	0	50	75	112.5	125	0	100	125

Patient 2

The next clinical vignette involves a typical case of Graves' disease in a 46-year-old woman. From a TFT done as part of a pre-insurance checkup, her stable euthyroid set point was a FT4 of 15.7 pmol/L and a TSH of 1.25 mIU/L, done when she was 32 years of age. At diagnosis, her FT4 was 57.4 pmol/L, and TSH was suppressed to 0.015 mU/L. She was initiated on carbimazole (CMZ) 30 mg daily till she attained clinical euthyroidism. However, her serum TSH remained persistently suppressed for another 5 months prior to finally becoming biochemically euthyroid after 6 months of antithyroid drug treatment (**Table 2**).

This TFT trajectory circumscribes a clockwise hysteresis, as illustrated below (**Figure 2**).

Patient 3

The final clinical vignette describes a 54-year-old woman with a strong family history of autoimmune thyroid disease. She presented to the clinic with progressive weight gain, cold intolerance, and constipation. Investigations confirmed Hashimoto's thyroiditis, and she was put on lifelong L-T4 replacement. She had a normal TFT result taken during a previous medical screen as part of a staff benefit of her employment done about 10 years ago that showed a FT4 of 16 pmol/L and TSH of 1.98 mU/L. The **Table 3** shows the TFT trend and the associated graph (**Figure 3**).

EXPERIMENTAL EVIDENCE OF HYSTERESIS IN LOWER VERTEBRATES

In a study where healthy euthyroid C57BL/6 mice were rendered thyrotoxic with intraperitoneal triiodothyronine (T3),

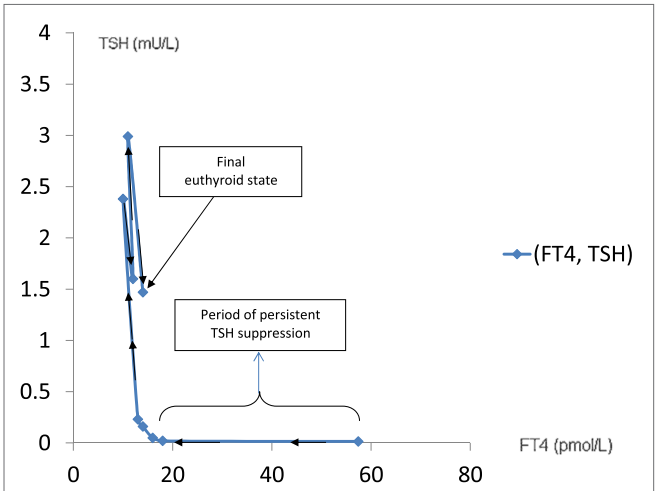

FIGURE 2 | The course of recovery from hyperthyroidism follows a clockwise hysteresis as illustrated in this graph. This is typically observed in patients with Graves' disease treated with antithyroid drugs.

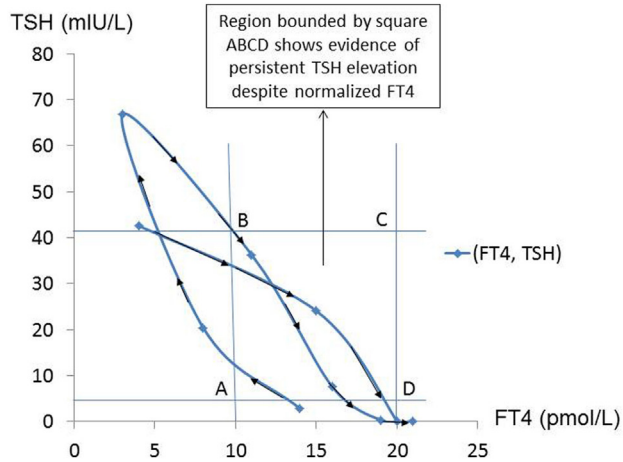

FIGURE 1 | Arrows trace the trajectory of FT4–TSH, as the patient transited from TSH suppression to persistent TSH elevation, showing hysteresis in operation.

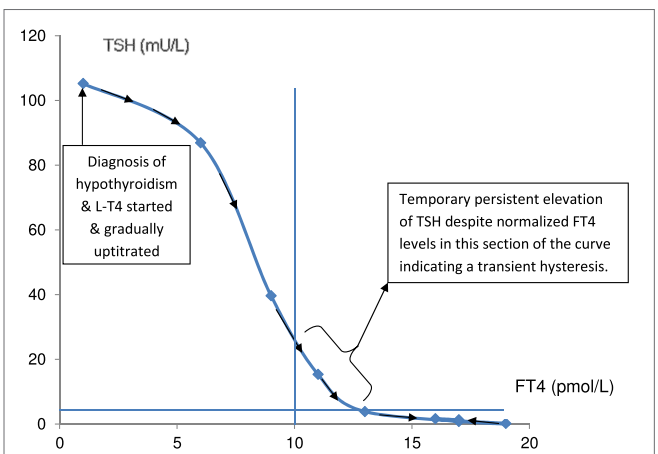

FIGURE 3 | Graph illustrating the hysteretic path of FT4–TSH in a patient with severe hypothyroidism recovering to a euthyroid state following L-thyroxine replacement.

TABLE 2 | Change in thyroid function tests of "Patient 2" over time.

Day	Age 32 (euthyroid set point)	Age 46 (diagnosis – Graves')	Day 42 (CMZ started 6 weeks ago)	84	120	170	215	300	420	600
FT4 (pmol/L)	15.7	57.4	18	16	14	13	10	12	11	14
TSH (mU/L)	1.25	0.015	0.02	0.05	0.16	0.23	2.38	1.60	2.99	1.47
CMZ (mg/day)	0	0	30	20	20	15	10	5	5	2.5

TABLE 3 | Change in thyroid function tests of "Patient 3" over time.

Day	Age 29 (normal euthyroid set point)	Age 44 (diagnosis: Hashimoto thyroiditis)	Day 14 (L-T4 started 2 weeks ago)	30	90	210	350	450	530	620
FT4 (pmol/L)	16	1	6	9	11	13	19	17	16	17
TSH (mU/L)	1.98	105.21	86.92	39.64	15.33	3.86	0.17	0.79	1.65	1.32
L-T4 (μg/day)	0	0	25	25	50	75	100	87.5	87.5	87.5

it was observed that serum TSH was suppressed below the normal limit and remained low for a few days despite recovery of serum FT3/FT4 to normal (21). Taking into consideration of the fact that the time scale in small mammalian vertebrates, such as a mouse or rat, is significantly compressed relative to a human being (22–24), this brief period of delayed recovery of TSH is an evidence that the hysteresis phenomenon also occurs in other mammalian species. This demonstrates that hysteresis of the HPT axis occurs most likely through an evolutionarily conserved mechanism and that hysteresis confers a survival advantage (25). Interestingly, it was found that a number of genes were also suppressed to levels below their pre-thyrotoxicosis baseline expression despite normalization of serum T3 and TSH. This implied that thyrotoxicosis is a state that not only leads to a lag time in recovery of TSH but also a delayed recovery of other genes that are regulated by thyroid hormones especially since thyroid hormones regulate an enormous spectrum of genes throughout the body (26, 27). We also discovered that the expression of target thyroid hormone-responsive genes vary according to whether the state of thyroid hormone excess was acute or chronic. Even more interestingly, we have shown for the very first time that epigenetic histone modifications are involved in these differential gene expressions triggered by the thyrotoxic state and that the type of histone mark mediating this was different in acute (H3 acetylation) vs. chronic (H3K4 trimethylation) thyrotoxicosis (**Figure 4**). Moreover, upon withdrawal of T3 and during the transition from thyrotoxicosis to euthyroidism T3 levels, we showed that about 10% of genes showed incomplete recovery despite normalization of serum T3 and TSH, with some persistently above or below baseline expression (**Figure 5**). Also, the same pattern was observed among the negatively regulated genes. Hence, at least one of the molecular mechanisms governing the prolonged suppression of the TSH gene is likely to be due to epigenetic histone modifications.

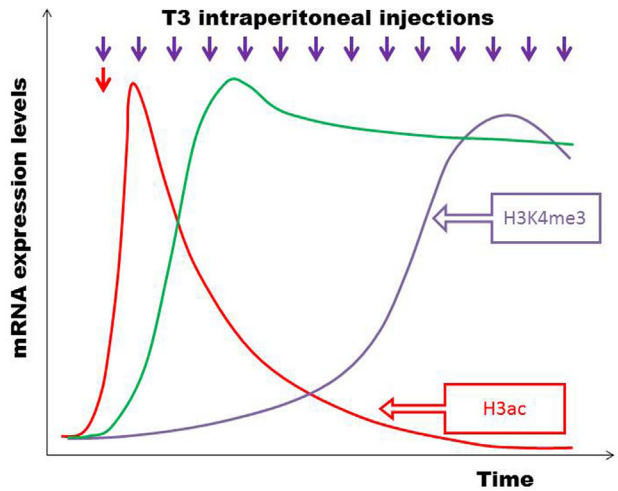

FIGURE 4 | C57BL/6 mice were divided into two groups – one given a single acute T3 injection (red arrow) and another group given multiple T3 injections intraperitoneally over 14 days (violet arrows). Microarrays on liver tissue to study gene expression patterns were done 6 h after acute and chronic T3 injections. Certain target genes responded acutely with a brief increase in mRNA expression, which subsequently became desensitized to T3 and declined (e.g., *Bcl3, Thrsp*) (red line). There were also target genes positively regulated only by chronic T3 exposure, but initially unresponsive to T3 (e.g., *Fgf21*, *Cyp17a1*) (violet line). A third group of genes were positively regulated by both acute and chronic T3 exposure (e.g., *Dio1, Fndc5, Idh3a*) (green line). Different histone modifications influence differential temporal expression patterns during the development of thyrotoxicosis, with H3 acetylation regulating acute T3 responses and H3K4 trimethylation regulating chronic T3 stimulation (21).

MODELING

The mathematical modeling of the hysteresis phenomenon is complex and is rarely applied in the field of biology. Hysteresis modeling has a long history dating back to the landmark Preisach paper published in 1935 (28). The Preisach model introduces a hysteresis operator denoted by $\gamma_{\alpha\beta}$ that represents a rectangular input–output loop, where α and β refer to switch in inputs from "up" to "down," respectively. As an input $u(t)$ is monotonically increased, the function proceeds according to an ascending path while the function switches along a descending path distinct from the ascending path when $u(t)$ is monotonically decreased. Factoring an arbitrary weighted Preisach function, $\mu(\alpha,\beta)$, this hysteresis operator is given by the double integral as follows:

$$f(t) = \hat{\Gamma}u(t) = \iint_{\alpha \geq \beta} \mu(\alpha,\beta)\hat{\gamma}_{\alpha\beta}u(t)d\alpha d\beta$$

where Γ is the Preisach hysteresis operator.

It is instructive to consider various models of HPT axis negative feedback regulation, which can then be modified to include an element of hysteresis as a modeling approach. One such model is exemplified by Pandiyan et al (29). Goede and Leow in 2013 (30) described a simplified hysteresis model of the HPT axis

formed by generalization of a negative exponential model. This was based on a clinically validated HPT axis model, represented by this equation (31):

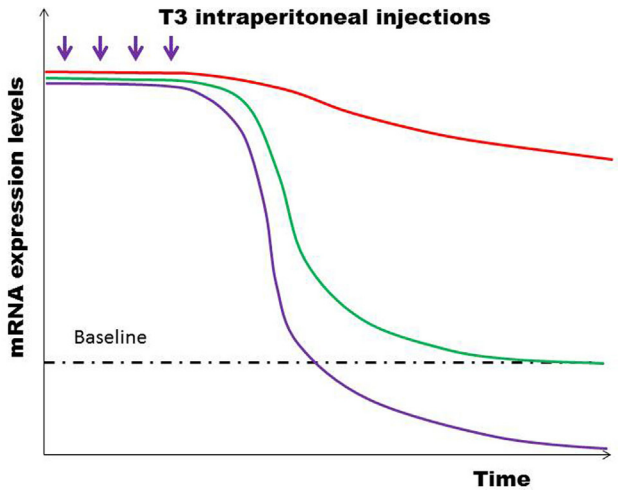

FIGURE 5 | After the final T3 dose injected, most genes returned rapidly to baseline expression levels (green line), while some genes remained persistently upregulated (red line). Still other genes were suppressed below the baseline levels (violet line). Similar patterns of differential temporal expression were also observed in negatively regulated genes (21).

$$[\mathrm{TSH}] = S \exp(-\varphi\,[\mathrm{FT4}])$$

When remodeled by incorporating a hysteresis factor, ψ, the above model can be expressed as:

$$[\mathrm{TSH}] = S/[\psi S + \exp(\varphi\,[\mathrm{FT4}])]$$

This results in saturation effects at the extrema of [FT4] with displacement of the [TSH] function such that the sigmoidal curve is translated horizontally to the left when [FT4] recovers from severe thyrotoxicosis, while the original curve is shifted to the right when [FT4] recovers from severe hypothyroidism. This can be illustrated by the **Figure 6**. Such a simplified hysteresis model assumes that the maximum TSH response of any individual is known. Obviously, it is difficult in reality to know what the maximum TSH response of any given person is. Based on clinical experience, the [TSH] level of those patients who are severely hypothyroid can range from anywhere between 100 mU/L and well above 400 mU/L or so, giving an idea of the usual maximal magnitude of TSH responses in humans (32). On the contrary, many clinicians have encountered how [TSH] can be suppressed to levels practically close to zero or undetectable (e.g.,<0.005 mU/L) in severe hyperthyroidism. In practice, a realistic value that this hysteresis factor ψ will take that applies to the majority of [TSH] responses is therefore about 0.01. Using this value, it is theoretically possible to deduce what a likely normal euthyroid set point of a patient will be in the absence of hysteresis (i.e., when the effect of hysteresis has fully resolved).

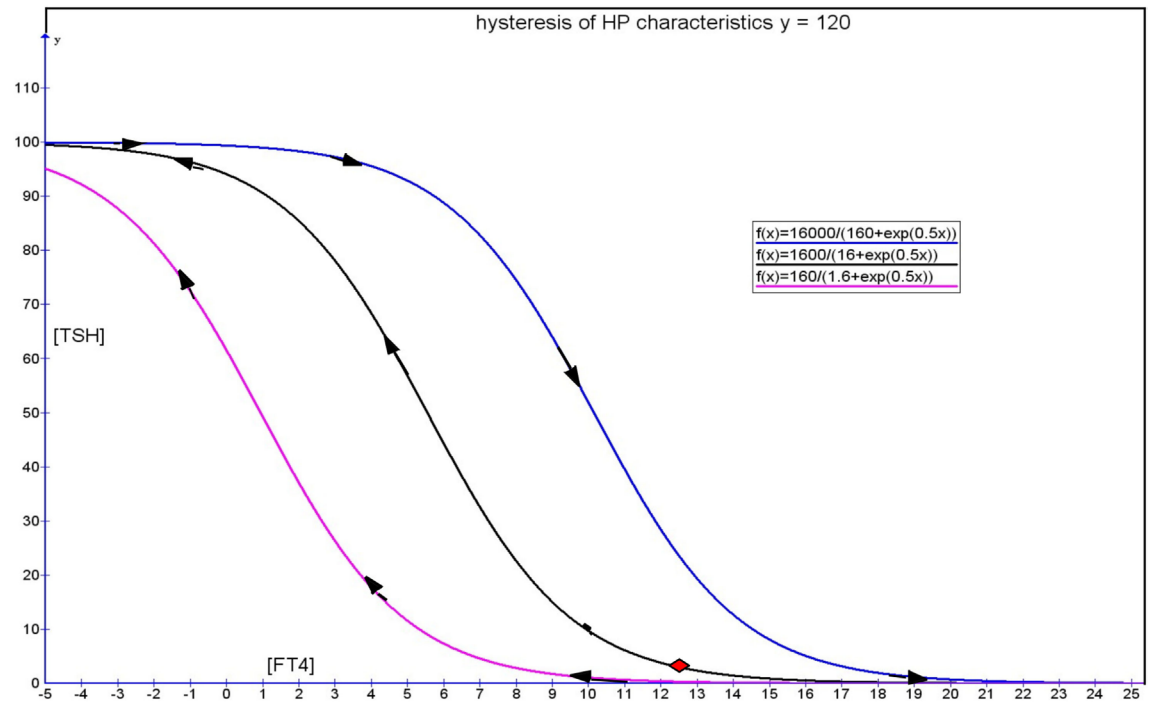

FIGURE 6 | Hysteresis model showing a clockwise loop formed by an ascending limb represented by the pink curve during recovery of severe thyrotoxicosis and a blue descending limb during recovery from severe hypothyroidism. The black curve represents the TSH response trajectory that can be followed with more gentle deviations of FT4 from the euthyroid set point indicated by the red diamond. [Figure adapted from Ref. (30)].

This is understandably a simplified model depicting two hysteresis curves – the forward (recovery from hypothyroidism in blue) and reverse (recovery from hyperthyroidism in red) in a "symmetrical" fashion that are horizontally translated from the original HP curve. In reality, these hysteresis curves are often asymmetric with a more "refractory" reverse hysteresis limb and a relatively more transient forward hysteresis limb. For the purpose of illustration of the concept of hysteresis, a simple model stripped down to the bare essence that contains the elements of delayed recovery to euthyroid TSH levels is shown. This is mainly useful for simulation and teaching purposes. More complex mathematical models of hysteresis will be required to mimic the delayed recovery of TSH for application to individualized patient care. However, such an endeavor is beyond the scope of this article, which is meant to be a brief overarching review of the hysteresis phenomenon in the HPT axis.

BIOLOGICAL SURVIVAL ADVANTAGES OF HYSTERESIS

An intriguing question facing physicians is whether the rapid restoration of suppressed or overexpressed [TSH] to normal in thyrotoxic and hypothyroid patients, respectively, who recently achieved normal [FT4] is necessarily a desirable outcome. Occasionally, this poses a concern to anesthesiologists who wondered if it is possible for physicians to normalize [TSH] rapidly in addition to normalizing [FT4] as an optimization of perioperative risk prior to major surgery. Additionally, once [FT4] has been rendered to normal levels but associated with an elevated or suppressed [TSH], it is arguable if this state is pathologically similar to subclinical thyrotoxicosis or subclinical hypothyroidism. Unlike the latter states, what is clear is that the abnormal [TSH] during the recovery of thyrotoxicosis and hypothyroidism is temporary and will ultimately resolve when given time.

Although it may require preclinical and clinical research to elucidate the factors influencing the duration to recovery from prolonged elevated or suppressed [TSH], an important clue comes from questioning the reason why nature has engineered such a response to cope with swings in hormones with great potency. Thyroid hormone belongs to this category in which adequate levels are critical to survival and yet life threatening when excessive or deficient. Is it any wonder then that [TSH] should remain suppressed for weeks that sometimes dragged to months or years in someone suffering from severe hyperthyroidism whose [FT4] has been brought down effectively by antithyroid drugs? When one analyzes this situation, it becomes apparent that the hysteresis with a lagging recovery in [TSH] helps to protect the individual from accelerated rebound hyperthyroidism in case antithyroid drugs are suddenly discontinued prematurely for whatever reason because the persistent suppressed [TSH] implies negligible TSH stimulation on the unrestrained overactive thyroid. Had [TSH] been normalized rapidly following severe hyperthyroidism, then sudden cessation of antithyroid drugs leading to rapid escalation of [FT4] will be compounded further by extra TSH stimulation on the thyroid follicles to generate even greater [FT4]. In the same vein, for an individual who requires L-thyroxine replacement for severe primary hypothyroidism, the prolonged elevation in [TSH] meant that there is an attempt by the body to continue maximally stimulating the thyroid, in case L-thyroxine should be unexpectedly stopped.

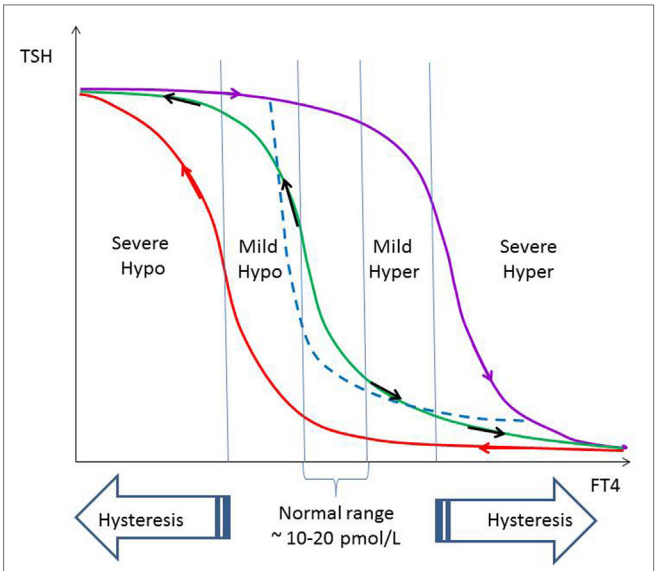

FIGURE 7 | The relationship of FT4 and TSH is a negative exponential function with saturation characteristic, as shown by the green curve (12). Within the normal range of FT4 and slightly beyond on either sides denoted by mild hypothyroidism and hyperthyroidism, respectively, recovery of TSH is more rapid and not subjected to hysteresis. The FT4–TSH relationship within the non-hysteresis boundaries may be approximated by the dashed blue negative exponential curve without saturation (10). In severe hypothyroidism and hyperthyroidism, recovery of TSH lags behind recovery of FT4 significantly due to hysteresis (violet and red lines, respectively).

Therefore, hysteresis of the HPT axis serves as a buffering mechanism to reduce the magnitude of the biological impact of severe hyperthyroidism or hypothyroidism on the organism, especially when thyroid hormones escalate to extreme levels at either side of the normal (**Figure 7**). While speculative, this buffering capacity offered by the hysteresis phenomenon probably confer a survival advantage and is thus expected to be evolutionarily conserved among all vertebrate species depending on a thyroid system for development, metabolism, and survival. Although the TSH gene is the focus of this treatise on the hysteresis of the HPT axis, it is likely that the multitude of other crucial genes governed by thyroid hormones are also potentially subjected to this hysteresis phenomenon and may thus take a variable period of time to return back to baseline following severe hypothyroidism or hyperthyroidism.

CLINICALLY RELEVANT CONSIDERATIONS AND APPLICATIONS

Although suppressed or elevated [TSH] in a real situation of hysteresis is often obvious, it is important to consider the

possibility that TSH abnormalities can occasionally be the result of drug or antibody interferences with assay platforms (33–35). In this respect, the presence of certain heterophile antibodies, such as human anti-mouse monoclonal antibodies (HAMA), can lead to artifactual elevations [TSH] depending on the reactivity of these heterophile antibodies with the detection antibodies in the assay systems. Patients taking biotin supplements can also face the issue of falsely elevated or suppressed [TSH] in biotin-streptavidin affinity-based assays (36). In addition, the prolonged suppression of TSH itself may be contributed by the regulation of TSH secretion *via* ultrashort autocrine loop at the hypothalamic–pituitary level, as supported by the expression of TSH receptors in the folliculo-stellate cells in the anterior pituitary (37–39). Rate-dependent "hysteresis" due to a dynamic lag between input and output such as turnover kinetics of hypothalamic TRH biosynthetic enzymes coupled with varying degrees of enzymatic induction or repression in response to signals establishing new homeostatic equilibria may potentially contribute to the overall observed hysteresis as well. Finally, there is a theoretical possibility among those on L-thyroxine (L-T4) replacement that the timing of blood sampling relative to their L-T4 dosing may pose a confounding suppressive effect on [TSH], as an increase of up to 14% in [FT4] would be expected if L-T4 was ingested prior to blood sampling, assuming a half-life of 7 days for [FT4]. In practice, this is probably insignificant, which means patients need not withhold L-T4 prior to the blood draw as TSH secretion rate does not respond so quickly to such degrees of changes in ambient [FT4] (40, 41).

CONCLUSION

The relationship of [FT4] and [TSH] is a reciprocal one best described by a negative exponential model. Hyperthyroidism and hypothyroidism lead to temporary suppression and overexpression of TSH out of the normal reference range. Even fluctuations of [FT4] within its normal reference range are associated with perceptible reciprocal changes in [TSH]. Mild displacements of [FT4] off the normal limits seldom result in any lagged recovery in TSH. However, in more extreme cases of hyperthyroidism or hypothyroidism, TSH is often appropriately suppressed or overexpressed for a protracted period of time despite adequate treatment that renders [FT4] into the normal range. This phenomenon is now recognized as hysteresis of the HPT axis and probably represents an adaptive response that confers a biological survival advantage for the organism. Hence, HPT axis hysteresis may be evolutionarily conserved and could well operate in vertebrates other than humans, as has been demonstrated in a mouse model. The implication of hysteresis acting as protective buffer may imply that rapid restoration of [TSH] to normal during this lagging recovery phase is not necessarily desirable or advantageous in terms of optimization of the euthyroid state compared to recovery of [TSH] along a slower trajectory.

AUTHOR CONTRIBUTIONS

ML conceived this work, drafted the manuscript, critically reviewed its intellectual content, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Conflict of Interest Statement: Exploit Technologies Pte Ltd. (ETPL), A*STAR's tech-transfer arm, has filed a patent on the HPT axis set point algorithm that has been developed into a computer program (Thyroid-SPOT software), and ML is listed as one of the three coinventors. The patent is successfully granted in Singapore.

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Mathematical Modeling of the Pituitary–Thyroid Feedback Loop: Role of a TSH-T_3-Shunt and Sensitivity Analysis

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Despite significant progress in assay technology, diagnosis of functional thyroid disorders may still be a challenge, as illustrated by the vague upper limit of the reference range for serum thyrotropin (*TSH*). Diagnostical problems also apply to subjects affected by syndrome T, i.e., those 10% of hypothyroid patients who continue to suffer from poor quality of life despite normal *TSH* concentrations under substitution therapy with levothyroxine (L-T_4). In this paper, we extend a mathematical model of the pituitary–thyroid feedback loop in order to improve the understanding of thyroid hormone homeostasis. In particular, we incorporate a *TSH*-T_3-shunt inside the thyroid, whose existence has recently been demonstrated in several clinical studies. The resulting extended model shows good accordance with various clinical observations, such as a circadian rhythm in free peripheral triiodothyronine (FT_3). Furthermore, we perform a sensitivity analysis of the derived model, revealing the dependence of *TSH* and hormone concentrations on different system parameters. The results have implications for clinical interpretation of thyroid tests, e.g., in the differential diagnosis of subclinical hypothyroidism.

Keywords: thyroid hormones, pituitary–thyroid feedback loop, mathematical modeling, diagnosis, TSH-T_3-shunt, sensitivity analysis

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1. INTRODUCTION

In recent years, the mathematical modeling of human thyroid hormone homeostasis via the hypothalamic–pituitary–thyroid feedback loop has received an increasing amount of attention. Starting from early phenomenological models, more precise models have been developed based on molecular and pharmacokinetic data, see, e.g., Ref. (1–3, 4–6) for recent surveys on existing modeling approaches. These mathematical models can give important insight into the functionality of the hypothalamic–pituitary–thyroid axis and can be used to simulate the dynamic behavior of thyroidal hormone concentrations under different (euthyroid and non-euthyroid) conditions, and sometimes also for clinical decision-making (7). Furthermore, in Ref. (8), a method is proposed to compute personalized euthyroid setpoints that can be used for individualized diagnosis and treatment of thyroid diseases. While this static model is appealing due to its simplicity (only two

parameter values have to be estimated), it does not consider any dynamic phenomena in the HPT axis, which are, however, of great importance for a deepened understanding of the HPT axis and ultimately the development of personalized optimal medication strategies. Another drawback is the absence of any consideration of T_3, which has been shown to be significant not only as a key actor in the hypothalamic–pituitary–thyroid feedback loop (4, 9) but also in maintaining a good quality of life (5).

The main objective of this paper is an improved mathematical modeling of the HPT axis in order to obtain a more detailed understanding of the dynamic phenomena occurring in thyroid hormone homeostasis. In particular, as a first contribution, we extend the model originally developed in Ref. (1, 2) in order to incorporate new insights obtained through several recent clinical studies. In particular, we incorporate a direct *TSH*-T_3 path inside the thyroid, accounting for the central T_3 production by the thyroid. Existence of such a *TSH*-T_3-shunt was hypothesized and demonstrated in several experiments and clinical observations (10–15). In Ref. (10), it was shown that L-T_4-treated athyreotic patients exhibit decreased FT_3 concentrations despite normal free thyroxine (FT_4) levels, which would not be the case if peripheral FT_3 was mainly produced by deiodination of peripheral FT_4. Furthermore, the sum activity of step-up deiodinases (G_D) is positively correlated with the *TSH* concentration (11) and with the thyroidal volume (12) and significantly decreases after thyroidectomy. These observations suggest that besides the peripheral T_4/T_3 conversion, also *TSH*-stimulated deiodinases inside the thyroid contribute to the total T_3 production. In our work, we show that the extended model including such a *TSH*-T_3-shunt is in good accordance with various clinical observations. For example, we show that the FT_3 concentration shows a clear circadian pattern, as was observed *in vivo* in Ref. (16). Notably, this is not the case in the previous model, which did not include the *TSH*-T_3-shunt.

As a second main contribution of this paper, we perform a sensitivity analysis of the derived model. Loosely speaking, the (first-order) sensitivities are a measure for how "sensitive" certain system states (i.e., *TSH* or hormone concentrations) are with respect to changes in certain parameters (such as, e.g., the thyroid's secretory capacity G_T). These sensitivities reveal structural insight into the functionality of the hypothalamic–pituitary–thyroid axis and can provide explanations for certain clinical observations. For example, we show that the sensitivity of *TSH* with respect to G_T is much higher for low values of G_T (i.e., in hypothyroidism) than for high values of G_T (i.e., in hyperthyroidism). This fact can be used to explain why in clinical practice, *TSH* concentrations may significantly vary beyond the upper limit of the reference range despite normal thyroid function.

The remainder of this paper is structured as follows. Section 2 presents the extended mathematical model and discusses the identification of the required (additional) parameters. In Section 3, we show simulation results of the derived model and discuss the observed properties (such as the existence of a circadian rhythm in FT_3 concentrations). A sensitivity analysis of *TSH*, FT_4, and FT_3 concentrations with respect to different parameters is performed in Section 4. Finally, we conclude the paper in Section 5.

2. PRESENTATION OF THE EXTENDED MODEL AND PARAMETER IDENTIFICATION

As outlined above, several clinical observations have led to the hypothesis that a direct, *TSH*-stimulated path exists for T_3 production inside the thyroid, which we now incorporate into the mathematical model from Ref. (1, 2). The extended model including this *TSH*-T_3-shunt is illustrated in **Figure 1**, see Section S1 in the Supplementary Material for a mathematical description of the underlying differential equations. To this end, both intrathyroidal conversion of T_4 into T_3 via type 1 and 2 5′-deiodinases as well as a direct synthesis of T_3 are modeled (see upper three blocks in the "Thyroid" block in **Figure 1**). Both mechanisms are stimulated by *TSH* and modeled via nonlinear Michaelis–Menten–Hill kinetics, see Section S1 in the Supplementary Material for further details.

Most of the parameters of the extended model can be taken from the model in Ref. (1, 2), where the parameters have been estimated according to known physical quantities (such as the half-life period of certain substances, etc.) or have been identified using data measured *in vivo*. A detailed listing of these parameters can be found in the Tables S1–S3 in Supplementary Material. Some of the parameters were calibrated according to average population data, and hence the resulting model can be interpreted to be a functional model of some generic euthyroid subject. Clearly, personalized model identification would be highly valuable for individualized clinical decision-making and the development of personalized optimal medication strategies. For this, however, sufficient data such as individual dynamic trajectories of hormone concentrations would be needed to avoid overfitting. We note that, while the present report deals mainly with average population data, the observed phenomena are in good accordance with individual samples (9).

For the extended model, the new parameters G_{T3} and k have to be determined. Also, the sum activity of the type 1 5′-deiodinase, G_{D1}, has to be re-estimated. This is the case since the extended model considers the additional T_3 secretion inside the thyroid, while in the original model, G_{D1} was calibrated by only considering peripheral T_3 production, and hence G_{D1} had been estimated too high. In order to obtain the parameters G_{T3} and G_{D1}, a least squares estimation was performed, fitting the FT_3-output of the presented model to FT_3 measurements of a clinical study. Although there is no unique solution in case that only single FT_3 measurements are available, it provides a set of optimal parameters, which could be further reduced to a unique solution if additional measurements were available (compare the detailed discussion below). In order to perform the least squares estimation, the equilibrium FT_3 level predicted by the extended model, in the following denoted by $FT_{3,eq}$, can be computed in dependence of the parameters by solving a cubic polynomial (see Section S1 in the Supplementary Material for a more detailed description). For this computation, we set *TRH* to a constant value (later, for the dynamic analysis *TRH* is varying in a sinusoidal fashion). This equilibrium value is then fitted in a least-squares sense to real measurement data resulting from 1,121 untreated patients of the clinical study in Ref. (11). In particular, this is

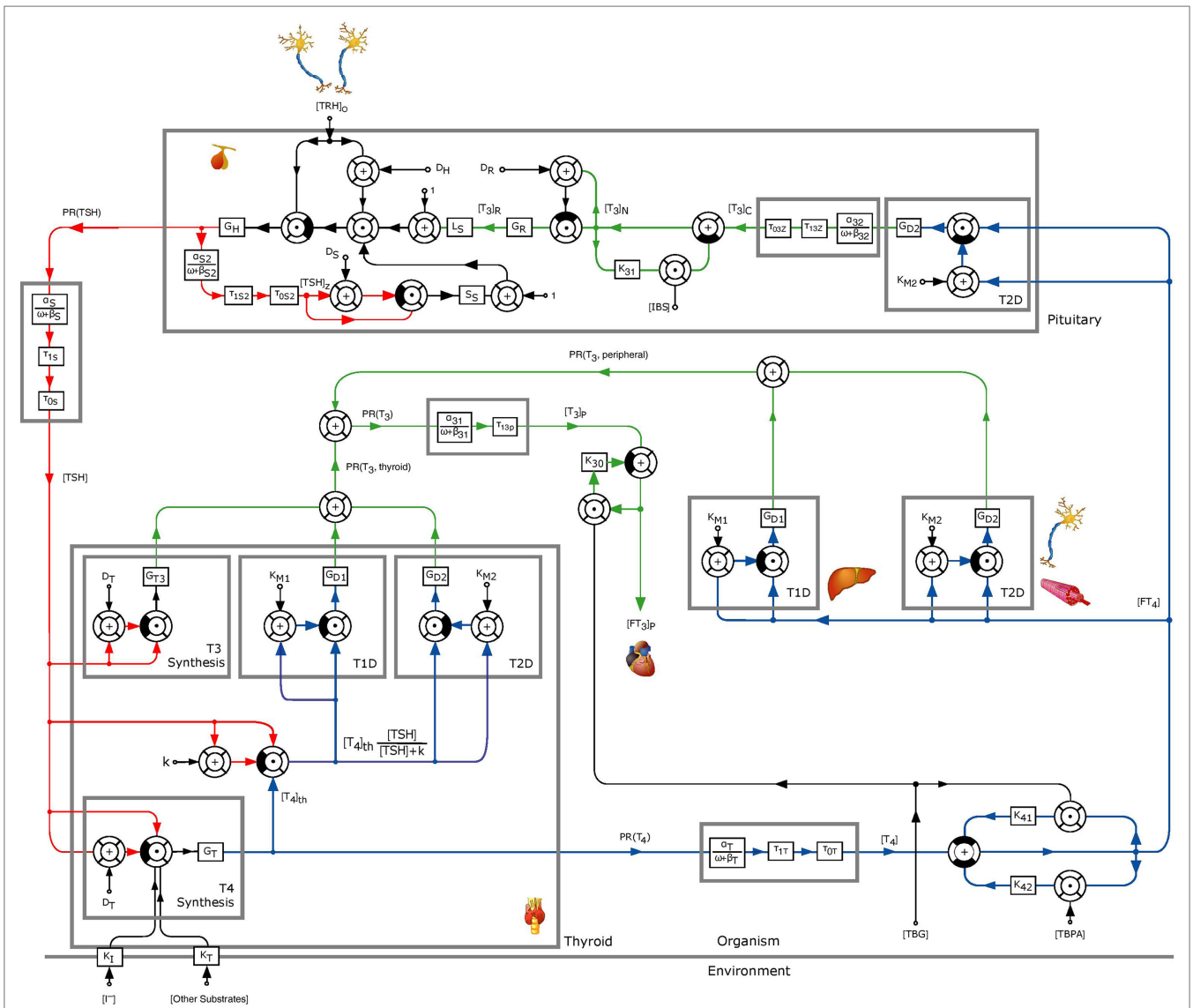

FIGURE 1 | Block diagram of the thyrotropic feedback control loop with an additional *TSH-T_3*-shunt, adapted from Ref. (1, 2). Except for G_{T3}, k, and G_{D1}, all parameters were adopted from the model in Ref. (1, 2). The parameters G_{D1} and G_{T3} were estimated to obtain an optimal (in a least squares sense) fit to measured *in vivo* FT_3-concentrations. To this end, the value of k was normalized to 1 mU/l.

achieved by minimizing the following cost function with respect to the parameters G_{T3}, k, and G_{D1}:

$$J(G_{T3}, k, G_{D1}) = \sum_{i=1}^{1121} \left(FT_{3,i} - FT_{3,eq}(G_{T3}, k, G_{D1})\right)^2 \quad (1)$$

Here, $FT_{3,i}$ denotes the measured FT_3-concentration of the i-th patient, and $FT_{3,eq}(G_{T3}, k, G_{D1})$ is the equilibrium FT_3-level predicted by the model depending on the parameters G_{T3}, k, and G_{D1}. The other parameters that are needed to compute $FT_{3,eq}$ are adopted from Ref. (1) (see Tables S1–S3 in the Supplementary Material). The optimal solution to the above optimization problem can be determined analytically and is given by $FT_{3,eq}(G_{T3}, k, G_{D1}) = \overline{FT_3}$, where $\overline{FT_3}$ is the mean value of the 1,121 FT_3 measurements. Using the derived formula for $FT_{3,eq}(G_{T3}, k, G_{D1})$ (see Section S1 in the Supplementary Material), this results in different (infinitely many) optimal parameter combinations for G_{T3}, k, and G_{D1}. For example, normalizing k to $1\frac{mU}{l}$ (which will be used in the following), the optimal parameter combinations for G_{T3} and G_{D1} can be seen in **Figure 2**.

Different (optimal) parameter combinations for G_{T3} and G_{D1} result in different fractions of thyroidal and peripheral T_3 production. For example, $G_{D1} = 22\frac{nmol}{s}$ and $G_{T3} = 394\frac{fmol}{s}$ approximately lead to 80% T_3 production from peripheral conversion of FT_4 and approximately 20% T_3 production from intrathyroidal secretion, corresponding to the values suggested by Ref. (17, 18). On the other hand, also, a higher or lower fraction of intrathyroidal T_3 production is possible, depending on the values of G_{T3} and G_{D1}. In particular, higher values of G_{T3} and lower values for G_{D1} result in a higher fraction of intrathyroidal T_3 production

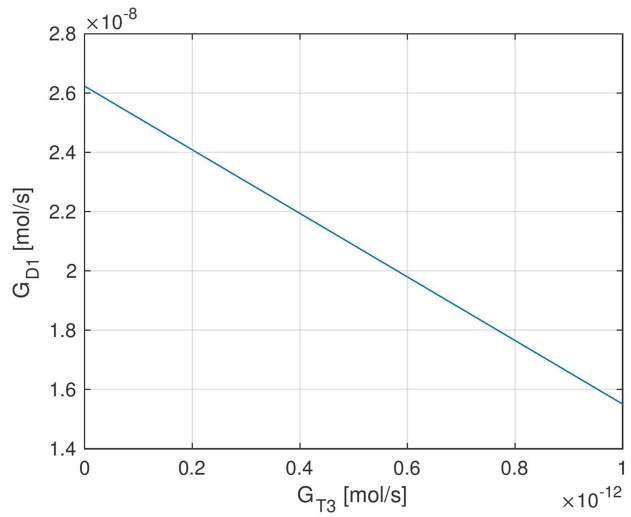

FIGURE 2 | Set of optimal (in a least-squares sense) parameters G_{T3} and G_{D1} when normalizing the parameter k to 1 mU/l. Due to the affine dependence of $FT_{3,eq}$ (G_{T3}, k, G_{D1}) on G_{T3} and G_{D1}, the set of optimal parameters is contained in a one-dimensional affine subspace of $\mathbb{R}^2$.

and vice versa. For the dynamic simulation of the model and the sensitivity analysis in the following sections, we (mostly) use the values $G_{D1} = 22\frac{nmol}{s}$ and $G_{T3} = 394\frac{fmol}{s}$, and we comment when certain results qualitatively change if other parameter values for G_{D1} and G_{T3} are used.

The above discussed non-uniqueness in the optimal parameter fit is due to the fact that the model is not fully identifiable given the measured data. Namely, FT_3 is the only hormone that is affected by the parameters G_{T3}, k, and G_{D1}, and we only have stationary measurements available. Furthermore, in the above estimation, we made the simplifying assumption that peripheral and thyroidal deiodinase activities (G_{D1} and G_{D2}) are the same, which might in general not be the case. Identifying the corresponding parameters separately would result in a possibly better parameterized model, which is, however, again not possible given only the stationary FT_3 measurements. On the other hand, if we had additional data such as dynamic hormone concentration trajectories or additional measurements (e.g., intrathyroidal hormone concentrations), the above described non-uniqueness in the parameter estimation could be removed and also different parameter values for thyroidal and peripheral deiodinase activity could be identified, allowing for a more exact parameterization of the model. This would be an interesting topic for future research, however, such *in vivo* data are difficult to obtain and are typically not available. Moreover, the presented model does not consider membrane transport processes between thyroidal and peripheral tissue. Incorporating such processes by means of a compartment model would further increase the quality of our model, yet, this would yield additional parameters, which had to be identified. Nevertheless, as we will show in the following sections, the extended model with the parameters as identified in this section is a clear improvement compared to the previous model, allowing for a better reproduction and interpretation of various clinically observed phenomena.

3. DYNAMIC PROPERTIES OF THE EXTENDED MODEL

In the following, we simulate the extended model and analyze and interpret the obtained results. First, some simulation runs are carried out to illustrate the role of the *TSH*-T_3-shunt in obtaining a circadian rhythm in the FT_3-concentration. Afterwards, we investigate the delay between *TSH* and FT_3, which has been observed in several clinical studies [e.g., Ref. (16, 19)].

3.1. Dynamic Simulation

As detailed in the previous section, the intrathyroidal T_3 secretion is composed of two mechanisms, namely intrathyroidal conversion of T_4 into T_3 via type 1 and 2 5′-deiodinases (upper middle and right block inside the thyroid in **Figure 1**) as well as a direct synthesis of T_3 (upper left block inside the thyroid in **Figure 1**). In the dynamic simulation using the parameters as identified in Section 2, the intrathyroidal contribution to the total T_3 secretion rate was composed as follows:

$$\frac{Output\ of\ block\ "T3\ Synthesis"}{PR(T_3, thyroid)} = 79.7\%$$
$$\frac{Output\ of\ block\ "T1D"}{PR(T_3, thyroid)} = 20.3\%$$
$$\frac{Output\ of\ block\ "T2D"}{PR(T_3, thyroid)} = 0.002\%$$

Hence, with the parameters identified in Section 2, the main thyroidal source to T_3-production is direct T_3-synthesis via Michaelis–Menten–Hill kinetics, represented by the block "T3 Synthesis" in **Figure 1**. On the other hand, deiodination by type 2 5′-deiodinases has a negligible effect only, since the sum activity of type 2 5′-deiodinases is much smaller compared to that of type 1 5′-deiodinases. In case that a different optimal combination of parameters G_{T3} and G_{D1} is used (compare Section 2), the above results change accordingly, i.e., a higher value of G_{D1} yields a higher contribution of the deiodination by type 1 5′-deiodinases to the thyroidal T_3-production. However, this also causes a change in the ratio between thyroidal and peripheral T_3 production, as discussed in Section 2.

Figure 3 shows simulated FT_3-plots, where we further investigated the effect of the *TSH*-T_3-shunt on the dynamic behavior of FT_3.[1] In particular, **Figure 3A** shows simulation results using the previous model from Ref. (1) without the *TSH*-T_3-shunt whereas in **Figure 3B**, the full *TSH*- T_3-shunt as described in the previous section is included. For each of the two scenarios (i.e., for the corresponding models), we separately identified the (in a least-squares-sense) optimal parameter(s): G_{D1} for the model corresponding to **Figure 3A** and G_{D1} as well as G_{T3} for the model corresponding to **Figure 3B**. The exact values of these parameters for the different model configurations can be seen in Table S4 in Supplementary Material.

[1]The initial hormone values for the simulations shown in **Figure 3** (and for all subsequent simulation runs) were chosen as the stationary mean values of the model, i.e. as the hormone values the model yields for a constant *TRH* input. Note, however, that the choice of initial values is not particularly important for the simulation, as long as they lie somewhere in the euthyroid range.

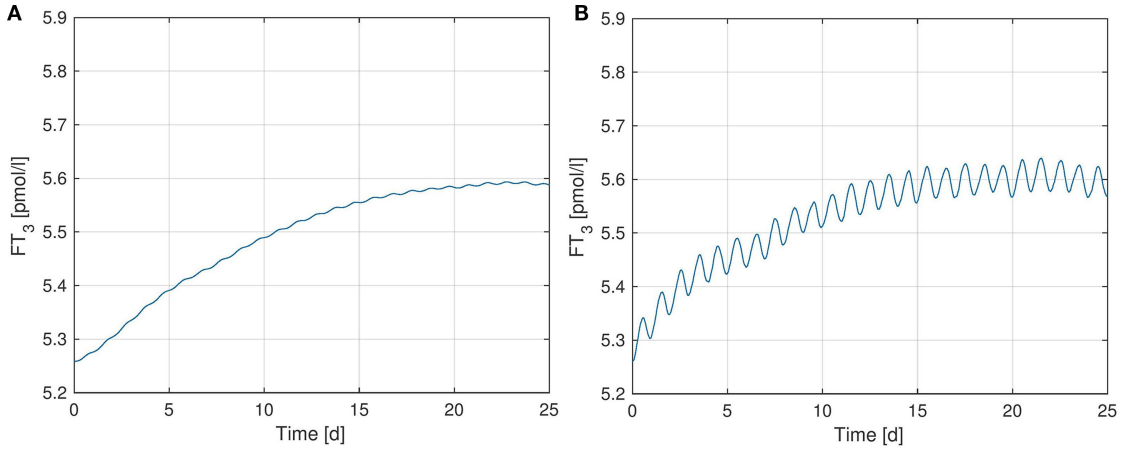

FIGURE 3 | FT_3-plots $[\frac{pmol}{l}]$ over a simulation horizon of 25 days for several configurations of the *TSH*-T_3-Shunt. The parameters G_{T3} and G_{D1} are identified via least squares optimization, separately for each model configuration. **(A)** No shunt included. **(B)** Full *TSH*-T_3-shunt.

We observe that the *TSH*-T_3-shunt causes a clear circadian oscillation in the FT_3 concentration, which is not (or only very weakly) present without considering intrathyroidal T_3 secretion. Such a circadian rhythm in FT_3 concentration has been observed *in vivo* in several clinical studies [see, e.g., Ref. (16, 19)], and hence our simulation results again support existence of the *TSH*-T_3-shunt.

Quantitatively, the oscillation amplitude of the measured *in vivo* FT_3 concentration in Ref. (16) is approximately six times as big as the amplitude observed in the simulated model (see **Figure 3B**). This difference might be due to the assumptions we made for the identification in Section 2 (same values for G_{D1}, G_{D2}, K_{M1}, and K_{M2} inside the thyroid and the peripheral tissue). Namely, if thyroidal deiodination activity and/or G_{T3} were higher than computed in Section 2, without increasing the peripheral deiodination activity as well, we would obtain a larger oscillation amplitude in FT_3 concentration. Nevertheless, the fact that a clear circadian pattern arises in the simulations when including the *TSH*-T_3-shunt into the model is a clear indicator supporting both its existence as well as the fact that thyroidal T_3 secretion is stimulated by *TSH*.

3.2. Delay of FT_3 w.r.t. *TSH*

The authors in Ref. (16) make the observation that *in vivo* FT_3-measurements follow a clear circadian pattern, which is approximately 90 min delayed w.r.t. *TSH*; this number can also vary between different individuals (19). As already mentioned in the previous section, the FT_3-level obtained by the model in **Figure 1** including intrathyroidal T_3-secretion shows a clear circadian pattern. In this section, we investigate how the delay between *TSH* and FT_3 in the presented model is influenced by this newly incorporated mechanism.

A dynamic simulation with the same setup as in Section 3.1 yields the following: when incorporating the *TSH*-T_3-shunt into the model, FT_3 is delayed w.r.t. *TSH* by approximately 6 h, whereas the delay amounts to 13 h in the previous model, which did not incorporate this mechanism. These observed values can be explained as follows. The phase shift between FT_3 and *TSH* in our model mainly results from the first order lag elements $\frac{\alpha}{i\omega+\beta}$ modeling peripheral T_3 and T_4 secretion (i.e., the ones with parameters α_{31}, β_{31}, and α_T, β_T, respectively in **Figure 1**). The phase shift of the output signal of such a first order lag element for a given sinusoidal input signal with frequency ω depends on the parameter β and is given as follows:

$$phase = arctan\left(-\frac{\omega}{\beta}\right). \tag{2}$$

In our case, $\omega = \frac{2\pi}{T}$ where $T = 86{,}400\,s$ is the circadian period of 1 day. The delay between the output and input signal is now computed by simply relating the phase shift to the period length T: $delay = -phase \cdot \frac{T}{2\pi}$. For the given parameter values α_{31}, β_{31}, and α_T, β_T of T_3- and T_4-generation, respectively, we obtain a delay of approximately 5.5 and 6 h, respectively.

The above observed delay of FT_3 w.r.t. *TSH* can now be explained as follows. In the previous model not including the *TSH*-T_3-shunt, the circadian oscillation has to pass through both first order lag elements for peripheral T_4 and T_3 production, resulting in a high delay w.r.t. *TSH*. On the other hand, the fraction of T_3 secreted inside the thyroid does not exhibit the delay caused by peripheral T_4 production and hence exhibits a much shorter delay w.r.t. *TSH*. Interestingly, the observed delay of total T_3 (approximately 6 h) mainly seems to be determined by the shorter one resulting from intrathyroidal T_3 production, although approximately 80% of the total T_3-production results from peripheral FT_4-deiodination and only 20% from intrathyroidal secretion. The reason for this is that as explained above, the circadian rhythm of FT_3 is mainly induced by intrathyroidal T_3 secretion. Namely, the ratio of the amplitude and the mean value equals 0.3% for the peripheral T_3 production rate $PR(T_3, peripheral)$ and 23% for the thyroidal T_3 production rate $PR(T_3, thyroid)$. Thus, the phase of FT_3 is almost solely characterized by the phase of thyroidal T_3-production, and hence the delay of FT_3 w.r.t. *TSH* is determined by the phase shift of only one first order lag element when the shunt is included, compared to two without the shunt. To conclude, the inclusion of the *TSH*-T_3-shunt into the model significantly reduces the delay of FT_3 w.r.t. *TSH*. While

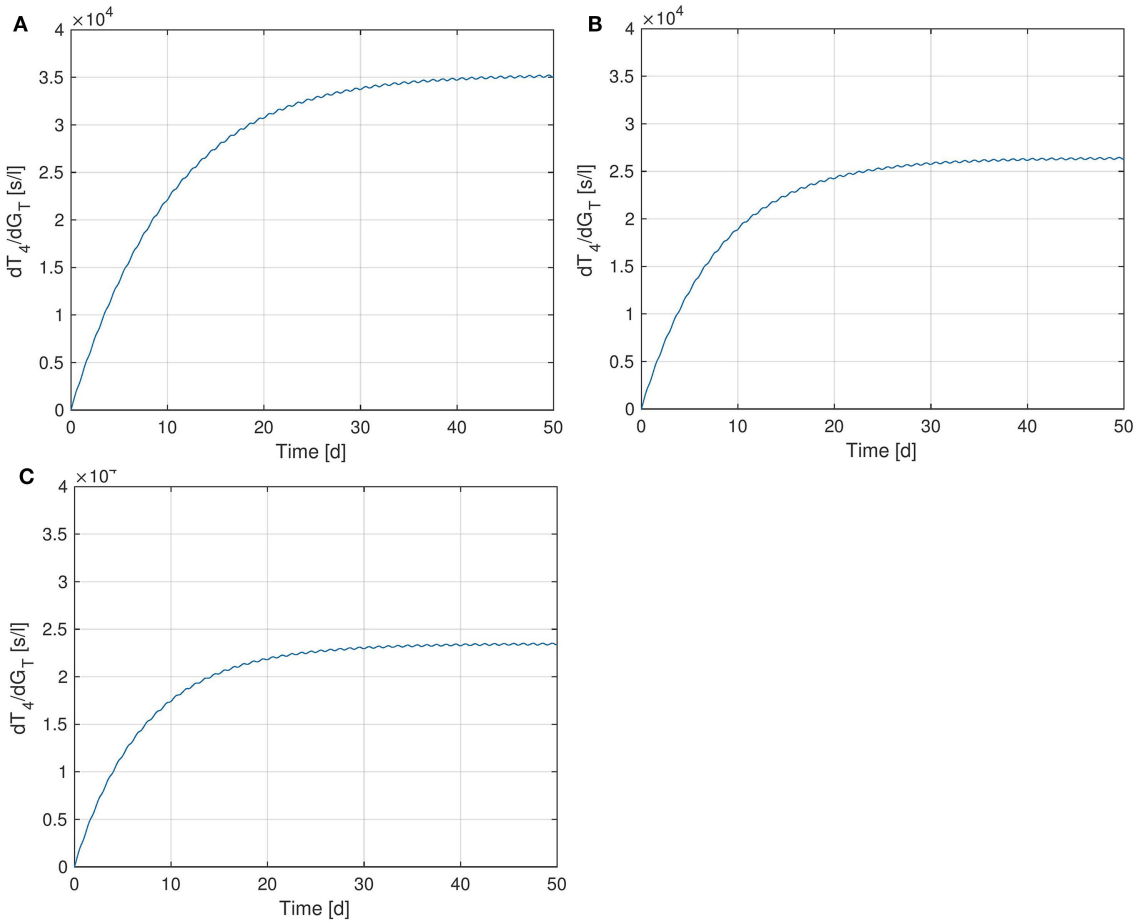

FIGURE 4 | Sensitivity of T_4 w.r.t. G_T for different values of G_T. **(A)** $G_T = 1.2 \cdot 10^{-12}\,\frac{mol}{s}$, **(B)** $G_T = 3.375 \cdot 10^{-12}\,\frac{mol}{s}$ - nominal value, **(C)** $G_T = 5 \cdot 10^{-12}\,\frac{mol}{s}$.

the absolute numbers are still too high compared to the observed *in vivo* delays (16, 19), this is again a clear indicator for the existence of the *TSH*-T_3-shunt.

4. SENSITIVITY ANALYSIS AND STATIONARY DEPENDENCIES

In this section, we perform a sensitivity analysis of the previously presented mathematical model of the hypothalamic–pituitary–thyroid feedback loop (see **Figure 1**). Sensitivity analysis is a tool for determining how a certain parameter influences the trajectories resulting from simulation of the model, i.e., from the solution of the underlying system of differential equations, and in particular, how "sensitive" these trajectories are with respect to certain parameter changes. In the following, we give a brief non-formal introduction to sensitivity analysis and refer to the Section S2 in Supplementary Material for a more complete and formal description.

To define sensitivities, consider the following vector-valued ordinary differential equation with parameter vector p:

$$\dot{x} = f(t, x, p), \quad x(t_0) = x_0. \tag{3}$$

The first-order sensitivity function[2] is now defined as $S(t) = \left.\frac{\partial x(t,p)}{\partial p}\right|_{p=p_0}$, where p_0 is some nominal (constant) parameter value. The sensitivity function $S(t)$ is a time-dependent matrix with as many rows as the dimension of x and as many columns as the dimension of p. Under some assumptions (smoothness, existence of solutions, ...), it can be shown that S satisfies the following differential equation, which is solved simultaneously with the state equation (3), see Ref. (20).

$$\begin{aligned} \dot{x} &= f(t, x, p_0) \\ \dot{S} &= \left[\frac{\partial f(t,x,p)}{\partial x}\right]_{p=p_0} \cdot S + \left[\frac{\partial f(t,x,p)}{\partial p}\right]_{p=p_0} \\ x(t_0) &= x_0, \quad S(t_0) = 0. \end{aligned} \tag{4}$$

The initial sensitivity, i.e., $S(t_0)$, is set to zero since the states' initial values are independent of the parameters. The above presented mathematical model of the hypothalamic–pituitary–thyroid feedback loop (see **Figure 1**) includes 36 parameters. With 5 states

[2]The definition of S is such that it measures the sensitivity locally around a given nominal parameter value p_0 along a solution trajectory of system (3), which is why it is typically called *first-order* sensitivity. In the following, for brevity, we just use the term sensitivity.

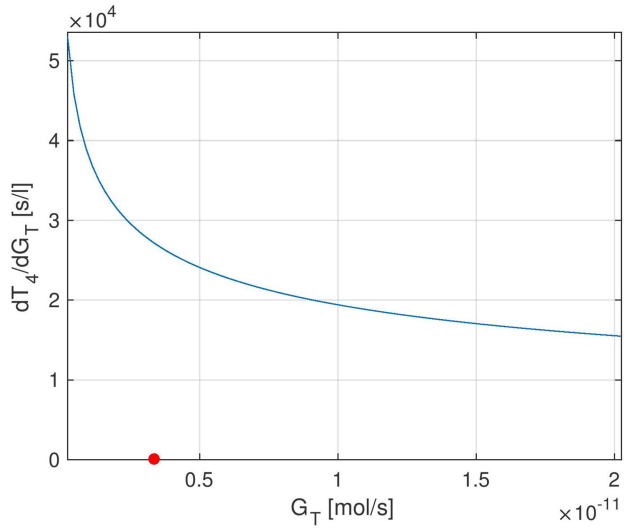

FIGURE 5 | Stationary sensitivity of T_4 w.r.t. G_T as a G_T-dependent function. The red point indicates the nominal G_T-value from Ref. (1).

(pituitary *TSH* and T_3 as well as peripheral *TSH*, T_4, and T_3 concentrations), this makes a total of 180 different sensitivity curves - for one specific nominal parameter configuration p_0. In the following, we only analyze a few interesting curves to obtain some new insights. Of course, if desired, one could analogously analyze further sensitivities of other state and parameter pairs. In order to be able to employ the standard sensitivity analysis tools described above, the time delays in the hypothalamic–pituitary–thyroid (HPT) axis model are neglected.

4.1. Sensitivity of T_4 w.r.t. G_T

We start by examining the sensitivity of peripheral T_4 with respect to the thyroid's secretory capacity G_T. In **Figure 4**, several plots of the sensitivity $\frac{\partial T_4}{\partial G_T}(t)$ are shown with different G_T-values, corresponding to different parameter values p_0 in equation (4).[3] Note that the sensitivity curves do not exhibit large variations over the day, i.e., only show a small circadian oscillation. It can be seen that different values of G_T result in different sensitivities of T_4 with respect to G_T. For example, a low value of G_T (which can be seen as a simple modeling of hypothyroidism) causes an increase of the sensitivity, whereas a high G_T value (which can be seen as a simple modeling of hyperthyroidism) causes a decrease of the sensitivity. This means that larger fluctuations in T_4 can be expected at the lower end of its euthyroid reference range (compare Section 4.3). This observation can compactly be expressed for a wide range of G_T-values by investigating the stationary sensitivity (i.e., $\lim_{t\to\infty} \frac{\partial T_4}{\partial G_T}(t)$). **Figure 5** shows it as a function of the parameter G_T. A comparison between **Figures 4** and **5** shows that the stationary sensitivity, indeed, is the limit of the sensitivity curve for $t \to \infty$.

[3]For the simulation runs shown in **Figure 4** and in all subsequent dynamic sensitivity curves, the initial sensitivity is set to zero [cf. also the initial condition $S(t_0)$=0 in (4)]

4.2. Sensitivity of *TSH* w.r.t. *TRH*

It is interesting to observe that the Ultra-Short-Feedback loop (i.e., the lower left part inside the pituitary in **Figure 1**, compare Ref. (2)) has a significant influence on the sensitivity of *TSH* w.r.t. TRH. **Figure 6** shows the curves of this sensitivity for different values of S_s. It can be seen that an increase in S_s causes a decrease in the sensitivity.

In the considered HPT axis model, *TRH* is treated as a time-dependent input that comes from the hypothalamus. A disturbance in the system could lead to a change in the *TRH* concentration arriving at the pituitary. Apparently, the Ultra-Short-Feedback increases the robustness of the *TSH* production w.r.t. changes in portal *TRH*. If the additional feedback is absent (i.e., $S_s = 0$), the sensitivity is significantly higher than in the nominal case $(S_S = 100\frac{l}{mU})$.

4.3. Stationary Dependencies of *TSH* and T_4 on G_T

In the following, the influence of the thyroid's secretory capacity G_T on the equilibrium concentrations of *TSH* and T_4 is analyzed. To this end, we solve the system's stationary equations (i.e., for $t \to \infty$) for the different hormones and plot the resulting equilibrium hormone levels as functions of G_T. The slopes of these functions are exactly the entries of the stationary sensitivity matrix $\lim_{t\to\infty} S(t)$. For example, the stationary sensitivity $\lim_{t\to\infty} \frac{\partial T_4}{\partial G_T}(t)$ for a given value of G_T is equal to the derivative of the curve $T_4(G_T)$ w.r.t. G_T, which we treat in the following.

The curves of T_4 and *TSH* depending on G_T are shown in **Figure 7**. One can see that the parameter G_T can be used as a measure of hypo- or hyperthyroidism (1). The equilibrium T_4-concentration increases almost linearly with G_T. Furthermore, we have high *TSH*-levels for low values of G_T, i.e., in hypothyroidism, and vice versa. This is a well-known fact, which is usually used in clinical decision-making for the determination of subclinical thyroid diseases. Another interesting fact is that the magnitude of the sensitivity of *TSH* w.r.t. G_T (which is the slope of **Figure 7B**) is high for low values of G_T and vice versa. This means that *TSH* is much more sensitive to fluctuations in the thyroid's secretory capacity if G_T is low. This fact can be used to interpret the following clinical observation. In practice, *TSH* concentrations may be misleading, especially, if located slightly above the vague upper limit of the reference range. A reason for this could be that as discussed above, *TSH* is much more sensitive to fluctuations in the thyroid's secretory capacity (e.g., due to different iodine supply and other influences) at the upper limit of its (euthyroid) reference range than at its lower limit.

4.4. Sensitivity of FT_3 w.r.t. G_T

Finally, we perform a sensitivity analysis for FT_3 w.r.t. the parameter G_T. **Figure 8** shows the sensitivity curve, where in **Figure 8A**, the extended model including the *TSH*-T_3-shunt was used, whereas **Figure 8B** uses the previous model without the shunt. It can be seen that the sensitivity of FT_3 w.r.t. G_T decreases when the shunt is included. This is to be expected since in the extended model, a direct synthesis of T_3 (upper left block inside the thyroid in **Figure 1**) is included, which is independent of

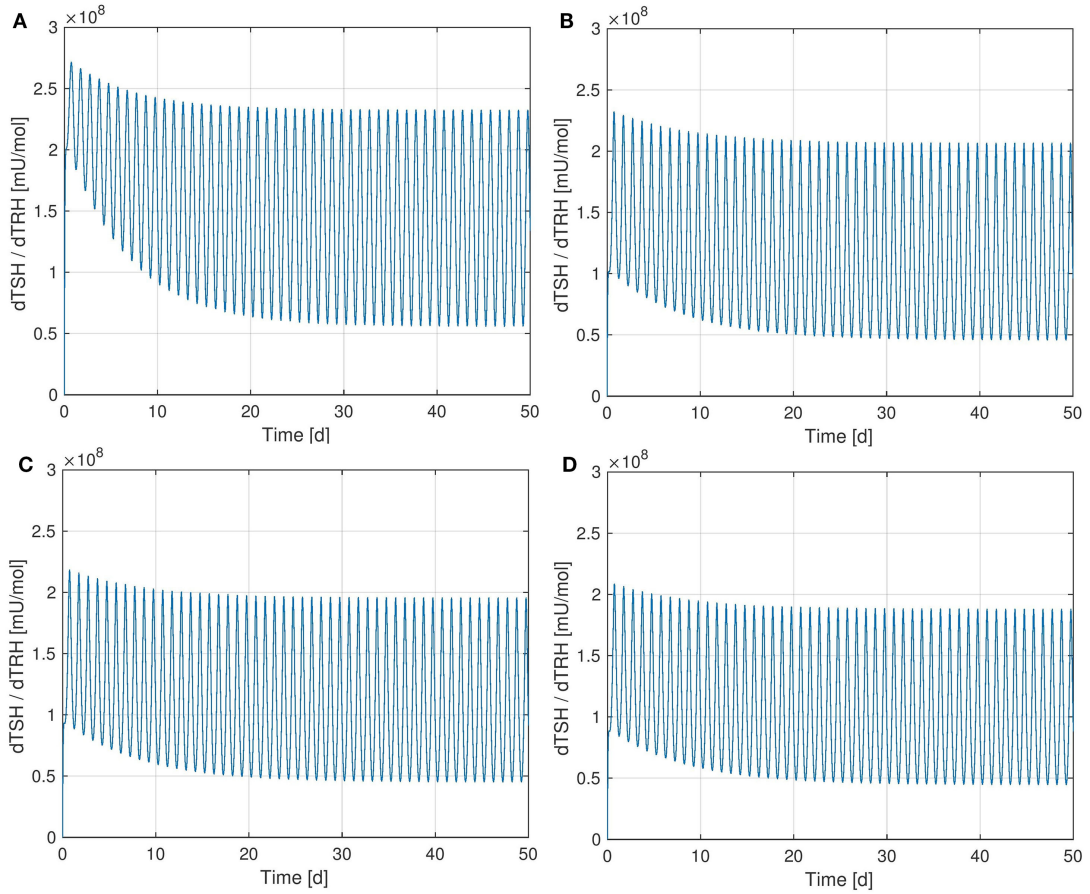

FIGURE 6 | Sensitivity of *TSH* w.r.t. *TRH* for different values of S_S. **(A)** $S_S = 0\frac{l}{mU}$, **(B)** $S_S = 50\frac{l}{mU}$, **(C)** $S_S = 100\frac{l}{mU}$ - nominal value, **(D)** $S_S = 200\frac{l}{mU}$.

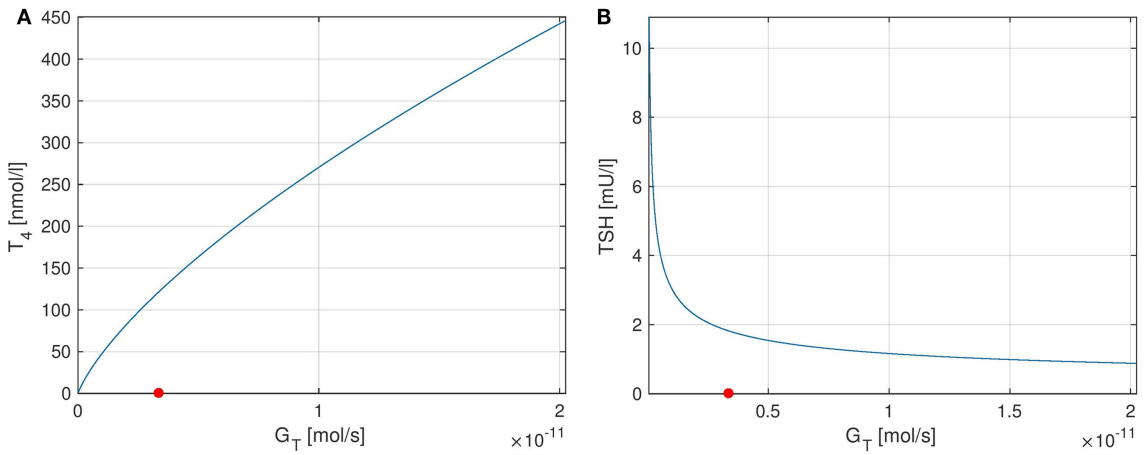

FIGURE 7 | Plots of equilibrium T_4 and *TSH* levels depending on the thyroid's secretory capacity G_T. The red point in the figures indicates the nominal G_T-value from Ref. (1). **(A)** Equilibrium T_4, **(B)** equilibrium *TSH*.

G_T, i.e., from the thyroid's secretory capacity for T_4. Another interesting fact is that we can observe a small circadian rhythm in **Figure 8A** whereas the plot (**Figure 8B**) seems not to be affected by this. This confirms the observations we made in Section 3.1, namely that incorporating intrathyroidal T_3-secretion causes a circadian rhythm in FT_3 and hence also in the sensitivity w.r.t. G_T.

As for T_4 and G_T, we can also analyze the stationary sensitivity $\lim_{t\to\infty} \frac{\partial FT_3}{\partial G_T}(t)$ for different values of G_T. In Ref. (10), the

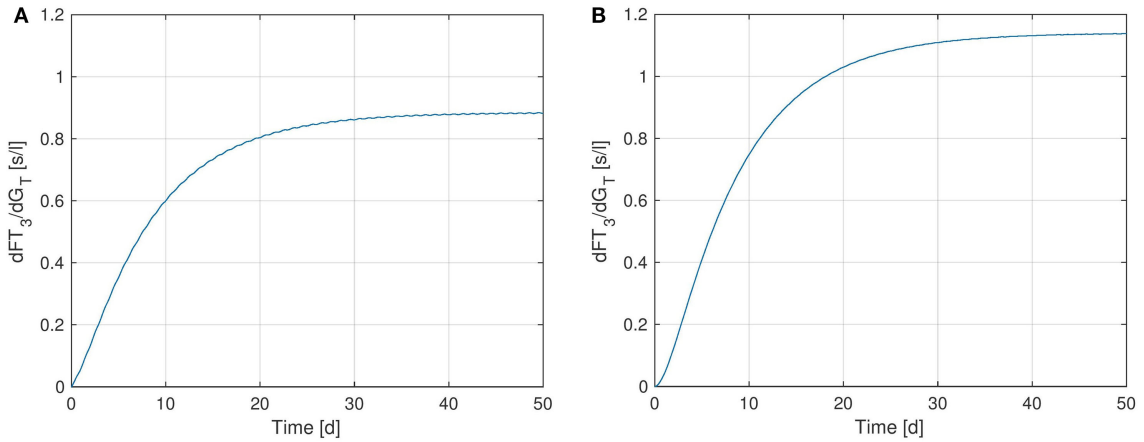

FIGURE 8 | Sensitivity of FT_3 w.r.t. G_T for two versions of the HPT axis model: one incorporating the *TSH*-T_3-shunt and one without this extension. **(A)** Full *TSH*-T_3-shunt, **(B)** no shunt included.

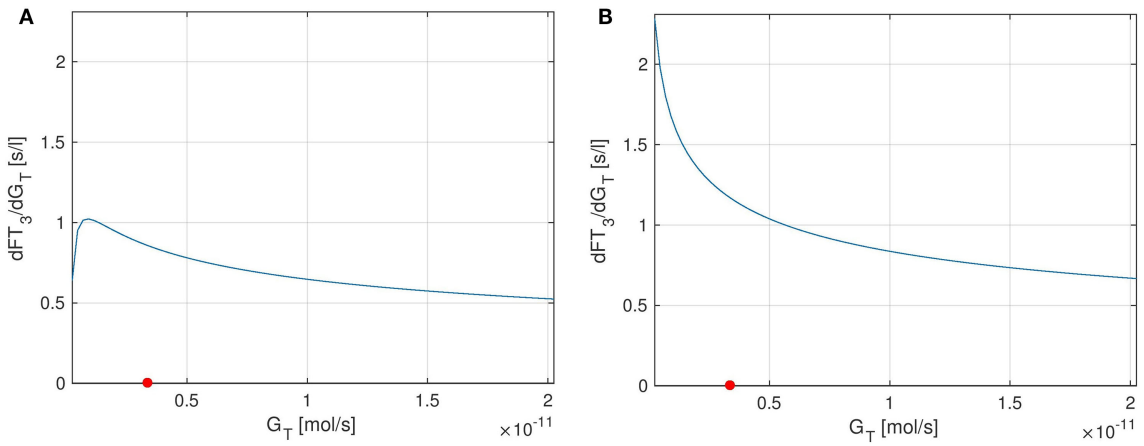

FIGURE 9 | Plots of the stationary sensitivity of FT_3 w.r.t. the parameter G_T as a function of the thyroid's secretory capacity G_T. Two configurations of the model are shown: one including the *TSH*-T_3-shunt and one without the shunt. The red point in the Figures indicates the nominal G_T-value from Ref. (1). **(A)** Full *TSH*-T_3-shunt, **(B)** no shunt included.

outcomes of a clinical study lead to the observation that the dependency of T_3-generation on G_T is lower than that predicted by a model, which does not include a *TSH*-T_3-shunt. Indeed, comparing **Figures 9A,B**, one can see that the sensitivity of FT_3 w.r.t. G_T significantly decreases when the shunt is incorporated into the model, i.e., T_3 production is less sensitive to fluctuations in the thyroid's secretory capacity if the shunt is included. As already mentioned above, this seems plausible since we now have a completely G_T-independent path from *TSH* to FT_3 (upper left block inside the thyroid in **Figure 1**).

5. CONCLUSION AND OUTLOOK

In this work, a mathematical model of the hypothalamic–pituitary–thyroid feedback loop was extended to include *TSH*-stimulated intrathyroidal T_3-secretion. The hypothesis of the existence of such a *TSH*-T_3-shunt has been brought forward in various recent clinical studies. Our results show that the hypothesized mechanism can indeed explain various clinical findings. In particular, we have shown that intrathyroidal T_3-secretion results in a clear circadian pattern of peripheral FT_3, which has been observed *in vivo* in, e.g., Ref. (16, 19), and which is not the case without the incorporation of such a *TSH*-T_3-shunt. Also, a sensitivity analysis revealed that the sensitivity of peripheral FT_3 with respect to the thyroid's secretory capacity for T_4 is indeed lower when including intrathyroidal T_3-secretion into the model, in accordance with the clinical study of Ref. (10).

While the present report deals primarily with technical aspects of the thyroid pituitary feedback regulation, a better understanding of the underlying control system is of high clinical interest and relevance. Currently, clinical diagnosis and treatment of thyroid disease heavily relies on an indirect approach assessing the pituitary *TSH* response rather than circulating free thyroid hormones, FT_3 and FT_4 (21). The application is based on the underlying assumption that pituitary *TSH* in equilibrium at all times provides an accurate mirror image of the peripheral hormones. However,

recent evidence has challenged this simplistic tenet suggesting that the HPT axis is a much more dynamic system than has been previously thought (5, 22). In particular, the interrelationships between FT_3, FT_4, and *TSH* are less constantly fixed, rather conditional and contextualy adaptive (5, 22). Mathematical modeling presented in this study confirms and advances the theoretical framework that is emerging from recent clinical studies. Given the high prevalence of subclinical thyroid disorders in the population, being as high as 10% in middle aged women, the epidemiological and therapeutic implications are substantial. From the performed sensitivity analysis in the present study, important insights into the functionality of the HPT axis have been obtained. These include the robustification of *TSH* production through the ultrashort feedback loop in the pituitary, as well as a possible explanation why in clinical practice, diagnosis of wrong subclinical hypothyroidism is much more common than diagnosis of wrong subclinical hyperthyroidism.

In particular, the upper reference limit for *TSH* has been a matter of fierce debate for a decade (23). According to our models, the issue appears to be more fundamentally rooted. This relates to a substantial error rate, depending on the statistical analytical technique used, in the conventional disease classification based solely on statistical *TSH* abnormality (24). The relative variability in *TSH* rises even further with higher *TSH* concentrations in subclinical hypothyroidism (25). Recent guidelines have raised the clinical threshold for therapeutic intervention in subclinical hypothyroidism to a *TSH* level of 10 mU/l, whereas the laboratory-based disease definition continues to rely on the upper reference limit of approx. 4 mU/l (21). A better understanding and refined mathematical expression of hypothalamic–pituitary regulation in allostatic reactions (22), in thyrotropic insufficiency (26), and in situations of imminent thyroid failure (9, 27) as well as in their interactions may help reconcile this discrepancy that poses a considerable challenge to clinical decision-making.

Future work should focus on the further extension of the model to include currently unmodeled phenomena and mechanisms, such as, e.g., non-classical thyroid hormone signaling (28) and compartment models for the incorporation of membrane transport processes, which are increasingly understood as a regulatory element in their own right (29, 30). We would also aim to define the steady-state more narrowly and precisely for individual subjects under different conditions in an attempt to reduce the high uncertainty surrounding *TSH* measurements at the upper limit of its reference range. In general, obtaining further insight into the overall functionality of the hypothalamic–pituitary–thyroid feedback loop and developing suitable and detailed enough mathematical models might eventually pave the way for designing optimal medication strategies for various non-euthyroid states of human hormone homeostasis.

AUTHOR CONTRIBUTIONS

JB and MM drafted the manuscript. JB performed the simulations using Matlab/Simulink. **Figure 1** was designed by JD and modified by JB, all other figures were created by JB. The deidentified data used for the parameter identification was provided by RH. All authors read and approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at https://www.frontiersin.org/articles/10.3389/fendo.2018.00091/full#supplementary-material.

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REVIEW
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Thyroid Allostasis–Adaptive Responses of Thyrotropic Feedback Control to Conditions of Strain, Stress, and Developmental Programming

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The hypothalamus–pituitary–thyroid feedback control is a dynamic, adaptive system. In situations of illness and deprivation of energy representing type 1 allostasis, the stress response operates to alter both its set point and peripheral transfer parameters. In contrast, type 2 allostatic load, typically effective in psychosocial stress, pregnancy, metabolic syndrome, and adaptation to cold, produces a nearly opposite phenotype of predictive plasticity. The non-thyroidal illness syndrome (NTIS) or thyroid allostasis in critical illness, tumors, uremia, and starvation (TACITUS), commonly observed in hospitalized patients, displays a historically well-studied pattern of allostatic thyroid response. This is characterized by decreased total and free thyroid hormone concentrations and varying levels of thyroid-stimulating hormone (TSH) ranging from decreased (in severe cases) to normal or even elevated (mainly in the recovery phase) TSH concentrations. An acute versus chronic stage (wasting syndrome) of TACITUS can be discerned. The two types differ in molecular mechanisms and prognosis. The acute adaptation of thyroid hormone metabolism to critical illness may prove beneficial to the organism, whereas the far more complex molecular alterations associated with chronic illness frequently lead to allostatic overload. The latter is associated with poor outcome, independently of the underlying disease. Adaptive responses of thyroid homeostasis extend to alterations in thyroid hormone concentrations during fetal life, periods of weight gain or loss, thermoregulation, physical exercise, and psychiatric diseases. The various forms of thyroid allostasis pose serious problems in differential diagnosis of thyroid disease. This review article provides an overview of physiological mechanisms as well as major diagnostic and therapeutic implications of thyroid allostasis under a variety of developmental and straining conditions.

Keywords: thyroid allostasis, non-thyroidal illness syndrome, thyroid hormone metabolism, hypothalamus–pituitary–thyroid feedback control, TACITUS syndrome

INTRODUCTION

Contemporary diagnosis of thyroid disorders relies predominantly on point measurements of thyrotropin [thyroid-stimulating hormone (TSH)] concentration (1). While some guidelines recommend combining TSH measurements with free thyroxine (FT4) determination (2, 3), others constrain diagnostic workup on TSH measurements as a first-line diagnostic test and only recommend determining peripheral thyroid hormones, if TSH concentrations fall outside of their respective reference ranges (4–6). This strategy rests upon the assumptions of a log-linear relationship between TSH and FT4 (7–10), a long plasma half-life of thyroid hormones (11, 12) and tight coupling of all involved control elements of the feedback loop (13, 14). While TSH-based diagnostic interpretation may be inexpensive (at least at the beginning of the decision-making process) it is over-simplifying and involves considerable risks of both false positive and false negative results. Restrictions of TSH-based protocols include circadian and ultradian variation of TSH and thyroid hormones (15–18), the plasticity of central components of the feedback loop under substitution therapy with levothyroxine (19–22) and, as we will subsequently outline, reactive adjustments of thyroid homeostasis in certain phases of development and conditions of strain and stress (23–29).

The hypothalamic-pituitary-thyroid (HPT) axis acts as an adaptive, dynamic system, functioning in two distinct operating modes. The system operates as a homeostatic regulator in unstrained resting conditions, aiming at constant value control (30–33) and maintaining serum concentrations of thyroid hormones in the vicinity of a fixed set point (17, 18, 34–38). The stable situation in equilibrium permits the use of TSH measurement for diagnostic purposes in thyroid disease. However, concentrations of TSH and thyroid hormones may be altered in other physiological and pathological situations in the absence of any dysfunction of the thyrotropic control system or any of its elements (23–25, 39–42). The feedback control mechanism is able to modify its transfer parameters, if a need arises, to tune consumption to available supply with oxygen, energy, and glutathione. The operating mode then shifts to a system of tracking control, which features a dynamically changing set point (26, 43–45). Clinical patterns emerging from this kind of adaptation are well known to physicians. They typically include, but are not limited to, altered, either low or high, T3 concentrations, changes in binding of thyroid hormones to plasma proteins and adjustment of the central control input. In humans, allostatic operation of the HPT axis was initially described in exhausting exercise, starvation, and systemic illness (46–52). Similar patterns were later observed under such diverse conditions as fetal life, major depression (MD), and space flight. However, adding further to the complexity of the constellation, opposite changes have been described in other situations such as pregnancy, endurance exercise, and certain psychiatric diseases (**Figure 1**) (27–29).

The characteristic adaptive constellation of thyroid homeostasis to severe illness is referred to as low-T3 syndrome, nonthyroidal illness syndrome (NTIS), euthyroid sick syndrome (ESS) or thyroid allostasis in critical illness, tumors, uremia, and starvation (TACITUS). About 30% of hospitalized patients (54) and more than 60% of patients affected by critical illness (55, 56) experience transient changes in serum concentrations of TSH and thyroid hormones. Characteristic patterns are low levels of free and total 3,3′,5-triiodothyronine (T3) (39, 40, 56), impaired plasma protein binding of thyroid hormones (57, 58) and, in more severe cases, thyrotropic adaptation with a downward shift of the set point characterized by paradoxically low TSH levels in the presence of normal or even low concentrations of FT4 (59, 60). Conversely, serum concentrations of 3,5,5′-triiodothyronine (rT3) and 3,5-diiodothyronine (3,5-T2) are typically increased (39, 40, 61–63).

In severe illness, the presence of NTIS predicts poor prognosis (54, 60, 64–66). It is still a matter of fierce debate if patients

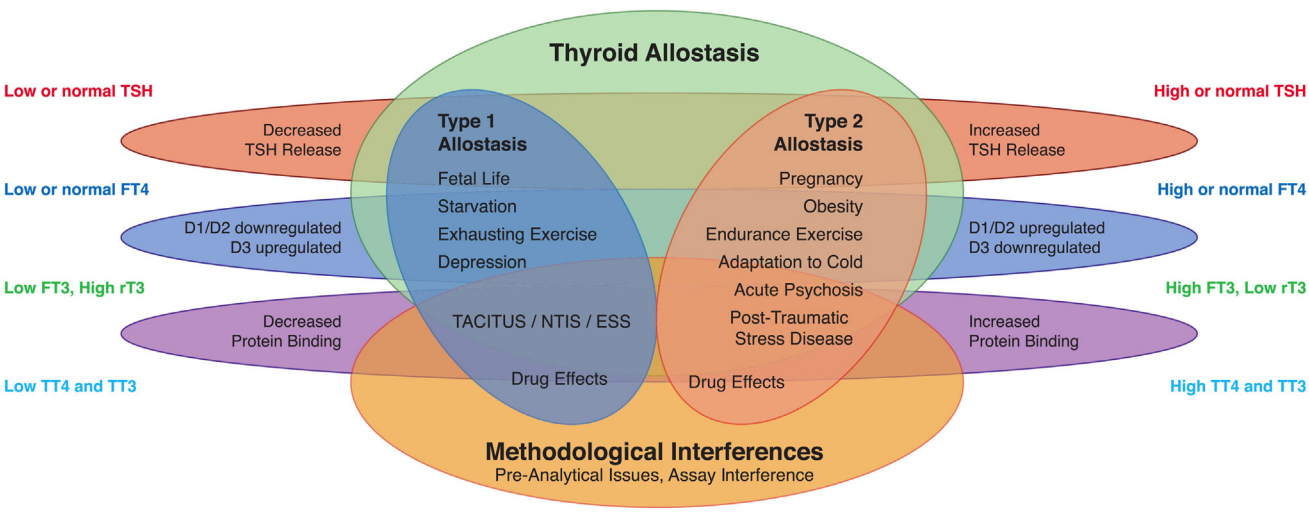

FIGURE 1 | Altered concentrations of thyroid hormones in certain life situations may result from type 1 allostatic load (comprising thyrotropic adaptation, hypodeiodination, and decreased protein binding of thyroid hormones), type 2 allostatic load [showing increased thyroid-stimulating hormone (TSH) release, hyperdeiodination, and augmented binding of thyroid hormones to plasma proteins], and non-homeostatic mechanisms including methodological interferences (53).

affected by the syndrome may benefit from substituting thyroid hormones (67–70). Importantly, significant problems in differential diagnosis may arise from both considerable overlap of hormone concentrations in NTIS with those in primary or secondary thyroid disorders and by methodological interference with thyroid hormone assays (71–73).

This review article provides an overview of adaptive responses of thyroid homeostasis in type 1 and type 2 allostatic situations. It is based on a broad literature search executed with the search formulas "non-thyroidal illness OR non-thyroidal illness OR NTI OR NTIS OR TACITUS OR euthyroid sick OR low T3 OR low triiodothyronine OR Euthyroid Sick Syndromes [MeSH]," "(thyroid OR thyroxine OR triiodothyronine) AND (allostasis OR allostatic)" and "amygdala AND TRH" in PubMed, the authors' own collections of literature and secondary publications referenced there. Where not otherwise specified information provided refers to the human organism. Data from animal research are reported, where information on the human metabolism is lacking.

HISTORICAL OVERVIEW

Perhaps the first description of NTIS dates back to the tenth century BC, when King David was on his deathbed: "*Now King David was old, and advanced in years: and when he was covered with clothes he was not warm.*" [1 Kings 1:1]. Of note, David was not mentally impaired, since in the same time he had managed to defend Solomon, his designated successor, against a subtle conspiracy. Therefore, the situation described by the unknown author seems to represent an exclusively peripheral reduction of thyroid hormones, perhaps in the context of senescence and/or multi-morbidity.

In the human organism, transient alterations of thyroid hormone metabolism unrelated to pituitary or thyroid disease were first explicitly described in 1968, when Clifford Irvine reported reduced half-life of T4 in athletic training, which was reversible after three days' rest (74). The same author had made similar observations in horses, where thyroxine secretion rate increased in training and adaptation to cold, and half-life decreased in trained animals (75). Shortly later, Harland and Orr described a significantly decreased half-life of T4, when rats were exposed to a cold environment (76). Transiently changed concentrations of thyroid hormones were first described in 1971 by Terjung and Tipton, who reported increased concentrations of free and total T4 during bicycle ergometer training and reduced total T4 levels 24 h later (46).

In 1973, Rothenbuchner et al. reported decreased serum concentrations of T3 in the starving organism (47). Nearly simultaneously another group confirmed this observation in a different population (49). Shortly thereafter T3 concentrations were observed to be reduced in patients with critical illness requiring intensive care, in tumors and in uremia (48, 51, 52, 77).

The last four decades witnessed the discovery of many more pathologies that are associated with the low-T3 syndrome or other patterns of NTIS, including sepsis, circulatory arrest, stroke, myocardial infarction, pulmonary embolism, inflammatory bowel disease, renal failure, and gastrointestinal fistulae (24, 25, 39, 56, 66).

A pattern typical of "NTIS" was also observed specifically in non-pathological conditions, such as the fetal period, torpor in poikilotherm animals, and hibernation in certain mammalian species (78, 79). These observations suggested that ESS is not a dysfunction of the feedback loop, rather an allostatic reaction and potentially useful adaptation of the pituitary–thyroid feedback control system to reduced supplies in energy, oxygen, and glutathione. We therefore recently coined a new term of TACITUS to provide a more neutral designation that encompasses several non-pathological conditions with adaptations of TSH and thyroid hormones (18, 35).

MECHANISMS OF THYROID ALLOSTASIS

In situations of current or anticipated strain central and peripheral mechanisms interact to ensure a coordinated adaptation of thyroid hormone signaling (43). This is associated with a variety of alterations at the molecular level in nearly all tissues.

Cybernetic Principles of Integrative Thyroid Control

Homeostatic control of thyroid function represents a classical example of a hypothalamic–pituitary-mediated endocrine feedback mechanism (**Figure 2**) (18, 35). Its principal mediators are hypothalamic thyrotropin-releasing hormone (TRH), pituitary thyrotropin (TSH), thyroxine (T4), and triiodothyronine (T3).

Thyroid-stimulating hormone is a glycoprotein hormone with a rather short half-life of 50–60 min, which stimulates synthesis and release of T4 and to a lesser degree T3 from the thyroid gland *via* binding to a specific TSH receptor (35). With respect to classical thyroid hormone actions (effects mediated *via* nuclear thyroid hormone receptors) T4 is a prohormone requiring activation to the highly agonistic hormones T3 and 3,5-T2 to be effective. However, other non-classical actions of T4, acting, e.g., *via* integrin receptors, do not require prior activation, rendering T4 an active hormone with respect to non-classical effects (80).

Plasma T3 is derived from several sources, including direct formation in the thyroid gland and release by proteolysis of thyroglobulin (Tg), deiodination from T4 in the thyroid and deiodination in peripheral organs (12). T4 and T3 display prolonged plasma half-lives of 1 week and 1 day, respectively, resulting from intracellular accumulation and a high proportion of plasma protein binding (17, 35, 81). T3 affects most tissues in a pleiotropic manner. It also closes the feedback loop by inhibiting both synthesis and release of TSH from the pituitary gland (17, 18, 35).

This "short" feedback loop is augmented by additional control motifs, including an ultrashort feedback loop of TSH release (17, 82), a long feedback loop, where thyroid hormones inhibit TRH release in the hypothalamus (83, 84), and a direct stimulatory effect of TSH on T3 formation. A TSH–T3 shunt has been predicted in animal and cell culture experiments (85–90), and we recently demonstrated its existence in the human organism (19, 22, 91–94).

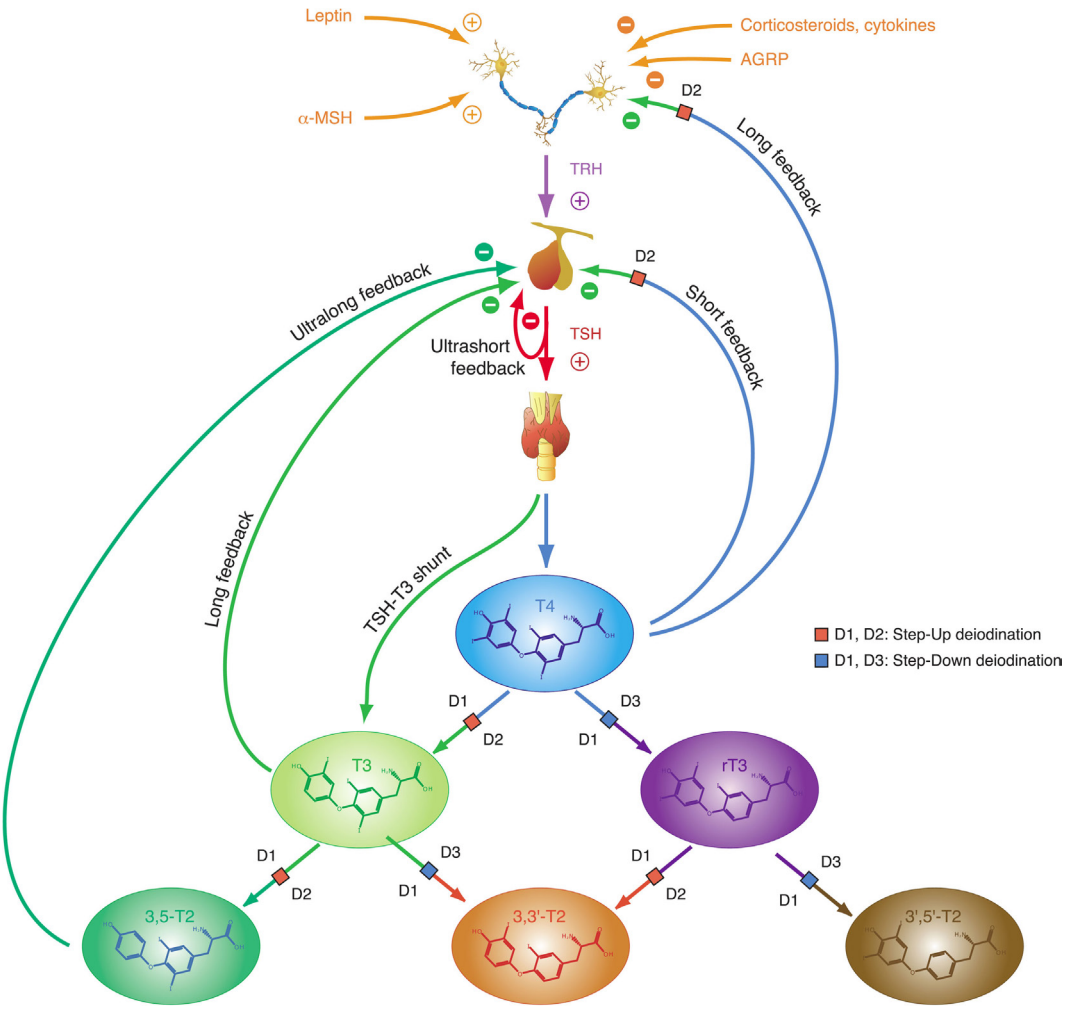

FIGURE 2 | Thyroid homeostasis comprises ultrashort, short, and long feedback mechanisms. In addition, conversion between iodothyronines is adaptively mediated by three distinct deiodinases (18, 35). Deiodination is controlled by multiple local and global mediators including thyroid-stimulating hormone (TSH).

The integrative control at the hypothalamic level is mediated by parvocellular hypophysiotropic paraventricular nucleus (PVN) TRH neurons. Their activity provides an interface between thyroid hormone feedback, nutritional status, and stimulatory or inhibitory influences of the circadian rhythms (**Figure 3**) (95). In this respect, tanycytes lining the third ventricle play a pivotal role in central homeostasis. They are able to fine-tune the sensitivity of PVN *via* provision of central T3 and to degrade TRH *via* pyroglutamyl peptidase II (PPII) at the level of the median eminence (**Figure 3**) (95, 96).

Allostasis and Allostatic Load

In 1988, Sterling and Eyer extended the classical paradigm of homeostasis with the theory of allostasis (100). Briefly, an allostatic response is defined as a dynamic stress reaction that maintains stability through change (101). This distinct operating mode of homeostatic systems becomes apparent in straining and occasionally life-threatening situations. Allostasis both contains and extends the homeostatic principles by adapting set points and other boundaries of control (**Table 1**) (101). The primary mediators of allostatic response include but are not limited to catecholamines, hormones of the hypothalamo-pituitary–adrenal (HPA) axis and cytokines (102). Of note, allostasis deals with a trade-off situation. It ensures survival in extreme situations, where, e.g., the demand of energy exceeds supply, but this protective reaction occurs at the expenses of a stress reaction (referred to as *allostatic state*), which, in turn, may have adverse consequences of its own. The cumulative result of an allostatic state is referred to as *allostatic load* of the organism (**Figure 4**). If allostatic load remains excessive or persists over a period of time it may confer pathology and turn out to be life threatening by its own nature (*allostatic overload*).

Usually, two types of allostatic load are distinguished. *Type 1 allostatic load* occurs, if energy demands exceed the sum of energy intake and the amount of energy that can be mobilized from stores. Typical examples are breeding birds exposed to inclement weather conditions and the conflict of homeothermic animals resulting from reduced availability of food in cold

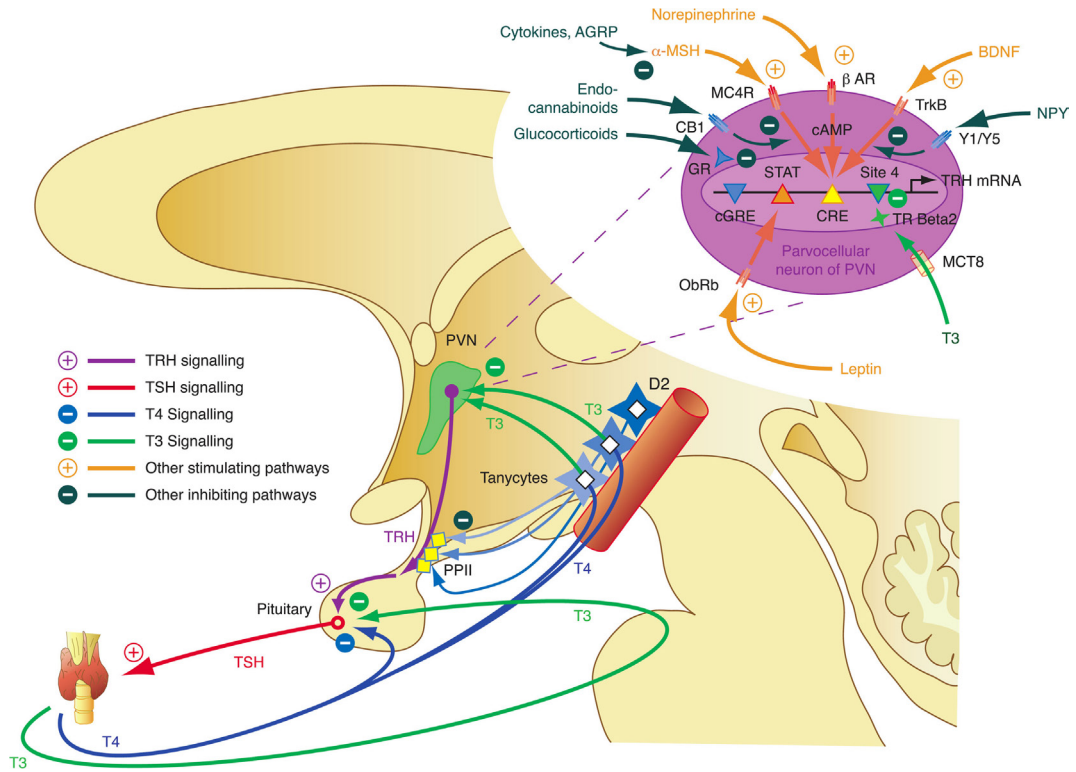

FIGURE 3 | Critical components of the central governor of thyroid homeostasis include parvocellular thyrotropin-releasing hormone (TRH) neurons, which integrate multiple afferent signals relaying information on nutrition and stress, and tanycytes lining the third ventricle at the blood–brain barrier, which are able to control both synthesis and degradation of thyrotropin-releasing hormone (TRH) *via* type 2 deiodinase (D2) and pyroglutamyl peptidase II (PPII) (95, 97–99).

TABLE 1 | Key concepts of homeostasis and allostasis (103, 104).

Homeostasis	Allostasis
Constant or oscillating set point	Changing set point
Physiologic equilibrium	Compensated equilibrium
No or little anticipation[a] of demand	Extensive anticipation of demand
No adjustment based on history	Adjustment based on history
Adjustment carries no price	Adjustment and accommodation carry a price (allostatic load)
No pathology	Potentially leads to pathology

[a]*Anticipatory components of homeostatic control are usually restricted to small effects of, e.g., circadian rhythms, while allostasic anticipation results in profound adaptation in the awaiting of major strains of threats.*

seasons, when they have both to save energy and to increase energy spending on maintaining body temperature (101). *Type 2 allostatic load* results from *expected* increase in energy demand, although the cumulative energy balance is still sufficient. This constellation is typical of psychosocial stress situations, e.g., in predominating competitive social structures within animal populations and, as applied to humans, conflicts arising from differences in socioeconomic status (101). Multiple components of an unhealthy lifestyle including overnutrition, poor sleep, and toxic chemicals are also able to contribute to the phenotype of allostasis (107). Long-term consequences of type 2 allostatic load include obesity, hypertension, type 2 diabetes mellitus, endothelial cell damage, and dyslipidemia, i.e., classical components of metabolic syndrome.

Our group has proposed to extend the concept of allostasis to the adaptive response of thyroid function in defined straining situations (35). This extension goes beyond the simple issue of energy balance, although thyroid hormones have an intricate relationship to energy homeostasis. While stimulating the mobilization of energy for metabolic usage thyroid hormones also increase its consumption. Their activation is coupled with a depletion of reduced glutathione stores involved in regeneration of NADH and NADPH acting as cofactors of deiodinases (86, 87, 108–110). It may therefore be expected that when energy or glutathione availability does not meet their consumption, active thyroid hormones are selectively downregulated. By analogy to classical concepts we will subsequently refer to this situation as *type 1 thyroid allostasis*. Conversely, in situations, where energy stores have to be mobilized to meet *anticipated* demands (e.g., in pregnancy, endurance training and adaptation to cold weather conditions), upregulation of active thyroid hormones will be beneficial. Since these situations bear a resemblance to classical type 2 allostatic load, we will sum them up as *type 2 thyroid allostasis*. This is further justified because a high-T3 constellation in the absence of hyperthyroidism is also observed in obesity (111) and psychosocial stress (112), i.e., type 2 allostatic load according to the classic definition by McEwen and Wingfield.

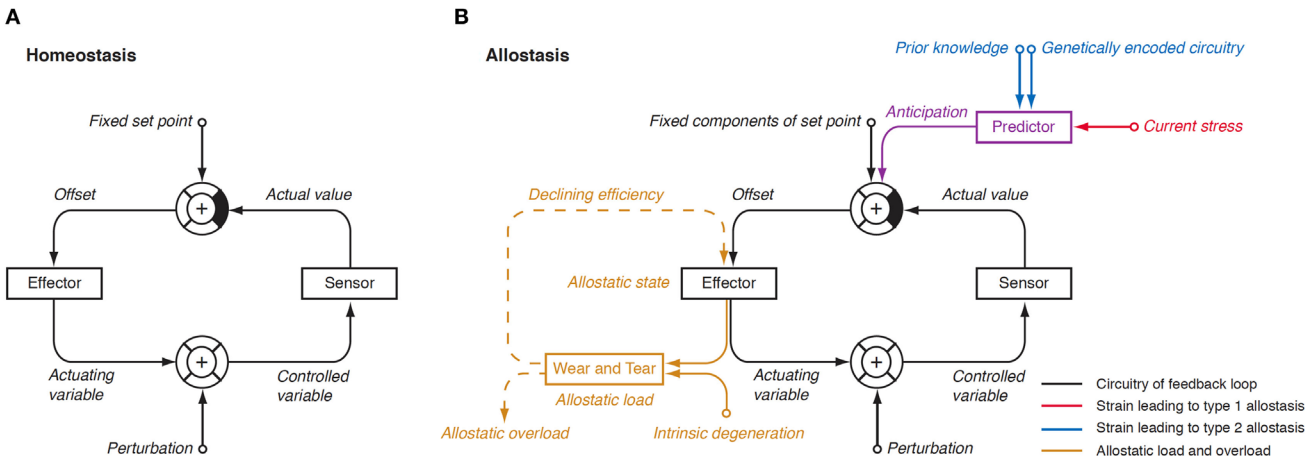

FIGURE 4 | **(A)** In a physiological homeostatic system, afferent information is compared with a fixed set point, and the sensed discrepancy leads to counter-regulatory activity of the effector. Negative (degenerative) feedback ensures static stability of the system. **(B)** In situations of allostasis, stress signals are looped in at a central level, thus resulting in ongoing offset signaling. Due to saturation of receptors and enzymes, this discrepancy reduces the efficiency of the effector and, if ongoing, gives rise to "wear and tear" reactions. Both mechanisms combine as allostatic load, which may be a source of pathology on its own (105, 106).

In summary, unlike classical stress transduction systems the HPT axis produces two phenotypically distinct types of allostatic load if strained: in type 1 allostasis production of thyroid hormones, especially T3, is downregulated, while it is upregulated in type 2 allostasis. In that respect, the thyroid stress reaction differs sharply from that of the HPA axis, the prime example of an allostatically controlled system, as the latter responds invariantly with increased release of cortisol.

Molecular Mechanisms of Thyroid Allostasis

In situations of starvation, inflammation, and oxidative stress, a variety of mediators (including nitric oxide, hydrogen peroxide, proteolysis-inducing factor, angiotensin II, TNF-α, and other cytokines) converge in the NF-κB pathway, a key regulatory system of immune response, cell proliferation, and apoptosis (113). Among multiple other effects the NF-κB/IL6 signaling pathway (114, 115) inhibits T3-induced expression of peripheral type 1 deiodinase. Downregulation of D1 and peripheral type 2 deiodinase (D2) results in reduced concentrations of free and total T3 (41, 116).

Recent research revealed a complex interaction of insulin and thyroid hormone signaling in skeletal muscle, which might also extend to lung and liver tissue (117, 118). *Via* the PI3K–mTORC2–Akt pathway insulin and IGF-1 inactivate FOXO1 by phosphorylation at Ser256, which leads to increased D2 activity (117). T3 again inhibits Akt activity, thereby closing a negative feedback loop (118). This mechanisms might play a pivotal role in linking reduced concentrations of insulin in fasting state (119) as well as decreased IGF-1 levels in a subgroup of critically ill (120) to hypodeiodination and consecutive low-T3 syndrome.

In isolation, decreased peripheral step-up deiodination would lead to increased (disinhibited) TSH release, which would result in elevated serum thyrotropin concentration. This, in turn, would reset the concentrations of T3 to their previous levels, thus neutralizing the effect of hypodeiodination. However, concomitantly with reduced peripheral step-up deiodination, D1 and D2 located in hypothalamic tanycytes (95), and the anterior pituitary gland are upregulated. This change is mediated by bacterial lipopolysaccharide (121, 122), alterations in cytokines, e.g., IL-12 and IL-8 (43) and, possibly, increased concentrations of 3,5-diiodothyronine (61–63), triiodothyroacetate, and tetraiodothyroacetate (123, 124) during the acute phase response. The upregulation of central step-up deiodination results in increased central thyroid hormone signaling and, consequently, suppressed release of TRH and TSH. The seeming paradox that low-T3 syndrome may ensue from hyperdeiodination is resolved by the spatial diversity of deiodinase activity (**Figure 5**). This was investigated *in silico* by computer simulations (20) and confirmed by means of animal experiments *in vivo* (83, 84, 121, 122, 125–127).

A coordinated interaction of central and peripheral deiodinases in the lead-up to low-T3 syndrome is further supported by upregulation of type 3 deiodinase (D3) in TACITUS (129, 130), giving rise to elevated serum concentrations of rT3, a iodothyronine with inhibiting effects on thyroid hormone signaling. Increased D3 activity in starvation may be caused by decreased leptin concentrations as shown in mice experiments (23, 131). During prolonged critical illness, decreased food intake might be an important factor in regulating the activity of liver deiodinases (132). D3 in peripheral organs might also be upregulated by hypoxia due to decreased tissue perfusion during illness (133).

In addition, TSHb is decreased through IL-1b and TNF-α independently of T3 uptake and action in pituitary cells (134, 135). Moreover, supraphysiological concentrations of IL-1a and IL-1b suppress cAMP accumulation, thus inhibiting the TSH-induced Tg mRNA expression and Tg release in human cultured thyrocytes (136, 137).

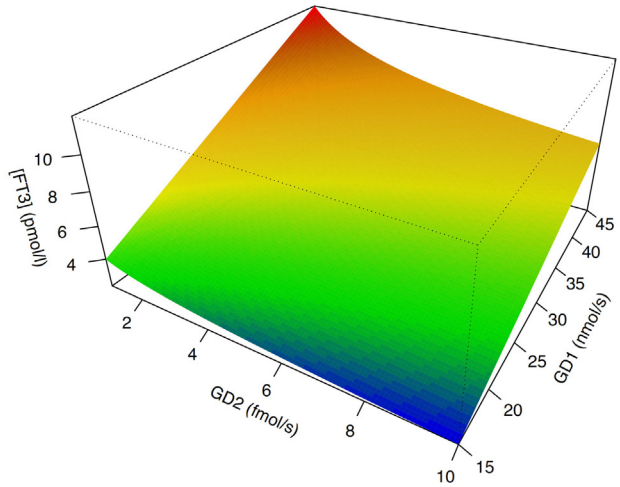

FIGURE 5 | The phenotype of low-T3 syndrome may result from both peripheral hypodeiodination and central hyperdeiodination. Although FT3 concentrations rise with increasing sum activity of peripheral type 1 deiodinase (GD1), they descend with increasing activity of central type 2 deiodinase (GD2). This seeming paradox is explained by feedback effects (20, 21). Despite research in humans being hindered by ethical and methodological barriers, results of computer simulations [shown is sensitivity analysis based on SimThyr 4.0 (128)] and animal experiments (126, 127) are consistent.

Thyrotropin-releasing hormone neurons originating from the hypothalamic PVN, the major autonomic output area of the hypothalamus (95, 138–140), play a key role in the central control of thyroid homeostasis by providing a set point for the short loop control (141). The release of TRH is inhibited by central T3, which is predominantly generated by D2 expressed in tanycytes lining the third ventricle (142–145) (**Figure 3**). This circuit forms an additional long feedback mechanism of thyroid homeostasis (83, 84). Apart from the so defined cascade control mechanism, TRH neurons contribute to the coordination of global energy metabolism by integrating multiple afferent signals (146) including catecholamines (147, 148), cocaine- and amphetamine-regulated transcript (149, 150), leptin (151), and alpha-MSH (146, 152) (all stimulating) as well as neuropeptide Y (NPY) (153–155), agouti-related peptide (141, 146, 156), and glucocorticoids (146) (all inhibiting). Moreover, endocannabinoids have been shown to exert inhibitory effects on TRH neurons *via* the type 1 cannabinoid receptor (97). Animal experiments revealed that the integration of all afferent projections has profound effects on secretion of TRH and consecutively TSH in straining situations (95).

Depending on the origin of stress (physical or psychogenic), its duration and the animal's endocrine and energetic status TRH release may be upregulated or downregulated (157) subsequently affecting the set point of the overall homeostatic system. Downregulation with consecutive thyrotropic adaptation, i.e., low or normal TSH levels despite low concentrations of T4 and/or T3, may occur for instance in cases of systemic infection and sepsis, where lipopolysaccharides induce D2 activity in tanycytes (126). Moreover, low TRH expression in the PVN characterizes the NTIS (95). In addition to the effects of NPY as neurotransmitter, elevated fasting serum concentrations of NPY have a stimulatory effect on hepatic thyroid hormone degradation *via* increased glucuronoconjugation (facilitating biliary clearance) and sulfoconjugation (enabling step-down deiodination to rT3S) (140, 158).

A high proportion of the circulating iodothyronines is bound to thyroxine-binding globulin (TBG), transthyretine, and albumin. This mechanism contributes to the exceptionally long half-lives of thyroid hormones. In case of rapid onset of stress situations, e.g., severe illness, the time frame of the described control mechanisms would be too long to be effective, if plasma protein binding of thyroid hormones remained unchanged. However, in critical illness the extent of plasma protein binding is reduced owing to decreased concentrations of binding proteins and the existence of certain binding inhibitors (57, 58, 159). This effect is putatively mediated *via* cytokines (160). Consequently, degradation of iodothyronines is considerably accelerated, which represents another underlying mechanism toward the adaptation of the feedback loop to conditions of type 1 allostatic load (161–163).

In an animal model for prolonged critical illness the iodothyronine membrane transporters MCT10 and OATP1C1 (but not MCT8) were increased, suggesting some adaptation at the level of transmembrane transport, however, with uncertain clinical relevance (164).

Finally, alternative metabolic pathways of thyroid hormones in peripheral tissues such as sulfation, conjugation to bile acids and glucuronide, and ether link cleavage may affect the concentrations of thyroid hormones in critical illness (165–169).

THYROID ALLOSTASIS IN VARIOUS PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

A variety of scenarios associated with type 1 and type 2 allostatic load have been recognized to result in adaptive changes in thyroid homeostasis. **Table 2** provides a short summary, and the associated conditions are subsequently described in more detail.

Energy Restriction and Starvation

Early reports that serum concentrations of T3 are reduced in states of low caloric intake (47, 49) gave rise to the concept of what we now call the Low-T3 syndrome. Reduced T3 concentrations have been described in various conditions associated with energy deprivation, including anorexia nervosa (183–185), calorie-free diet in obesity (47), military combat training with caloric restrictions (186, 187) and other energy-deficient situations (188). Even moderate weight loss may result in hypodeiodination with consecutive decreased T3 concentrations (189). Today, at least three mechanisms explaining this finding are known (**Figure 6**) (23). In the fed state, peripheral step-up deiodination is stimulated by insulin (119) and bile acids (190–192). In addition, increased leptin concentrations facilitate release of TRH and TSH *via* the hypothalamic melanocortin pathway (23, 83, 146, 193). Together, these different mechanisms enhance conversion of T4 to T3, thus mediating postprandial thermogenesis. Conversely,

TABLE 2 | Characteristic phenotypical changes of thyroid-stimulating hormone (TSH) and various classical and non-classical thyroid hormones in certain allostatic situations show nearly opposing changes in type 1 and type 2 allostatic load (61, 63, 111, 123, 170–182).

	TSH	FT4	TT4	FT3	TT3	rT3
Type 1 allostasis in fetal period, acute and chronic critical illness, and in deprivation of energy						
Fetal life	↓, → or ↑	↓	↓	↓	↓	↑
Caloric deprivation	→ or ↓	→	↓	↓	↓	↑
Exhausting exercise	→ or ↓	→	↓	↓	↓	↑
Critical illness (general)	→ or ↓	→	→ or ↓	↓	↓	→ or ↑
Chronic heart failure	→ or ↓	→	→ or ↓	↓	↓	→ or ↑
Renal diseases	→	→ or ↓	→ or ↓	→	↓	→
Liver diseases	→	→ or ↓	↑	↓	→ or ↑	→ or ↑
Pulmonary diseases	→	→	→	↓	→	→ or ↑
Diabetes mellitus	→ or ↓	→ or ↑	↓	↓	↓	→ or ↑
Sepsis	↓	→	→ or ↓	↓	↓	→ or ↑
HIV infection	→	→ or ↓	→	→ or ↓	→	→ or ↓
Depression	→	→ or ↑	↑	↓	↓	→ or ↑
Type 2 allostasis-related conditions						
Pregnancy	→ or ↓	→	↑	→	↑	→
Endurance training	↓, → or ↑	↑	↑	↑	→ or ↓	↑
Obesity	↑	→ or ↓	→ or ↑	↑	↑	→ or ↓
Adaptation to cold	↓, → or ↑	↑	↓, → or ↑	↑	↑	→ or ↓
Acute schizophrenia	→ or ↑	→ or ↑	↑	→ or ↑	↑	→
Post-traumatic stress disorder	→	→	↑	↑	↑	?

FT4 and FT3, free T4 and T3, respectively; TT4 and TT3, total (free + protein-bound) T4 and T3, respectively; rT3, reverse T3; TSH, thyroid-stimulating hormone. Hormone concentration unchanged (→), increased (↑), decreased (↓), or not reported (?). Small studies also reported increased concentrations of 3,5-diiodothyronine (3,5-T2) (61–63), triiodothyroacetate and tetraiodothyroacetate (123, 124), and decreased concentrations of 3-monothyronamine (63) in critical illness and chronic heart failure (not shown in table). See text for definition of type 1 and type 2 allostasis in the context of pituitary–thyroid function.

in fasting conditions concentrations of insulin, bile acids, and leptin are low, which results in decreased step-up deiodination and thyrotropic adaptation, and eventually in low-T3 syndrome. Additional mechanisms leading to impaired TSH release include increased expression of neuromedin B, a bombesin-related peptide, which is an inhibitor of TSH secretion, and upregulation of hypothalamic D2 expression during fasting (23), resulting in low TRH expression in the PVN (25, 95, 98, 194).

Obesity

Obesity is a classical consequence of type 2 allostatic load (101). It is linked to multiple metabolic and endocrine responses (111) including thyroid function. The interconnection between thyroid hormones and body weight is bidirectional, and both hypothyroidism and hyperthyroidism are known to result in changes of body mass. Conversely, obesity may result in adaptive responses of thyroid homeostasis: a variety of studies, as recently reviewed by Pacifico et al. (195) and Fontenelle et al. (111), described elevated TSH levels and increased total step-up deiodinase activity (although predominantly within the reference range) in patients with weight gain, while concentration of rT3 has been reported to be decreased. Reversibility of these alterations after weight loss indicates that they are consequence rather than cause of overweight (196). A recent study (42) described a significant rise in TSH in the absence of peripheral hypothyroidism in men with non-metastatic prostate cancer undergoing androgen deprivation therapy. The effect was mediated by body composition changes and by the fat-associated hormone leptin rather than androgen deficiency. Another study interrelated non-alcoholic fatty liver disease with higher fT3 concentrations in euthyroid subjects, probably consequent to central obesity (197). Both central and peripheral components of the feedback loop are apparently involved in the reactive adjustments to obesity (**Figure 7**). Increased concentrations of adipokines such as leptin have been proposed to be a key element of obesity-related thyroid allostasis, but mitochondrial dysfunction (195, 198), chronic inflammation, and insulin resistance (199) as well as both central and peripheral resistance to thyroid hormone may play additional roles (111).

Since thyroid hormones are potent stimulators of adaptive thermogenesis (202–204), upregulation of TSH release and deiodinase activity stimulates dissipation of energy and therefore may be part of autoregulatory mechanisms of body mass and fat storage. On the other hand, some of the obesity-related changes in thyroid function may contribute to the unfavorable metabolic phenotype of overweight (111). This and significant cardiovascular side effects of hyperthyroidism are the main reasons that intake of thyroid hormones is strongly discouraged as an adjunct in the treatment of obesity (205).

Adaptive Thyroid Responses to Thermoregulatory Challenge

In mammals, thyroid hormones are potent mediators of efficient thermoregulation. This ensues from a complex mechanism tightly integrating deiodinase activity with sympathetic signals (206), which leads to upregulation of the protein UCP1 in mitochondria of skeletal muscle and brown adipose tissue (207–209). This results in uncoupled oxidative phosphorylation and finally non-shivering thermogenesis (202–204, 210, 211). It is therefore not surprising that mammals, typical examples for homeotherm animals (endotherm thermoregulators), usually exhibit elevated serum concentrations of T4 and T3 in winter and during hypothermia as long as they are sufficiently fed (212, 213). Increased concentrations of TSH and/or thyroid hormones in cold seasons and during hypothermia have also been described in humans (214–217). Although the mechanism of thyroid hormone-mediated expression of UCP1 plays a pivotal role in efficient thermoregulation of mammals (202, 218, 219) it has not been described in non-mammal homeotherm vertebrates, e.g., in birds, and it is probably inexistent in homeotherm arthropods. Of note, although some insect species, e.g., *bombus* and *apis*, are homeotherm (220, 221), their temperature regulation is less potent and less energy efficient than that of mammals. This may be in part due to the fact that they probably lack endogenous production of thyroid hormones (222–224).

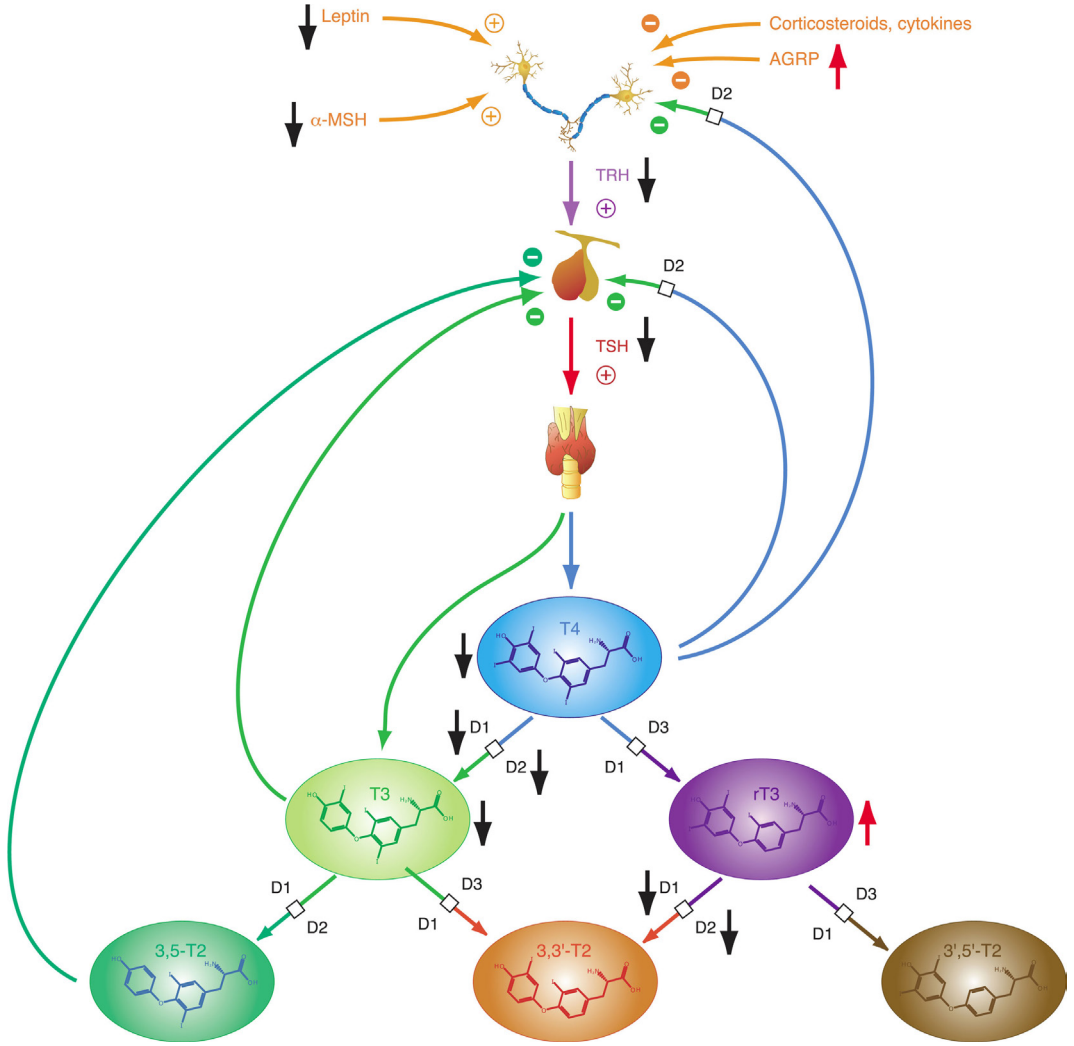

FIGURE 6 | In starvation, both step-up deiodination (*via* D1 and D2) and thyroid-stimulating hormone (TSH) release are reduced, leading to low-T4 and low-T3 constellations. rT3 concentrations may be increased. Black and red arrows indicate the direction of change from normal, homeostatic conditions in fed state.

In hibernating mammals, the situation is different to that of non-hibernating mammals, since here concentrations of T4 and T3 are downregulated during hibernation (78, 225–228). This also applies to the endocrine response to cold in starving mammals (229) and poikilotherm vertebrates during torpor (79).

In summary, during cold periods T3 and T4 are upregulated in fed non-hibernating homeotherm mammals, but in an NTIS-like pattern downregulated in hibernating mammals, starving non-hibernating mammals and poikilotherm vertebrates. Both mechanisms support conservation of energy: the first one by making thermoregulation more efficient, and the second one by partly tuning down the metabolism in periods of lower demand and supply.

Fetal Life

Iodothyronines are critical for development in the embryonal and fetal periods. Both hypothyroidism (230) and oversupply with thyroid hormones (231) may result in fetal loss and severe developmental disorders. In humans, the fetal thyroid gland starts to secrete hormones in the beginning of the 12th week of gestation (29). However, the feedback loop begins to be functional in the 20th week, i.e., in mid-gestation (29). In the first half of pregnancy, the fetus is largely dependent on maternal supply with thyroid hormones, probably explaining, why production of iodothyronines is upregulated in the mother (230). Despite this anti-NTIS-like pattern in the maternal metabolism, which is mainly mediated *via* human chorionic gonadotropin (hCG) and estrogens (230), concentrations of free and total T3 are low in the fetus throughout gestation, and concentrations of TBG, free and total T4 are, although rising with increasing gestational age (230), lower in the fetal than in the maternal circulation (**Figure 8**) (232). TSH levels attain a maximum of about 15 mIU/L in the 20th week, when the feedback loop maturates, and then again at birth (29). Both in fetal serum and in amniotic fluid, concentrations of rT3 are markedly increased (230), which probably results from high activity of type 3 deiodinase in placental tissue and multiple fetal

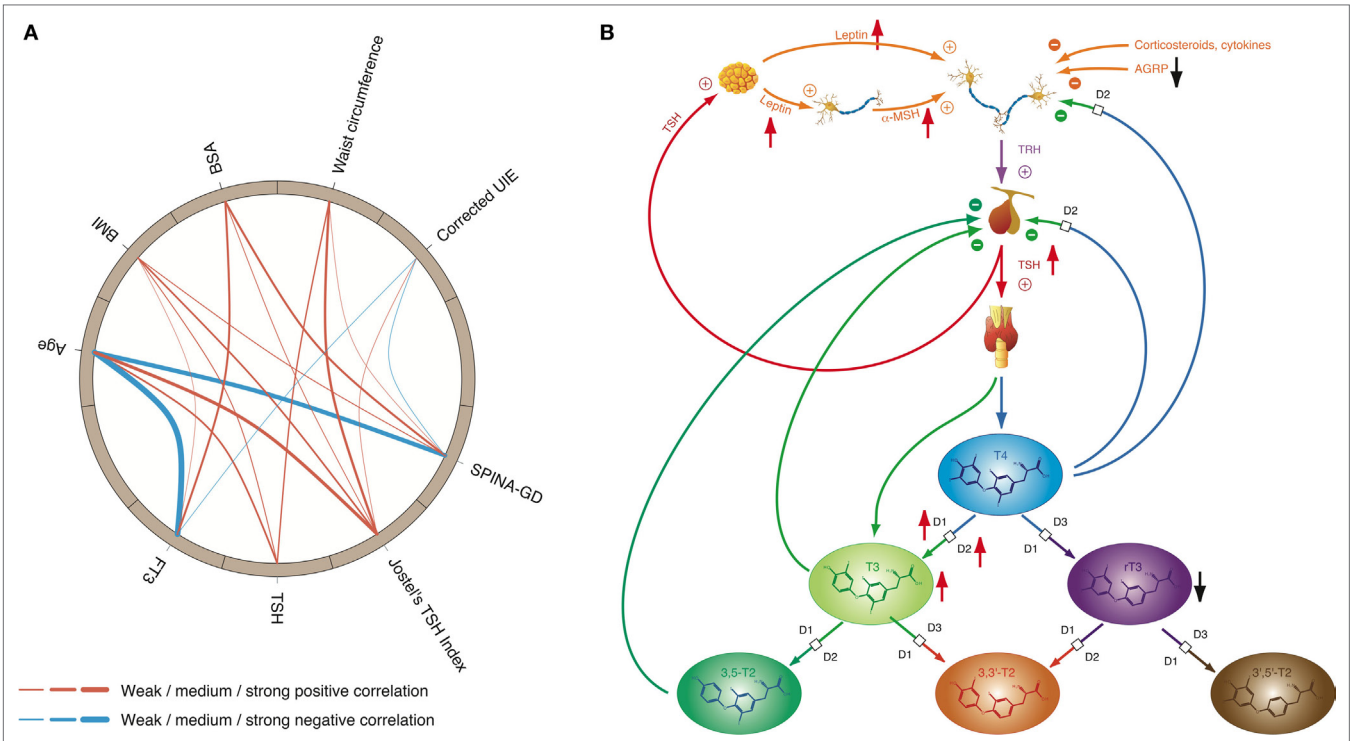

FIGURE 7 | High-T3 concentrations (although in most cases within the reference interval), reduced rT3 concentrations, increased activity of type 1 and type 2 deiodinase, and comparatively high thyroid-stimulating hormone (TSH) levels are the typical signature of obesity, a classical phenotypical sequela of type 2 allostatic load. **(A)** In healthy participants of the NHANES program (200), concentrations of TSH and FT3 as well as Jostel's TSH index (a measure for the set point of thyroid homeostasis) and SPINA-GD (an estimate for total deiodinase activity) show a significantly positive correlation to body mass index (BMI) and waist circumference, and with the exception of TSH also to body surface area (BSA). In addition, FT3 and SPINA-GD correlate negatively to age, and minor associations exist to creatinine-corrected urinary iodine excretion (UIE). In this circular map, positive correlations are marked in red and negative correlations in blue. The widths of the splines represent the correlation coefficient to denote the strengths of association (201). **(B)** Mechanisms of adaptive responses in obesity are mediated by elevated leptin concentrations and increased alpha-MSH signaling, while activity of AGRP terminals is reduced.

organs (230). Increased concentrations of sulfated metabolites of iodothyronines result from low type 1 deiodinase activity in fetal tissues and because T4 sulfate and T3 sulfate are not substrates for placental type 3 deiodinase (230). At birth, concentrations of TSH, T4, and T3 sharply spike to attain slightly elevated levels in the neonatal metabolism (29).

These patterns of thyroid hormones in the normal fetus closely resemble the constellation of NTIS. The conclusion that the normal fetal concentrations of thyroid hormones are beneficial despite being markedly different from that of both healthy adults and healthy newborns is supported by the observation that this constellation is actively defended in situations of thyroid disorders (233): in hypothyroid fetuses D2 activity increases and activities of D1 and D3 decrease, thus providing support for shunting of T4 to brain tissue (230), while, on the contrary, elevated concentrations of iodothyronines stimulate the activity of D3, which results in increased degradation of active thyroid hormones to rT3, 3,3′-T2 and 3′,5′-T2 (234–236).

Pregnancy

The adaptive endocrine response in pregnancy manages a trade-off situation, where the maternal organism is faced with the dual challenge of optimizing conditions for the developing fetus and its own survival. Allostatic changes are mediated both by the central hypothalamic–pituitary unit and the fetal-placental unit. The latter secretes protein and steroid hormones that modify the function of endocrine organs in the mother's organism throughout pregnancy (237). Due to its high structural similarity with TSH, the glycoprotein hormone hCG stimulates the human TSH receptor, which enables the placenta to gain parallel control over the thyroid system in early gestation (238). In extreme situations, e.g., starvation, the adaptive gestational responses control type 1 allostasis. Mostly, however, resources are sufficient to permit an anticipatory endocrine response of type 2 allostasis. Typical responses of the pituitary–thyroid axis in pregnancy include enhanced secretion of thyroxine from the thyroid gland and increased step-up deiodination. Unlike in obesity or other states of type 2 allostasis, TSH concentrations are low-normal or slightly decreased (230) (**Figure 8B**). This is a consequence of both elevated concentrations of T4 and hCG (stimulating TSH receptors in the anterior pituitary gland). In addition, plasma protein binding of thyroid hormones is increased. The majority of these effects are mediated by hCG, which displays, in addition to its gonadotropic action, mild TSH-mimicking effects in its sialylated form and TSH-antagonistic effects in a desialylated variant (239). Thyroid overstimulation by hCG is possible in pregnant women in the first trimester resulting in a distinct entity of gestational hyperthyroidism or hyperthyroidism in trophoblastic diseases,

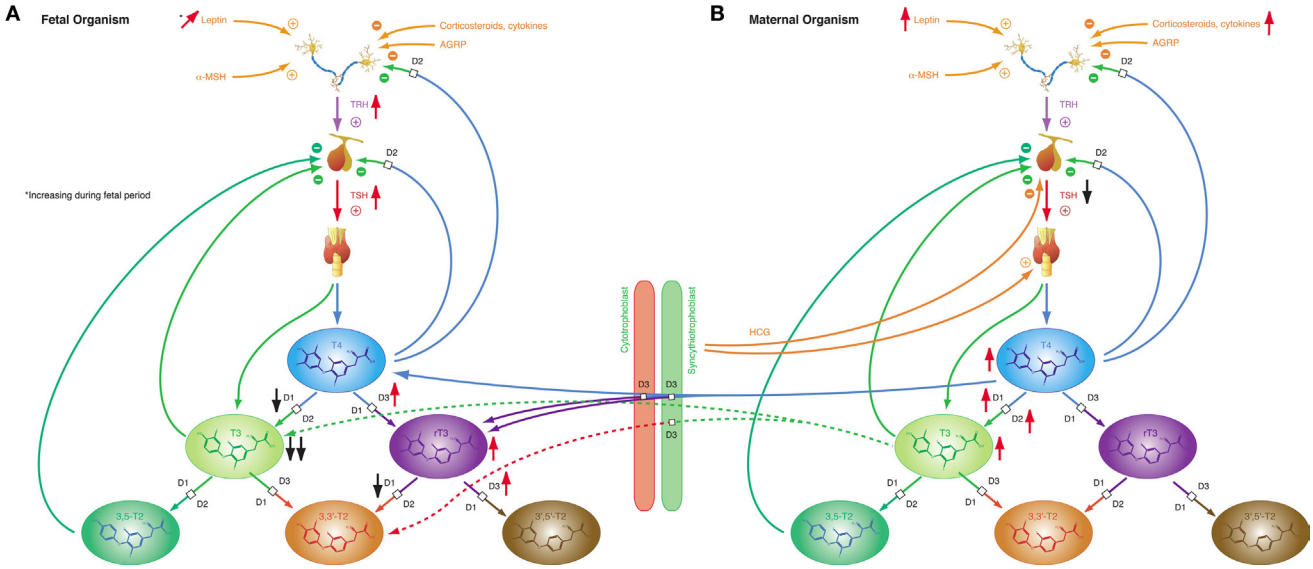

FIGURE 8 | Fetal and maternal thyroid homeostasis are dovetailed to optimize conditions for both organisms. **(A)** After maturation of the feedback loop in the 20th week of gestation, fetal thyroid-stimulating hormone (TSH) concentrations and step-down deiodination *via* D3 are temporarily increased, while step-up deiodination is decreased. This results in a pattern of markedly reduced T3 concentrations and elevated rT3 levels. Black and red arrows indicate the difference compared to normal, homeostatic conditions in healthy newborns and adults. **(B)** Pregnancy is accompanied by a characteristic "anti-NTIS"-like constellation of thyroid homeostasis including high concentrations of T3 and T4, step-up hyperdeiodination and increased binding of thyroid hormones to plasma proteins.

which may also affect men with testicular cancer (238, 240–247). This scenario exceeds physiological adaptation and translates into a specific disease entity. It demonstrates the strength of hCG-mediated effects, which confer a not so rare risk of subclinical hyperthyroidism in otherwise normal pregnancies (238).

The "anti-NTIS"-like pattern of maternal thyroid homeostasis in pregnancy results in increased availability of thyroid hormones for the developing fetus, which is especially necessary in the early phases of gestation, when the fetal thyroid is still unable to produce sufficient amounts of thyroid hormones, the more as the transport capacity through the placental barrier is limited.

Pregnancy-related allostatic changes in thyroid function are frequently causing problems in the differential diagnosis of thyroid disease as described below in the Section "Methods of Assessment and Differential Diagnosis." Because a sufficient supply with levothyroxine is critical for the development of the fetus, thresholds for initiation of substitution in subclinical hypothyroidism and dosage adjustment in hypothyroid women taking L-T4 prior to pregnancy had been lowered by professional societies (248–251). Based on evidence the American Thyroid Association and the Endocrine Society had recommended to lower the upper range of the TSH reference range to 2.5, 3.0, and 3.5 mIU/L in the first, second, and third trimester of pregnancy, respectively. Although several observational studies reported adverse pregnancy outcome in even mild hypothyroidism (252–261), two recent interventional trials did not confirm a beneficial effect of substitution therapy in subclinical hypothyroidism and hypothyroxinemia with respect to children's IQ at age of 5 years and secondary outcome markers (262, 263). Consequently, the recommended upper limit of the TSH reference range has been raised back to 4 mIU/L in the most recent guideline issued by the American Thyroid Association (264). However, thyroid peroxidase antibodies should be measured in pregnant women with TSH concentration above 2.5 mIU/L and treatment be considered, if antibody titers are positive, even if TSH levels are between 2.5 and 4 mIU/L (264).

The reason for these discrepancies and the lack of success by interventional studies is unknown. Ethnic differences among study populations (265) and the inverted U shape of the relation between maternal FT4 concentration and child IQ (266–268) as well as the relatively late onset of substitution therapy after the 10th week of gestation in the reported substitution trials (262, 263) may play a role. This uncertainty warrants further research.

Exercise

Intensive muscle activity in sports and training is associated with profound changes in endocrine control (27) and cytokine patterns (269, 270). This suggests modifications of thyroid homeostasis during or after exercise.

The response of thyroid hormones to exercise varies (**Figure 9**). With a few exceptions (271–273), most studies investigating thyroid hormones during or in a short-time interval after training found elevated concentrations of TSH, T4 and/or T3 (46, 274–276). After resting or in prolonged training programs with repeated heavy strain, however, the majority of studies described reduced concentrations of TSH, T4, and T3 (186, 187, 277–281). This seeming contradiction was attributed to hemoconcentration in or after exercise leading to falsely elevated hormone concentrations in short-time exercise (27). This assumption is supported by trials, where thyroid hormones have been measured both on short and long timescales. Hormone concentrations, while elevated during physical strain or at exhaustion decreased at rest

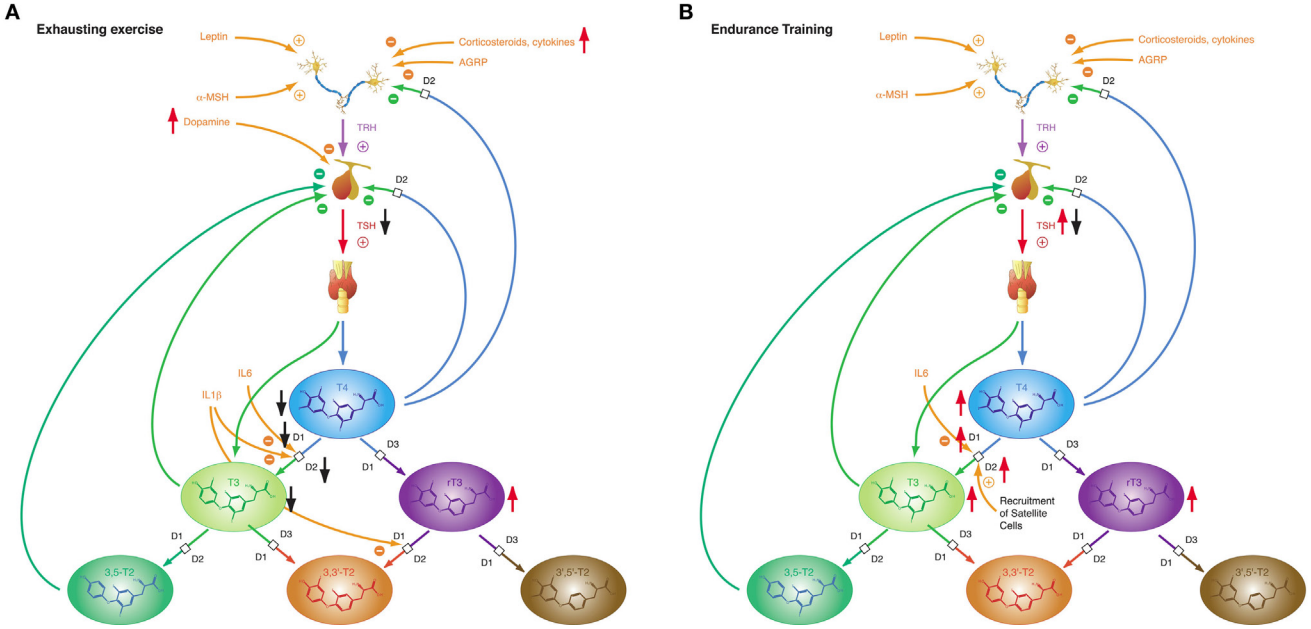

FIGURE 9 | The adaptive response of the hypothalamic–pituitary thyroid axis to exercise is heterogeneous, depending on duration and intensity of training and on the interval between exercise and laboratory investigations (27). This diversity may result from pre-analytical factors (e.g., hemoconcentration) and from an overlap of type 1 and type 2 allostatic load. **(A)** Exhausting exercise. **(B)** Endurance training.

after exercise (46, 186, 275–277). Resting allows for rehydration and represents a more realistic situation. As a consequence it has been recommended to allow for a 24-h recovery period before participants report for laboratory testing (282).

Of note, TSH and thyroid hormone concentrations are elevated in endurance exercise and in the beginning of military combat training before exhaustion (186, 277, 283), even if allowance was made for resting and rehydration before investigation (283). With the onset of exhaustion, the pattern changes and hormone concentrations decrease to subnormal values (186, 187, 277). This suggests that the variable homeostatic response to exercise may possibly result from a second mechanism, where type 1 allostasis ensues from exhaustion and deprivation of energy, thereby leading to downregulation of TSH and peripheral thyroid hormones, whereas in endurance training and before exhaustion allostasis is shifted to type 2 and stimulated release of TSH, T4 and T3.

The NTIS-like pattern of thyroid hormones after exhausting training is confirmed by two studies showing increased rT3 concentrations (187, 283) in physical strain. The type 1 allostatic endocrine responses were more pronounced in military combat training programs, when participants were subject to additional deprivation of sleep and energy (186, 187, 277).

Acute and Chronic Critical Illness

Characteristic patterns of NTIS have been described in a multitude of acute and chronic somatic illnesses including states of shock (284), circulatory arrest (285, 286), respiratory failure (65), community-acquired pneumonia (287), sepsis (288), chronic respiratory (289, 290) and cardiovascular (61, 64, 66, 291, 292) disease, renal failure (293–298), COPD (289), gastrointestinal diseases (299–301), autoimmune diseases (71, 302, 303), and cancer (130, 290, 304). Phenotypes of NTIS with high and high-normal FT4 concentrations have been described in dementia and frailty in elderly persons (305).

While most forms of acute critical illness may be interpreted as a state of starvation, the chronic form of severe illness—a result of modern critical care—represents most commonly a state of adequate nutrition (306). Hence it is not surprising that acute and chronic critical illness elicit different phases of NTIS: the acute phase (**Figure 10A**) and the chronic or prolonged phase (**Figure 10B**), also referred to as *wasting syndrome* (307). The former seems to beneficially affect outcome, the latter to have an impairing effect (308).

Non-thyroidal illness syndrome is a disease-independent risk factor for survival, so it is important to understand the underlying mechanisms (60). Patients with low free T3 show a significantly higher mortality and a significantly longer duration of mandatory ventilation (56, 309). Furthermore, low free T3 is a strong prognostic predictor in B-cell lymphomas (310). The alterations of the acute phase of NTIS in critical illness occur within hours or days and are defined by increased release of anterior pituitary hormones, low levels of anabolic peripheral effector hormones, reduced thyroid hormone-binding protein concentration, reduced binding affinity, reduced expression of thyroid hormone transporters, decreased thyroid hormone uptake and altered expression of D1 and D3 activity and the thyroid hormone receptor alpha1 (TRα1). The prolonged phase, on the other hand, is characterized by a suppression of the hypothalamic-anterior-pituitary-peripheral-hormone axis and low levels of anabolic peripheral effector hormones. Peripheral tissues respond by reactively increasing the expression of monocarboxylate transporters, upregulating D2 activity, reducing D1

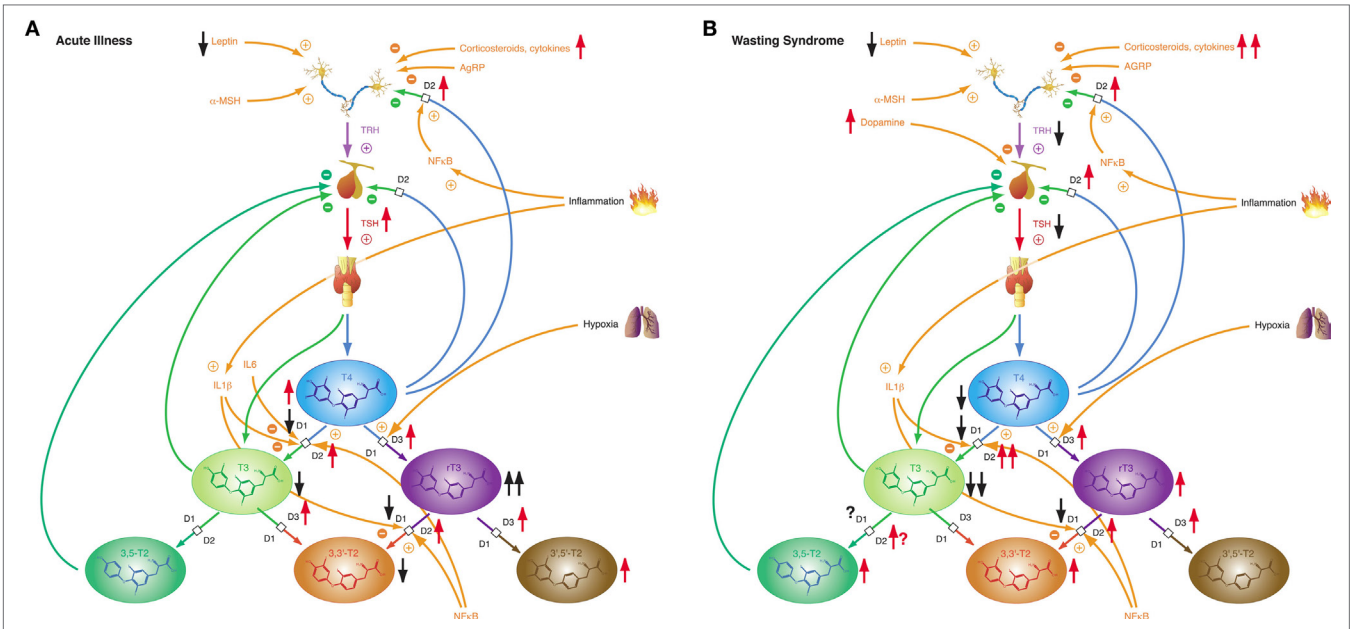

FIGURE 10 | Depending on severity and duration of disease, non-thyroidal illness syndrome presents with two related, but distinct phenotypes. **(A)** Allostatic reactions of the pituitary–thyroid feedback control system in acute illness lead to slightly decreased T3 and 3,3′-T2 concentrations, slightly elevated T4 and 3′,5′-T2 levels and markedly increased rT3 concentrations. **(B)** Medium-term and long-term adaptations in ongoing illness ("wasting syndrome") and chronic disease result in thyrotropic adaptation and slight increases of rT3, 3,3′-T2, and 3,5-T2 concentrations concomitant to low-T3 syndrome.

activity, and increasing sensitivity to thyroid hormone receptors. The chronic phase is characterized by a loss of pulsatility of TSH secretion, a reduced TRH-gene expression in the hypothalamic PVN, and suppressed hypothalamic stimulation. However, the pathogenesis is unclear. Altered D3 activity and MAPK and hedgehog pathway seem to play a pivotal role in the whole process (311). Despite our current knowledge, critical questions remain unanswered.

Psychosocial Stress and Psychiatric Diseases

The relationship between thyroid hormones and psychological phenomena is a paramount example of mutual and reciprocal influences of mind and body. It had been recognized for more than 150 years that diseases of the thyroid are frequently accompanied by psychiatric symptoms (312, 313). Although depression is a classical symptom of hypothyroidism, and psychosis may result from thyrotoxicosis, the linkage is clearly bidirectional (314). A great number of studies over the last decades showed that characteristic changes of thyroid hormone concentration may arise from mental or psychological disorders (28, 314) in the absence of thyroid disease. This assumption is confirmed by interventional studies that show normalization of formerly changed hormonal parameters after the underlying psychiatric disease has been successfully treated.

As early as in 1968 John Mason predicted thyroid hormone concentrations to rise in response to psychosocial stress (315). Subsequent research revealed that the interaction is complex and non-linear and that it is additionally dependent on the nature of the underlying psychiatric disease. A recent study demonstrated that stress could trigger the onset and the recurrence of hyperthyroidism in patients with Graves' disease (316). However, hyperthyroxinemia is indeed a nearly universal observation in different expressions of psychiatric disorders (317–322).

A large body of studies reported a characteristic pattern of thyroid hormones in MD (314, 323). Concentrations of T4 or FT4 are commonly increased during depression (324–329) and revert after recovery from MD, irrespective of the modality of treatment (330–339). Despite the presence of elevated T4 concentrations TSH levels tend to be normal in depressive patients, but circadian variation of TSH concentration is impaired (339–342) and the response to TRH test is blunted (323). Both total and free T3 concentrations are reduced in MD (175, 176, 343), but elevated in bipolar I disorder (237, 344). Concomitantly, rT3 concentrations are temporarily increased in both MD and manic disorder; however, not in bipolar I disorder (334, 345, 346).

Similar to the situation in bipolar I disorder, concentrations of free and total T3 are frequently elevated in post-traumatic stress disorder (PTSD) (177–179, 181, 182, 347), another classical example of a type 2 allostatic reaction (112). Except for concomitant borderline personality disorder (348), the common high-T3 syndrome in PTSD is at least partly due to increased step-up deiodination (179, 181, 182). Two studies in combat veterans of World War II and the Vietnam war revealed levels of total T4 and both total and free T3 to significantly correlate to severity of PTSD (181, 349). This anti-NTIS-like pattern is complemented by elevated TBG concentrations and increased plasma protein binding in these patients compared to healthy controls (179). Despite an elevated set point of thyroid homeostasis the response to TRH stimulation is blunted in PTSD (350).

A small number of studies reported elevated concentrations of FT4, TT3, and FT3 in schizophrenia spectrum disorders. This observation was, however, not reproducible in all studies and apparently dependent on severity of symptoms and the time after admission (351, 352).

In summary, MD is accompanied by a partly NTIS-like pattern, whereas bipolar I disorder and PTSD as well as severe and newly diagnosed schizophrenia involve a hormone constellation typical of type 2 allostasis. A relatively high set point for T4 is shared by all four disorders, as evidenced by unsuppressed TSH levels despite high T4 concentrations, whereas the response to the TRH test is mitigated.

Although our knowledge of the precise mechanisms mediating the endocrine response in this class of affective disorders is still limited, recent research revealed some elements that may play a key role in this scenario. For a long time, it was assumed that central TRH, which has in addition to its endocrine function widespread neurotransmitter and neuromodulatory effects, has a pivotal function in the link between depression and altered thyroid hormones. While TRH concentrations in CSF are increased in depressed patients (353, 354) and TRH levels are particularly high in subjects with violent suicidal behavior (353), the results after treatment and in recovery are inconsistent (323). A decisive influence possibly lies in the spatial distribution of neuromodulators and the complex interaction of positive and negative feedback loops between the limbic system and hypothalamus (**Figure 11**). TRH expression is upregulated in the amygdala in response to stress (355) and amygdala kindling (356), but downregulated in hippocampus (357). *Via* two pathways, such as the stria terminalis and the ventral amygdalofugal pathway, the amygdala stimulates the PVN, the origin of hypophysiotropic TRH neurons, with cholinergic and glutamatergic terminals (358). As a consequence, TSH release increases in situations of stress-induced type 2 allostasis (359). Of note, amygdala activity is inhibited again by means of feedback loops mediating the anxiolytic-like effect of TRH (360). In addition, the activity of the feedforward path is sensitive to context and circadian conditions, too (361). This complexity warrants further research.

From a teleological perspective, the type 1 allostatic pattern in depression makes sense. Depression and starvation represent sickness behavior, a common final path of the sickness syndrome, which may be beneficial by promoting social immunity (362).

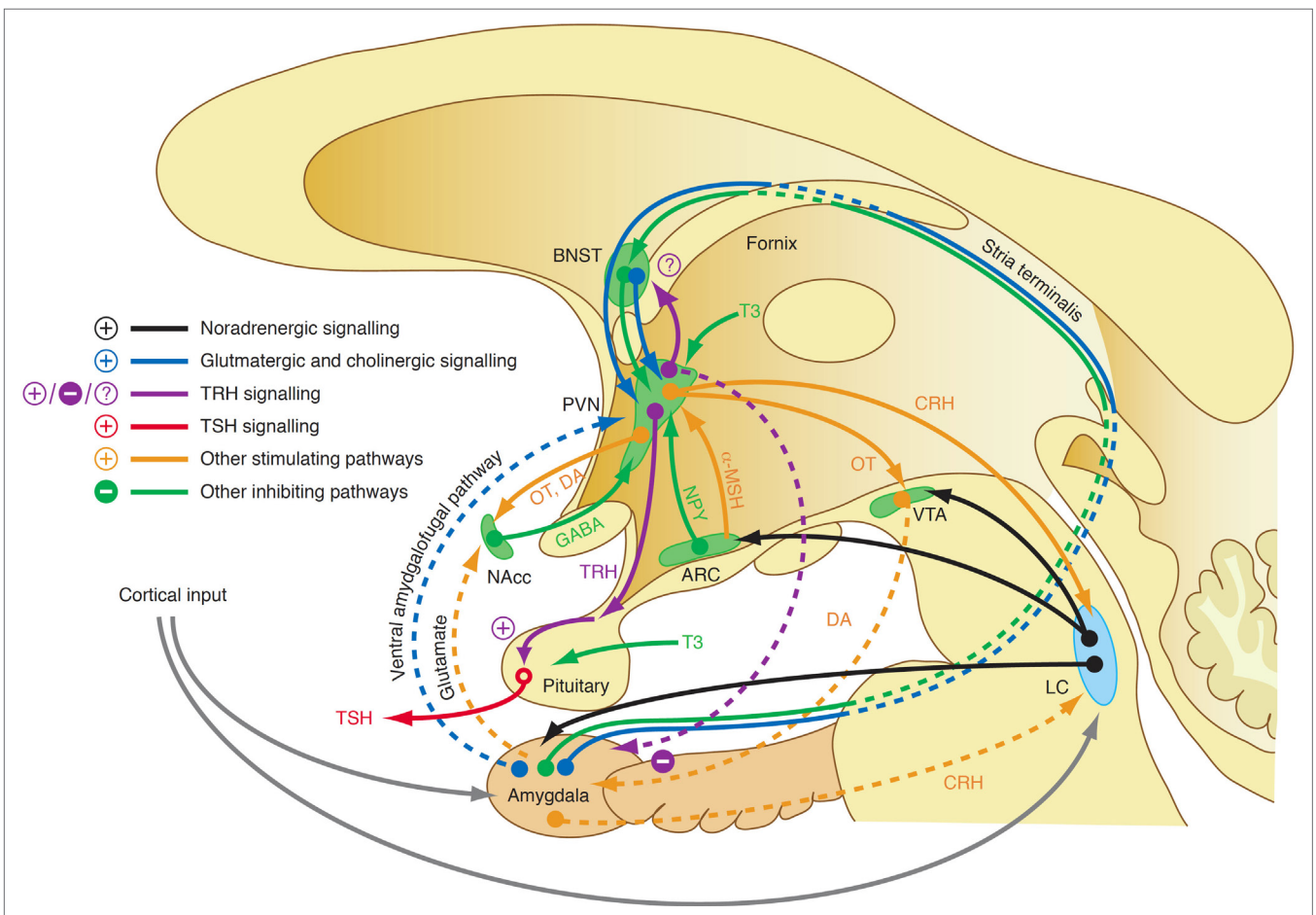

FIGURE 11 | A complex interaction of positive and negative feedback mechanisms linking centers of the limbic system to hypothalamic nuclei explains the adaptive response of the hypothalamic–pituitary thyroid loop in type 2 allostasis resulting from psychosocial stress situations (355, 356, 358, 359). ARC, arcuate nucleus of the hypothalamus; BNST, bed nucleus of the stria terminalis; CRH, corticotrophin-releasing hormone; DA, dopamine; GABA, gamma-aminobutyric acid; LC, locus coeruleus; NAcc, nucleus accumbens; OT, oxytocin; VTA, ventral tegmental area.

In this model, depression is the mediator between inflammation and NTIS.

NON-HOMEOSTATIC MECHANISMS

In illness, changes of measured hormone concentration may result from exogenous factors including pharmacological effects of drugs and assay interference. They have in common that they do not represent an adaptive homeostatic reaction of the organism. As these effects are common in TACITUS, overlap with the phenotype of NTIS and can be confused with allostatic reactions, the following section delivers a short overview over triggering scenarios and their consequences.

Drug Effects

A large number of drugs is known to influence thyroid function interfering with various mechanisms of thyroid hormone metabolism (363). Lithium and aminoglutethimide decrease thyroid hormone secretion. A high iodine load, as it ensues from amiodarone and/or radiocontrast dye application, decreases both central and peripheral deiodinases activity (364–368). In addition to causing hypodeiodination, amiodarone has antagonistic actions on T3 signaling, presumably due to its molecular similarity to thyroid hormones (369, 370). Dopamine, glucocorticoids, and somatostatin analogs suppress TSH release (371). Thyroxine absorption is altered by multiple substances including caffeine, bile acid sequestrants, sucralfate, ferrous sulfate, and aluminum hydroxide (372). This results in disruption of the enterohepatic circulation of thyroid hormones, thus contributing to reduced half-life. Moreover, many drugs alter thyroxine and triiodothyronine transport in serum such as estrogens, tamoxifen, heroin, methadone, mitotane, androgens, anabolic steroids, furosemide, NSAIDs, and salicylates by either increasing or decreasing TBG concentration or displacing them from protein-binding sites (363, 373). Antiepileptic drugs such as phenobarbital, phenytoin, and carbamazepine increase hepatic metabolism of thyroxine and triiodothyronine. In addition to amiodarone propylthiouracil, macrolides, and unselective beta-adrenergic blockers inhibit the activity of type 1 deiodinase (374), while sorafenib is able to increase D3 activity (375).

Assay Interferences

Significant problems in FT4 and FT3 assay interpretation can arise in the varying performance of tests from different manufacturing sources (376, 377). For example many tests are affected by residual interference from effects of albumin concentrations that are often considerably reduced in NTIS and thereby lead to artificially low estimates of FT3 or FT4. In addition, some tests are insufficiently robust to the lower concentrations of thyroid hormone-binding proteins overall found in these conditions. Some drugs used in severe illness can also distort the results directly by displacing bound thyroid hormones. FT3 tests appear to suffer more in this regard than FT4 tests, though both can be compromised (377).

This can significantly affect conclusions as to the exact magnitude of allostatic effects on thyroid function and may hinder comparison of any findings from studies using different methods of measurement. The most convincing results will therefore be found using assays with minimal interference and closest adherence to the Mass Action criteria governing the working of such assays.

EFFECTS OF HETEROGENEOUS OR UNKNOWN ORIGIN

The subsequently described influences originate from human civilization. They therefore fail to trigger a natural adaptive response of thyroid homeostasis. Since their action is complex, it is possible, however, that some of the associated mechanisms elicit an allostatic reaction by physiological mimicry of natural factors.

Endocrine Disruptors

Multiple industrial substances in the environment are able to profoundly modify thyroid function (378–380). The mechanisms are as heterogeneous as the substances, and there is some overlap to drug effects (see above). This applies, e.g., to perchlorate, which inhibits iodine uptake into the thyroid and may therefore cause primary hypothyroidism (381, 382). Primary hypothyroidism may also result from exposure to polyhalogenated aromatic hydrocarbons (383) and bisphenol A (384). The effects of polychlorinated biphenyls (PCBs) include increased hepatic degradation of thyroid hormones and inhibition of deiodinase activity, which applies predominantly to cerebral D3 (385–387). Typical high-T3 syndromes under PCB exposure are therefore probably caused by decreased D3 activity rather than by stimulated D1 or D2 activity. Some plant-derived substances have thyromimetic effects and lead to decreased concentrations of TSH, FT4, and FT3 (388). In reality, endocrine disruptors are rarely present in isolated forms. Interactions among different disruptors are complex and may be additive, sub-additive, and super-additive, depending on the individual combination of substances and their respective concentrations (389–391). This makes the effects difficult to predict in the individual situation. Endocrine disruptors may result in clinical thyroid disease whose etiology is hard to pinpoint.

Space Flight

Space journeys expose the human organism to multiple challenges. They include low or zero gravity, radiation, and impaired circadian rhythm. It is, therefore, not surprising, that manned space exploration is associated with multiple hormonal changes (392). Among them, alterations of the HPT axis seem to play a minor, but potentially significant role. Crewmembers of the Spacelab D-2 mission had slightly increased TSH concentration during flight, suggesting a form of subclinical hypothyroidism (393). These results are compatible to observations in rats and rhesus monkeys (394–397). The most plausible reason for slightly impaired thyroid function during spaceflight is the utilization of iodinated water (398). Iodine was used as a bactericidal agent in US spacecraft water systems until 1997, when a device was implemented in Space Shuttles (and later the International Space Station) to remove iodine from water before consumption. As a result post-flight TSH elevations are no longer observed in astronauts (399). However, regardless of iodine removal, in male astronauts TT4 concentrations and FT4 index continue to be

higher after space flight and T3 concentrations are decreased after flight. Due to unknown reasons this NTIS-like pattern is observed in men only, but not in women (399).

METHODS OF ASSESSMENT AND DIFFERENTIAL DIAGNOSIS

Allostatic adaptations of the HPT axis frequently pose serious problems for diagnosis and differential diagnosis of thyroid disorders, because concentrations of both TSH and peripheral thyroid hormones may change considerably and fall widely outside their normal reference ranges. A diagnostic problem arises from the fact that reference ranges for thyroid parameters have been established for healthy non-pregnant adults under resting and fed conditions—a premise that is not invariably met, especially not in conditions accompanied by both severe thyroid dysfunction and systemic illness or pregnancy, where early diagnosis is essential. Moreover, even the uncompromised reference interval for TSH varies significantly by age, sex, hour of day, and ethnicity (400).

The endocrine features of TACITUS share overlapping elements with all of the following diseases, hypothyroidism (low-T3 concentration and, in chronic illness, reduced T4 concentration, and even elevated TSH in the recovery phase), hyperthyroidism (transiently increased FT4 levels and occasionally low TSH concentrations) and hypopituitarism (thyrotropic adaptation with low TSH levels despite low or normal FT4 concentrations) (73). This may present a huge challenge for differential diagnosis (24, 35). For instance, the combination of reduced TSH level and normal or even slightly increased FT4 concentration, which arises from markedly different half-lives of TSH and T4 in transitional periods of thyrotropic adaptation, may be confused with subclinical or overt hyperthyroidism (71). The situation is even more complex as the clinical spectrum of numerous severe diseases shares phenotypical features with hypothyroidism or thyrotoxicosis, respectively (72). As an example, sepsis is usually associated with fever and hyperdynamic circulation including tachycardia and peripheral vasodilatation, which are also characteristic for thyroid storm. Conversely, myxedema coma is marked by impaired vigilance, hypothermia, hypercapnia, and bradycardia, and it is therefore difficult to differentiate from critical non-thyroidal illnesses. Even subclinical hyperthyroidism may pose a risk to patients, especially to those, who are required to receive a high iodine load, e.g., in form of amiodarone or iodinated radiocontrast agents—procedures that are frequently necessary in the critically ill.

It is therefore both essential and difficult to distinguish TACITUS from diseases that are accompanied by decreased TSH levels, e.g., thyrotoxicosis and hypopituitarism. If FT3 and FT4 are both elevated or at least in the upper quintile of the respective reference ranges the diagnosis of thyrotoxicosis is straightforward. The differential diagnosis between TACITUS and subtle forms of subclinical hyperthyroidism or hypopituitarism may be more difficult, especially if an isolated thyrotropic dysfunction is present. A history of prior symptoms and signs of pituitary dysfunction may be helpful. It is also useful to evaluate the function of the corticotropic axis in patients with critical illness and possible TACITUS. In case of dysfunction treatment with glucocorticoids should be commenced prior to thyroid hormone substitution.

Hypothyroidism associates mostly with elevated TSH levels. Positive antithyroid antibodies support the diagnosis of Hashimoto thyroiditis, however without proving the diagnosis of hypothyroidism and in most cases with significant delay.

Thyroid dysfunction induced by amiodarone therapy may lead to laboratory findings similar to TACITUS. Medical and drug history may help to approach the right diagnosis. Differential diagnosis is more complex, if typical amiodarone-induced changes of thyroid function are to be distinguished from amiodarone-induced thyrotoxicosis (e.g., in tachyarrhythmia), since in both cases FT4 concentrations may be elevated (by impaired deiodination under amiodarone or by hypersecretion of thyroxine, respectively).

Today, most intensive care units are equipped with ultrasound devices. Therefore, thyroid ultrasonography may be used as an inexpensive and non-invasive method to visualize size and internal structure of the thyroid gland. Thyroid enlargement, the presence of nodules (especially of TIRADS class 2 and colloid types 2 and 3) or diffuse hyperperfusion of the thyroid are indicative of hyperthyroidism.

Challenges in the assessment of thyroid function during pregnancy result from the normal gestational changes in thyroid activity and increased prevalence of conditions that cause hyperthyroidism in pregnancy (401). Additional uncertainty arises from an ongoing controversy, if levels of total (264) or free thyroid hormones (401, 402), measured either *via* immunoassays (403) or LC/tandem mass spectrometry (404), are the preferred targets for diagnostic interpretation (248, 405). There is no doubt, however, that laboratory investigations must always be accompanied by careful clinical evaluation of the patient's symptoms and history (401). Some professional guidelines suggested trimester-specific reference intervals for the concentrations of TSH and peripheral thyroid hormones (248, 264, 406), while the recent guideline of the American Thyroid Association recommended elevating the upper limit of the reference range back to 4 mIU/L (264). Considering the significant differences among guidelines and the conflicting results of clinical trials critical questions still remain unanswered.

Calculating model-based structure parameters of thyroid homeostasis may be helpful in differential diagnosis (407). Recent studies have used mathematical models for diagnosis and prognosis of thyroid disorders, such as Hashimoto thyroiditis and Graves' disease (408, 409). Multiple studies showed total step-up deiodinase activity (SPINA-GD) to be significantly reduced in subjects affected by NTIS (61, 299–301). In patients with heart disease, SPINA-GD negatively correlated to age, atrial conduction time, and concentrations of B-type natriuretic peptide as well as 3,5-diiodothyronine (3,5-T2), and it predicted atrial fibrillation after cardiac surgery (61). In a study with 219 obese patients SPINA-GT, an estimate of thyroid's secretory capacity, assisted in identifying subjects with mild secretory insufficiency of the thyroid (410). In chronic renal failure, SPINA-GT correlated to creatinine clearance, suggesting toxic effects of azotemia on thyroid function (411). Calculating the ratios of total to free T4 (TT4/FT4) (300, 301) and of total to free T3 (TT3/FT3) (300) may be helpful to screen for impaired plasma protein binding of

thyroid hormones in NTIS. Jostel's TSH index, a measure for thyrotropic anterior pituitary function (412), is decreased in patients with central adaptation in TACITUS syndrome (299). Despite accumulating evidence for the use of structure parameters in NTIS, their diagnostic utility is still insufficiently evaluated, and they have not been studied in other situations of thyroid allostasis including starvation, pregnancy, and psychiatric diseases. They have emerged, however, as valuable tools for clinical research (19–22, 24, 92, 94, 407).

TREATMENT OF LOW-T3 SYNDROME IN TACITUS—AN OPEN QUESTION

As noted above, low-T3 syndrome and other components of NTIS correlate to severity of disease and independently predict the outcome of affected patients (54, 60, 64–66, 413–416). Guided by the idea that NTIS represents a form of illness-mediated hypothyroidism, it was suggested to treat the condition with levothyroxine (L-T4) or liothyronine (L-T3) with the expectation to improve the prognosis of critically ill patients (39, 70).

In fact certain surrogate markers, e.g., hemodynamic parameters and other markers of cardiovascular function, were demonstrated to improve after initiation of treatment with L-T3 (417, 418). With one exception of preterm infants (419) hard endpoints including survival could not be ameliorated by thyroid hormone administration (25, 170, 420, 421). In contrary, some studies observed even detrimental effects of therapy (25).

IL6-induced oxidative stress decreases the catalytic activity of D1 and D2. Selenium supplementation failed to demonstrate a beneficial effect on NTIS, although it improves critical intracellular antioxidant functions, particularly of selenoproteins (115). By providing bacteriotoxic iodine atoms, increased step-down deiodination due to stimulated D3 activity might be beneficial by defending against bacterial infections (55).

Several studies have demonstrated that treatment of the underlying disease can aid in the resolution of NTIS (422, 423).

In conclusion, universal substitution therapy cannot currently be recommended in TACITUS (424). Vastly insufficient diagnostic methods, as noted above, hinder the development of valid laboratory-based decision criteria that would help to safely identify critically ill patients with mild hypothyroidism. Diagnosis of myxedema coma therefore still relies on score systems, which either incorporate TSH and FT4 concentrations among other parameters (425, 426) or completely renounce the use of hormone measurements (426, 427).

CONCLUSION

The hypothalamus–pituitary–thyroid feedback control mechanism is a dynamic adaptive system. In resting equilibrium conditions of healthy adults the behavior of all elements involved is sufficiently stable to be diagnostically interpretable. This changes dramatically in straining situations such as starvation, exhaustion, or non-thyroidal illness. In the latter situations requirements of energy, oxygen or glutathione exceed supply, prompting the control loop to switch to a different operating mode that helps to adjust consumption to available resources. This type of allostatic response is termed type 1 and it is marked by low-T3 syndrome, reduced plasma protein binding of thyroid hormones, thyrotropic adaptation, and high concentrations of rT3. A similar constellation is observed during the fetal life. An inverted response pattern is seen in cases of predictive adaptation marked by type 2 allostatic load (including obesity, endurance training, adaptation to cold and post-traumatic stress disease). Here, concentrations of TSH, FT3, and TT3 are elevated, as is the plasma protein binding of thyroid hormones. A partly similar pattern to type 2 allostasis is seen in pregnancy.

Is thyroid allostasis beneficial or harmful? Perhaps an answer can be found in the very extremes of thyroid function, thyroid storm and myxedema coma. Although both diseases are located in opposing edges of the functional spectrum, they share a special kind of interaction between dysregulation of thyroid homeostasis and increased sensitivity of the organism to altered thyroid hormone signaling. They also have a pathophysiological pattern in common: the preexisting thyroid dysfunction may remain oligosymptomatic and undiscovered for years, yet an unspecific trigger (e.g., infection, apoplexy, or myocardial infarction) may ignite a complex causal network that results in a life-threatening crisis (72, 426). Thyroid storm is a form of insufficient adaptation, since thyrotoxicosis prevents the development of TACITUS in cases of critical illness. On the other hand, myxedema coma represents a form of overcompensation, which is marked by a massive amplification of hypothyroidism by the development of NTIS in severe disease. Both forms of thyroid crisis are life threatening: thyroid storm by insufficient allostasis and myxedema coma by allostatic overload. These examples illustrate the Janus-faced character of allostasis: although lifesaving in many cases it may occasionally threaten survival through the burden of allostatic load.

Differential diagnosis of type 1 and type 2 allostasis from peripheral or central thyroid dysfunction may be difficult, the more as physiological reactions of the feedback loop overlap with non-homeostatic mechanisms including drug effects, pre-analytical factors and assay flaws in critical illness (**Figure 1**).

In an allostatic context correct interpretation of thyroid function cannot be based on simple diagnostic rules or laboratory tests. Rather it requires a deep understanding of physiology and a comprehensive diagnostic strategy that integrates the patient's history, clinical parameters, and laboratory findings. Developing and validating reliable algorithms that support the required level of integration is a fundamental task for future thyroid research.

AUTHOR CONTRIBUTIONS

AC, AA, JD, RH, and JM made the literature research and wrote the manuscript. BD wrote the part of the manuscript thyroid in psychiatric diseases. AU wrote the part of manuscript concerning thyroid and amiodarone. HK and SH critically read and revised the manuscript. All the authors have read and approved the manuscript.

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PERSPECTIVE
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Calculated Parameters of Thyroid Homeostasis: Emerging Tools for Differential Diagnosis and Clinical Research

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Although technical problems of thyroid testing have largely been resolved by modern assay technology, biological variation remains a challenge. This applies to subclinical thyroid disease, non-thyroidal illness syndrome, and those 10% of hypothyroid patients, who report impaired quality of life, despite normal thyrotropin (TSH) concentrations under levothyroxine (L-T4) replacement. Among multiple explanations for this condition, inadequate treatment dosage and monotherapy with L-T4 in subjects with impaired deiodination have received major attention. Translation to clinical practice is difficult, however, since univariate reference ranges for TSH and thyroid hormones fail to deliver robust decision algorithms for therapeutic interventions in patients with more subtle thyroid dysfunctions. Advances in mathematical and simulative modeling of pituitary–thyroid feedback control have improved our understanding of physiological mechanisms governing the homeostatic behavior. From multiple cybernetic models developed since 1956, four examples have also been translated to applications in medical decision-making and clinical trials. Structure parameters representing fundamental properties of the processing structure include the calculated secretory capacity of the thyroid gland (SPINA-GT), sum activity of peripheral deiodinases (SPINA-GD) and Jostel's TSH index for assessment of thyrotropic pituitary function, supplemented by a recently published algorithm for reconstructing the personal set point of thyroid homeostasis. In addition, a family of integrated models (University of California-Los Angeles platform) provides advanced methods for bioequivalence studies. This perspective article delivers an overview of current clinical research on the basis of mathematical thyroid models. In addition to a summary of large clinical trials, it provides previously unpublished results of validation studies based on simulation and clinical samples.

Keywords: thyroid hormones, homeostasis, SPINA-GT, SPINA-GD, set point, feedback control, thyroid's secretory capacity, sum activity of peripheral deiodinases

INTRODUCTION

Thanks to the advent of sensitive assays for TSH and free thyroid hormones, the diagnosis of classical forms of overt hypothyroidism and hyperthyroidism has become a straightforward task (1). Differential diagnosis may still be difficult, however, in some cases with subclinical forms of thyroid failure (2, 3), hypothalamic or pituitary dysfunction (4), and in situations of allostatic load, e.g., starvation and non-thyroidal illness syndrome (NTIS) (2, 5, 6). A therapeutic challenge arises from the fact that current standard treatment of hypothyroidism with levothyroxine (L-T4) fails to raise the quality of life (QoL) in patients to a level observed in a normal population (7). Rather, they display symptoms that are compatible with either hypothyroidism and hyperthyroidism, and a fraction of 5–15% of hypothyroid patients on L-T4 replacement continue to complain about impaired QoL, despite documented biochemical euthyroidism as defined by reference intervals (7, 8).

Reasons for low health-related QoL in treated hypothyroidism may include inadequate dosage of substitution therapy with L-T4, inadequate treatment modality, systemic sequelae of thyroid autoimmunity, concomitant other autoimmune diseases, and psychological phenomena, especially in form of a nocebo effect (7). Additionally, low reported QoL might ensue from some selection bias, since in most countries thyroid disease is treated by primary care physicians, who may refer "difficult cases" to academic centers, and since most functional thyroid disorders are diagnosed because patients report elements of lower QoL (9–11). According to the topic of this perspective article, we will focus our subsequent considerations to the former two possible mechanisms.

Inadequate treatment modality refers to potential adverse effects of monotherapy with L-T4, e.g. in a subgroup of hypothyroid patients, who are affected by reduced deiodination due to polymorphic variants with lower enzyme activity (12, 13). In this group, additional replacement with L-T3 (and also, possibly, low doses of other classical and non-classical thyroid hormones) may be beneficial. Due to disruption of the thyroid-mediated TSH–T3 shunt (14, 15), inefficient conversion of T3 from T4 may also arise in the subgroup of L-T4-treated athyreotic patients (16, 17). Narrow individual tolerance to hormone concentrations around the personal set point of thyroid homeostasis may also contribute to considerable variation in the treatment response (18–21). These observations have stimulated a recent debate, whether universal reference ranges for TSH and peripheral thyroid hormones are appropriate (14). Improved diagnostic efficiency has also been observed using multivariate analysis rather than the conventional univariate approaches (22). Based on recent research, we and others have propagated a more comprehensive systems-based approach. This includes the use of homeostatically defined structure parameters (6). Mathematical modeling of pituitary–thyroid feedback control has delivered functional insights beyond the scope of univariate reference ranges (14, 20, 23, 24).

This perspective article gives an overview of current methodology and established and possible future applications of modeling-based diagnostic investigation *in vivo*.

APPLYING CYBERNETIC MODELS OF THYROID HOMEOSTASIS

Over the past 60 years, a plethora of mathematical or simulative models of pituitary–thyroid interaction has been published (6, 14). Only a small subset, however, has been translated into applications for clinical decision-making or research (beyond the scope of modeling itself). These modeling platforms include the logarithmic standard model of thyroid homeostasis (25), compartment analytical models, which were originally developed at the Biocybernetics Laboratory of the University of California-Los Angeles (subsequently referred to as UCLA platform) (26–31), non-linear models combining Michaelis–Menten kinetics in the feedforward path and non-competitive inhibition in the feedback direction (aka MiMe-NoCoDI models) (32, 33), and a so-called "minimal model" that combines Michaelis–Menten kinetics with a logarithmic model of hypothalamic–pituitary function (20, 23, 24). Thanks to both sufficient empirical foundation and some physiological justification, models derived from these platforms are able to deliver meaningful measures of homeostatic function. Where biochemical knowledge is (or was) insufficient for the development of well-justified models, simple equations, e.g., ratios, have been introduced to deliver an estimate for basic processes of conversion or signal transduction.

APPLICATIONS OF THE UCLA PLATFORM

This family of models is based on separation of source and sink organ components, implemented as at least three source (organ) and three sink (distribution and elimination) subsystems. Dating back in its origin to 1966 (34–36), it was successively improved to incorporate current findings of basic and clinical research. The most recent implementations of this platform combine Michaelis–Menten kinetics (deiodination) with a three-parameter time-delay model (thyroid), a negative exponential model for feedback inhibition of TSH release, and a non-linear description of plasma protein binding (26–31).

Models of this platform were applied to pharmacokinetic (PK) and pharmacodynamic questions concerning substitution therapy with L-T4 (37). By mathematical modeling and computer simulations, it could be demonstrated that for the majority of hypothyroid patients standard L-T4-only therapy should be sufficient to reach normal triiodothyronine tissue concentrations (28, 29), but that substitution with L-T3 may be beneficial to reduce the withdrawal period before ^{131}I remnant ablation in patients with thyroid cancer (26).

Additionally, this platform paved the way for the development of an improved protocol for bioequivalence studies. Thyroid hormones are critical dosage drugs, i.e., small changes in concentration may exert major metabolic effects, and the absorption rate is highly sensitive to multiple influencing factors including meals, coffee, concomitant medication, and gastrointestinal disease (38). Moreover, L-T4 preparations of different brands cannot be considered bioequivalent (39, 40). Traditionally, bioequivalence is assessed by PK studies as required by the FDA and other regulatory authorities. Standard protocols are faced with the problem that they ignore the existence of functional feedback in healthy

volunteers, however. Models based on the UCLA platform delivered an improved baseline correction method that is less prone to this kind of interference (27, 28).

MEASURES OF THYROID FUNCTION AND PERIPHERAL HORMONE METABOLISM

Circulating T4 is actively taken up by cells and biologically activated by enzymatic monodeiodination before exerting (mostly genomic) intracellular effects. The molar T3/T4 ratio may therefore serve as a simple measure of deiodinase activity and conversion efficiency. Numerous studies investigated the T3/T4 ratio in various conditions. They found it to be increased in iodine deficiency (41) and other settings of hyperdeiodination (42–44) – possibly accompanied by intrathyroidal hypoiodination and representing an iodine recovery mechanism – and to be decreased in NTIS (45, 46), central hypothyroidism ensuing from thyrotropic insufficiency (47, 48), congenital thyroid hypoplasia (47), treatment with propranolol (49) and, compared with cases of true hyperthyroidism, in the acute phases of postpartum thyroid dysfunction, subacute, and painless thyroiditis (50–53). It is increased in Graves' disease compared to multinodular toxic goiter and toxic adenoma (51) and decreased in athyreotic patients receiving substitution therapy with L-T4 (54). An observation study found a negative correlation of T3/T4 ratio with age and positive correlation with serum selenium concentration (55). A report on strong negative correlation between FT4 index and T3/T4 ratio remains questionable, since the results were not corrected for spurious correlations (56).

Other measures related to conversion are FT3/reverse T3 (rT3) ratio, an estimate for the proportion of step-up to step-down deiodination, and 3,5-diiodothyronine (3,5-T2)/FT3 ratio. The former parameter is decreased in NTIS (TACITUS), while the latter is increased (57).

However, the simple ratios are conceptually incompatible with known kinetic properties of enzyme-mediated processes, as they wrongly assume linear relationships (6). Reference ranges for ratios are also more difficult to define than for non-fractions (58). These inherent deficiencies made it necessary to derive more robust structure parameters that describe the behavior of transfer elements in homeostatic models (33, 59). The novel parameters are based on the MiMe-NoCoDI platform, i.e., Michaelis–Menten functions and PK data to deliver a structure parameter inference approach (SPINA) that provides non-linear estimates of signal transduction (32).

To implement this approach, we estimated the sum activity of peripheral deiodinases ($\hat{G}_D$ or SPINA-GD), which reflects the maximum stimulated activity of step-up deiodination. It is calculated with

$$\hat{G}_D = \frac{\beta_{31}\,(K_{M1} + [FT_4])\,(1 + K_{30}[TBG])\,[FT_3]}{\alpha_{31}[FT_4]}$$

from equilibrium concentrations of FT4, FT3, and PK constants (**Table 1**) (32, 60). A simpler version employs the concentration of total T3 with

$$\hat{G}_D = \frac{\beta_{31}\,(K_{M1} + [FT_4])\,[TT_3]}{\alpha_{31}[FT_4]}.$$

TABLE 1 | Standard parameters used by the equations for SPINA-GT, SPINA-GD, and Jostel's TSH index (6, 32, 60).

Symbol	Explanation	Value
α_T	Dilution factor for thyroxine	$0.1\,L^{-1}$
β_T	Clearance exponent for T4	$1.1e{-}6\,s^{-1}$
D_T	EC_{50} for TSH	2.75 mIU/L
K_{41}	Dissociation constant of T4 at thyroxine-binding globulin	2e10 L/mol
K_{42}	Dissociation constant of T4 at transthyretin	2e8 L/mol
α_{31}	Dilution factor for triiodothyronine	$0.026\,L^{-1}$
β_{31}	Clearance exponent for T3	$8e{-}6\,s^{-1}$
K_{M1}	Dissociation constant of type 1 deiodinase	500 nmol/L
K_{30}	Dissociation constant of T3 at thyroxine-binding globulin	2e9 L/mol
[TBG]	Standard concentration of thyroxine-binding globulin	300 nmol/L
[TBPA]	Standard transthyretin concentration	4.5 µmol/L
β	Correction coefficient of logarithmic model	0.1345

Dilution factors are defined as the reciprocal of apparent volume of distribution (V_D).

The reference range for SPINA-GD is typically between 20 and 60 nmol/s (57), with some dependence on the assays used. Since the dissociation constant of type 1 deiodinase is beyond physiological plasma concentrations of FT4, SPINA-GD is nearly linear in the euthyroid range, so that it has similarities to the T3/T4 ratio. Its non-linear properties are advantageous especially in cases of high FT4 concentrations.

The thyroid's secretory capacity ($\hat{G}_T$ or SPINA-GT), also referred to as thyroid output or thyroid capacity, provides an estimate for the maximum secretion rate of the thyroid gland under stimulated conditions. It is defined with

$$\hat{G}_T = \frac{\beta_T\,(D_T + [TSH])\,(1 + K_{41}[TBG] + K_{42}[TBPA])\,[FT_4]}{\alpha_T[TSH]}$$

as a function of equilibrium concentrations of TSH, free T4, and constants or measured values for dissociation, protein binding, distribution, and elimination (**Table 1**) (32, 60). A simpler version utilizes total T4 concentration with

$$\hat{G}_T = \frac{\beta_T\,(D_T + [TSH])\,[TT_4]}{\alpha_T[TSH]}.$$

The reference range is usually between 1.4 and 8.7 pmol/s (57).

In silico evaluation with Monte Carlo simulations demonstrates that both SPINA-GT and SPINA-GD can be sufficiently reliably estimated, despite limited accuracy of laboratory assays (**Figures 1A,B**). *In vivo* validation confirmed that SPINA-GT is able to clearly differentiate between euthyroidism and functional thyroid disorders of primary origin (32, 61, 62). However, unlike TSH, it is unaffected by hypothalamic–pituitary dysfunction (**Figure 1C**; Table S1 in Supplementary Material). This translates to high specificity in thyroid disorders of secondary or tertiary origin. Physiologically, SPINA-GD correlates with the conversion rate of slow tissue pools (**Figure 1D**), as determined by isotope-based measurements in healthy volunteers (63).

SPINA-GT and SPINA-GD have been validated in numerous clinical trials. In a retrospective comparison with normal controls, SPINA-GT was significantly elevated in patients with toxic adenoma, Graves's disease, and even euthyroid diffuse and nodular goiter and significantly reduced in autoimmune thyroiditis (32).

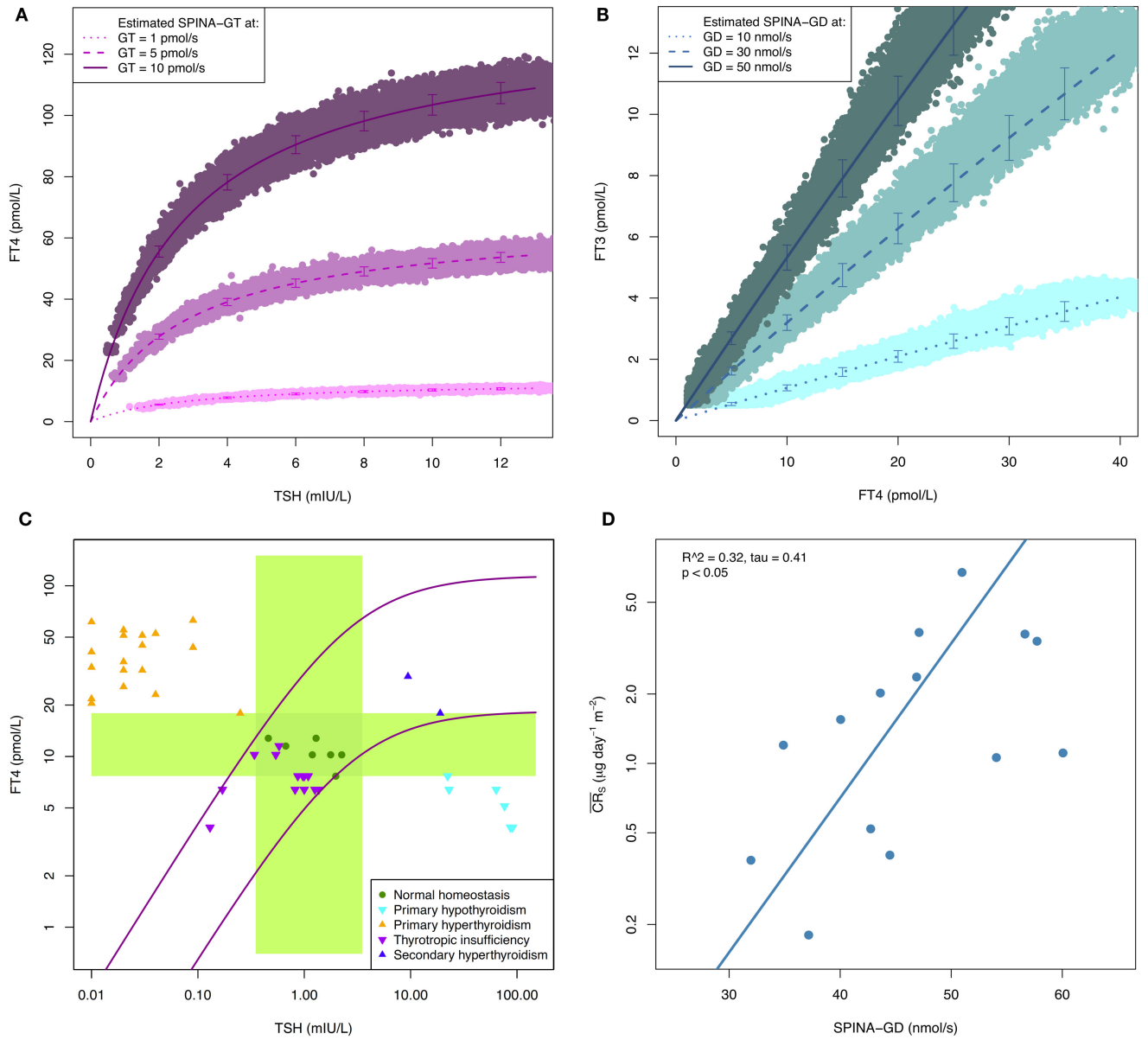

FIGURE 1 | (A,B) Reliability of SPINA-derived parameters is higher than that of measured hormone concentrations. Shown are results of Monte Carlo evaluation of SPINA-GT and SPINA-GD based on simulated imprecise hormone assays. Hormone concentrations were modeled in SimThyr 4.0 (64) with different pre-defined values for GT and GD, respectively. Subsequently, absolute hormone levels were converted to measurements by means of an S script (see supplementary code for an introductory example) that injected additive and multiplicative noise, in order to get vendor-reported concentration-dependent coefficients of variations (CV) (65, 66). The lines show mean ± SD of hormone concentrations predicted by structure parameters calculated from simulated noisy measurements. CVs as markers for measurement reliability (67) of SPINA-GT and SPINA-GD are below 10% in all cases, although CVs of corresponding hormone assays exceed 20% in low concentrations. **(C)** SPINA-GT is sensitive for thyroid disorders of primary origin and specific with respect to secondary dysfunction. The plot shows distribution of hormone concentrations in certain primary and secondary thyroid conditions compared to normal percentiles of SPINA-GT. The green crossing rectangles define univariate reference ranges for TSH and FT4, respectively. The purple lines represent FT4 concentrations at the 2 and 97% percentiles of SPINA-GT. Data from RUBIONERVE (registration number 4905-14 at RUB ethics committee) and NOMOTHETICOS studies (UTN U1111-1122-3273, ClinicalTrials.gov ID NCT01145040). **(D)** SPINA-GD is an estimate for deiodination. Shown is correlation between SPINA-GD and conversion rate in slow tissue pools. Data from Pilo et al. (63).

In the same study, it had a higher specificity for hyperthyroidism in toxic adenoma than TSH, FT4, or FT3 concentrations (32). A small trial with 20 healthy volunteers revealed the re-test reliability of SPINA-GT to be higher than that of every other parameter (6, 32). SPINA-GT was also shown to correlate with thyroid volume (32) and creatinine clearance (68).

Multiple trials demonstrated SPINA-GD to be reduced in NTIS (57, 69–71). One of these trials also reported that SPINA-GD predicts postoperative atrial fibrillation and correlates to age, total atrial conduction time (PA-TDI interval), as well as to concentrations of B-type natriuretic peptide (BNP) and 3,5-T2 (57). Two large trials together covering >3,500 participants independently

revealed SPINA-GD to correlate with TSH concentrations and to be significantly reduced after initiation of substitution therapy with L-T4 (16, 57, 72). Strong correlation with TSH levels seems to depend on the presence of residual thyroid tissue, since it was preserved in patients with autoimmune thyroiditis, but lacking in a cohort with thyroid cancer after surgery and radioiodine ablation (17). Conversely, FT3 concentrations correlated with L-T4 supply in treated cancer patients, while they remained constant over a broad range of SPINA-GT or L-T4 dosage in groups with remaining thyroid tissue. These observations suggest the existence of a thyroid-mediated TSH–T3 shunt, which might represent a compensatory mechanism, mitigating the effects of decreasing thyroid output in onset hypothyroidism (15, 17). In patients on L-T4 replacement therapy, SPINA-GD was an independent predictor of L-T4 dose (73).

If confirmed by sufficiently powered clinical trials, possible future applications of the SPINA methodology might include differential diagnosis of primary functional thyroid disorders from dysregulations of secondary or tertiary origin or from thyrotropic adaptation, i.e., transient alterations of TSH concentrations in cases of NTIS (5, 60), screening for iodine deficiency, and identification of patients who would benefit from additional substitution therapy with L-T3 (12, 13).

ESTIMATED PARAMETERS FOR PITUITARY FUNCTION

Jostel's TSH index (TSHI) was introduced as a quantitative marker for pituitary thyrotropic function (74). Based on the logarithmic standard model of thyroid homeostasis (25), it is calculated as

$$\mathrm{TSHI} = \ln([\mathrm{TSH}]) + \beta[\mathrm{FT_4}]$$

from measured concentrations of TSH and free T4 and a correction coefficient β (**Table 1**). The TSHI has been calibrated in a large sample of >9,500 subjects with and without anterior pituitary insufficiency. A *z*-transformed version of the parameter was defined as standardized TSH index (sTSHI) that results with

$$\mathrm{sTSHI} = \frac{\mathrm{TSHI} - 2.7}{0.676}$$

from mean (2.7) and SD (0.676) of the TSHI in a normal population. Accordingly, its reference range is between −2 and +2. In the original validation study, gonadotropic insufficiency and lower peak concentrations of growth hormone and cortisol in pituitary stimulation tests were associated with significantly diminished TSHI (74). Recently, it was demonstrated that the TSHI is also reduced in patients with NTIS and thyrotropic adaptation (69).

Another estimate for thyrotropic function, the thyrotroph thyroid hormone resistance index (TTSI, also referred to as thyrotroph thyroxine resistance index or TT4RI), results with

$$\mathrm{TTSI} = \frac{100[\mathrm{TSH}][\mathrm{FT_4}]}{l_u}$$

from equilibrium concentrations of TSH and free T4 and the upper limit of the reference interval of FT4 (l_u) (75). This screening parameter is elevated in cases of resistance to thyroid hormone due to mutations in the THRB gene (RTH Beta, Refetoff syndrome) (75). It may also be a valuable marker for monitoring central response to substitution therapy with triiodothyroacetate (TRIAC) in RTH beta (76). In a large cohort of twin pairs TTSI was strongly influenced by genetic factors (77). A variant of the TTSI (without correction for the upper limit of the reference range) was significantly increased in offspring from long-lived siblings compared to their partners (78). This observation suggests slight resistance to thyroid hormone to be beneficial with respect to longevity.

RECONSTRUCTING THE SET POINT OF THYROID HOMEOSTASIS

Intra-individual variation of TSH and T4 concentrations is considerably lower than inter-individual variation (18, 21). This observation gave rise to the set point theory of thyroid homeostasis, i.e., to the assumption that serum levels of TSH and FT4 are controlled to match a personal, genetically encoded reference. The region around the individual set point is the obvious target for substitution therapy with L-T4. Unfortunately, however, the location of the set point is unknown and inaccessible in the situation of hypothyroidism (6, 19). It may also vary in thyroid health, L-T4 treatment (79) and NTIS/TACITUS (2, 5, 80, 81). Recently, an algorithm was published that allows for reconstructing the set point, even in an open-loop situation (20, 23, 24). The method is based on the minimal model of thyroid–pituitary interaction and on the observation that, in healthy volunteers, the set point is located in the region of the highest curvature of the pituitary response curve. It requires a minimum of two TSH–FT4 pairs, which were obtained with a latency of at least 4 weeks. Then, two parameters, *S* (multiplier) and φ (slope of exponential function), are determined, either algebraically or *via* regression, to fit the negative exponential function

$$[\mathrm{TSH}] = Se^{-\varphi[\mathrm{FT_4}]}$$

to the data. The next step is to find the root of the third derivative of the pituitary function, where the curvature

$$K = \frac{\varphi^2 Se^{-\varphi[\mathrm{FT_4}]}}{\left(1 + \varphi^2 S^2 e^{-2\varphi[\mathrm{FT_4}]}\right)^{3/2}}$$

is at its maximum. From this, the set point components for FT4 and TSH can be obtained with

$$[\mathrm{FT_4}]_{\mathrm{SP}} = \frac{\ln\left(\varphi S\sqrt{2}\right)}{\varphi}$$

and

$$[\mathrm{TSH}]_{\mathrm{SP}} = \frac{1}{\varphi\sqrt{2}}.$$

The algorithm has been validated in a small trial, which revealed in all examined cases a goodness-of-fit between 95 and 99% (20). It has still not been investigated, however, if a set point-based dose titration regime leads to a better QoL compared

with the standard strategy on the premise of population-derived reference ranges.

CLOSING REMARKS AND FUTURE PERSPECTIVES

In this brief overview, we have described several calculated parameters derived from mathematical modeling that have emerged from recent clinical studies, as helpful tools in defining thyroid function. By extending the classical concept of separate measurements of thyroid hormone parameters these markers add new qualitative and quantitative dimensions to the evaluation of thyroid homeostasis.

Multivariate methods should improve diagnostic discrimination, as they account for interrelationships between thyroid parameters and permit determination of personal set points that are more narrowly defined than population-based reference ranges. Measuring conversion efficiency may particularly benefit the subgroup of patients with reduced QoL, despite normal TSH concentrations.

The use of structure parameters offers a more integrated and systemic view and has already delivered important insights into the physiology of pituitary–thyroid feedback control. Clinical applications are still experimental at present, and more trials are required to prove their utility for medical decision-making.

AUTHOR CONTRIBUTIONS

JD designed the validation study and developed Monte Carlo simulation software. JD, JM, and RH drafted the manuscript. GL-M, EW, HK, and AC recruited patients for formal evaluation of structure parameters. All authors read and approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://journal.frontiersin.org/article/10.3389/fendo.2016.00057

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Recent Advances in Thyroid Hormone Regulation: Toward a New Paradigm for Optimal Diagnosis and Treatment

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In thyroid health, the pituitary hormone thyroid-stimulating hormone (TSH) raises glandular thyroid hormone production to a physiological level and enhances formation and conversion of T4 to the biologically more active T3. Overstimulation is limited by negative feedback control. In equilibrium defining the euthyroid state, the relationship between TSH and FT4 expresses clusters of genetically determined, interlocked TSH–FT4 pairs, which invalidates their statistical correlation within the euthyroid range. Appropriate reactions to internal or external challenges are defined by unique solutions and homeostatic equilibria. Permissible variations in an individual are much more closely constrained than over a population. Current diagnostic definitions of subclinical thyroid dysfunction are laboratory based, and do not concur with treatment recommendations. An appropriate TSH level is a homeostatic concept that cannot be reduced to a fixed range consideration. The control mode may shift from feedback to tracking where TSH becomes positively, rather than inversely related with FT4. This is obvious in pituitary disease and severe non-thyroid illness, but extends to other prevalent conditions including aging, obesity, and levothyroxine (LT4) treatment. Treatment targets must both be individualized and respect altered equilibria on LT4. To avoid amalgamation bias, clinically meaningful stratification is required in epidemiological studies. In conclusion, pituitary TSH cannot be readily interpreted as a sensitive mirror image of thyroid function because the negative TSH–FT4 correlation is frequently broken, even inverted, by common conditions. The interrelationships between TSH and thyroid hormones and the interlocking elements of the control system are individual, dynamic, and adaptive. This demands a paradigm shift of its diagnostic use.

Keywords: setpoint, thyroid homeostasis, thyroid-stimulating hormone, levothyroxine treatment

INTRODUCTION

In the healthy body, multiple interlocking biochemical mechanisms interact homeostatically both to achieve biological equilibrium and to respond suitably to any challenges, internal or external, that might occur. However, the way in which different individuals maintain a steady homeostatic state varies considerably, including their appropriate reaction to any changes that

may be demanded of the system. In this regard, the possible biochemical expressions of a healthy framework within which viable variations of response can occur have finite limits (1). Appropriate reactions in individuals are defined by their own unique solutions both to homeostatic equilibrium and to biochemical challenges, which are much more closely constrained than those permissible over the whole population. This has profound implications as to how disequilibria are confronted by individual responses to the challenge or therapeutic interventions administered to obvert it.

The classical definitions of hypothyroidism and hyperthyroidism reflect this concept when referring to inadequate—either reduced or exaggerated—supply and response to thyroid hormones (2). Pathophysiological conditions or diseases may arise at various levels including deficiencies of hormone supply, alterations of control or resistance to cellular responses to the hormones (3–5). Thyroid gland failure represents a more restricted view, termed primary hypothyroidism. Current diagnostic definitions of thyroid disease are primarily laboratory based, and include subclinical dysfunctions that are dissociated from treatment recommendations (6–9). In this review, we revisit underlying principles of thyroid control, aligning or contrasting them with the current use of thyroid-stimulating hormone (TSH) in the diagnosis and treatment of thyroid diseases.

THE CURRENT PARADIGM OF THYROID DIAGNOSIS

Two events have shaped the current paradigm of thyroid function testing (1) the discovery of negative feedback by thyroid hormones on pituitary TSH and (2) methodological advances in sensitively measuring TSH (6, 10, 11). If TSH serum concentrations provided a more sensitive and accurate mirror image of thyroid hormone status than thyroid hormones themselves, this would be an ideal diagnostic tool. This argument emerged in the 1980s, and was readily accepted by clinicians (12, 13). TSH measurement subsequently gained a dominant role in thyroid function testing, facilitating cost effective disease screening, introducing new definitions of subclinical hypothyroidism or hyperthyroidism, and delivering biochemical treatment targets (14–17). A plethora of epidemiological studies then associated TSH concentrations with clinical outcomes (18–21). This resulted in a questionable paradigm elevating an indirect controlling parameter to such prominence and dominance as expressed in current guidelines for diagnosis and treatment of thyroid diseases (14–16). With the holy grail in the hand of scientists, clinically orientated approaches contradicting the TSH paradigm and noting discrepancies between a biochemically euthyroid and clinically hypothyroid status were readily dismissed (22–26). However, concerns have resurfaced in the light of recent basic and clinical studies revealing fundamental issues with the interpretation of TSH measurements and documenting unsatisfactory improvement in the quality of life of patients treated for hypothyroidism according to the current TSH paradigm (1, 27–29).

SHIFTING THE PARADIGM

Is the TSH concentration at all times a reliable mirror image of the thyroid hormone status of the patient?

Firstly, we must note that this question reverses causality of process. While TSH is subject to negative feedback by thyroid hormones predominantly FT4, thereby reflecting on the thyroid hormone status, its primary physiological role is to elevate the hypothyroid to the euthyroid state (1). Without appropriate stimulation by TSH, the basal hormone output by the thyroid gland remains insufficient, as becomes apparent in pituitary deficiency (secondary hypothyroidism) (4). Conversely, in thyroid health TSH raises the glandular hormone production to its physiological level, and it also enhances the formation of the biologically more active T3 and its conversion from T4 (30–32). The integration of the TSH–T3 shunt into the homeostatic control of the thyroid–pituitary axis is illustrated in **Figure 1A**. Feedback details have been discussed elsewhere (27). Clinical studies and theoretical modeling suggest that the shunt facilitates FT3 stability against variations in the glandular T4 output, and its absence may lead to T3 production instability in athyreotic patients (31, 33). This relational control loop operates still within the euthyroid TSH range (34). It also appears to mediate subtle expressions of central control over peripheral tissues, such as circadian or seasonal variation in FT3, but not FT4, the former lagging the TSH rhythm (35). In thyroid autoimmune disease, the feedforward control increases T4 to T3 conversion, compensating for latent thyroid failure until the disease has progressed beyond tolerance (34).

Pathophysiological challenges other than thyroid disease may disturb the equilibria between TSH and thyroid hormones, requiring setpoint adjustment of the hypothalamic–pituitary–thyroid axis (**Figures 1A–C**). Weight gain or loss, alterations in body composition, and aging may cause profound changes (**Figure 1C**), frequently switching the control mode from negative feedback to tracking FT4 rather than opposing it (36, 39, 40). This inversion of the TSH–FT4 correlation is clinically important to recognize, but it may be missed or misinterpreted as subclinical hypothyroidism when relying on TSH measurement as sole diagnostic test. Several mechanisms provide a physiological interface for shifting the control modes of the hypothalamic–pituitary–thyroid axis (36, 39–46). While detailed examination of the complex subject is beyond the scope of this article and dedicated reviews are available (36, 39, 41, 43, 45, 46), a brief explanation may be helpful. Hypothalamic tancytes strongly express the relatively more T3- than T4-affine thyroid hormone transporter MCT8 and deiodinase type 2 and 3, enabling them to sense FT3 and FT4 levels (41, 44–46). They also respond to alterations in the energetic and metabolic needs of the body and interact with TRH neurons (41, 42, 44–48). Fat cells release adipokines such as leptin into the circulation which directly or indirectly stimulate pituitary TSH secretion (36, 39, 40, 42, 45). In a vicious cycle, TSH may promote leptin release through TSH receptor activation on adipocytes (49). The controlling system stays informed on fat and energy reserves, and may act on adjusting energy expenditure accordingly. While rising with weight gain TSH decreases again after weight loss

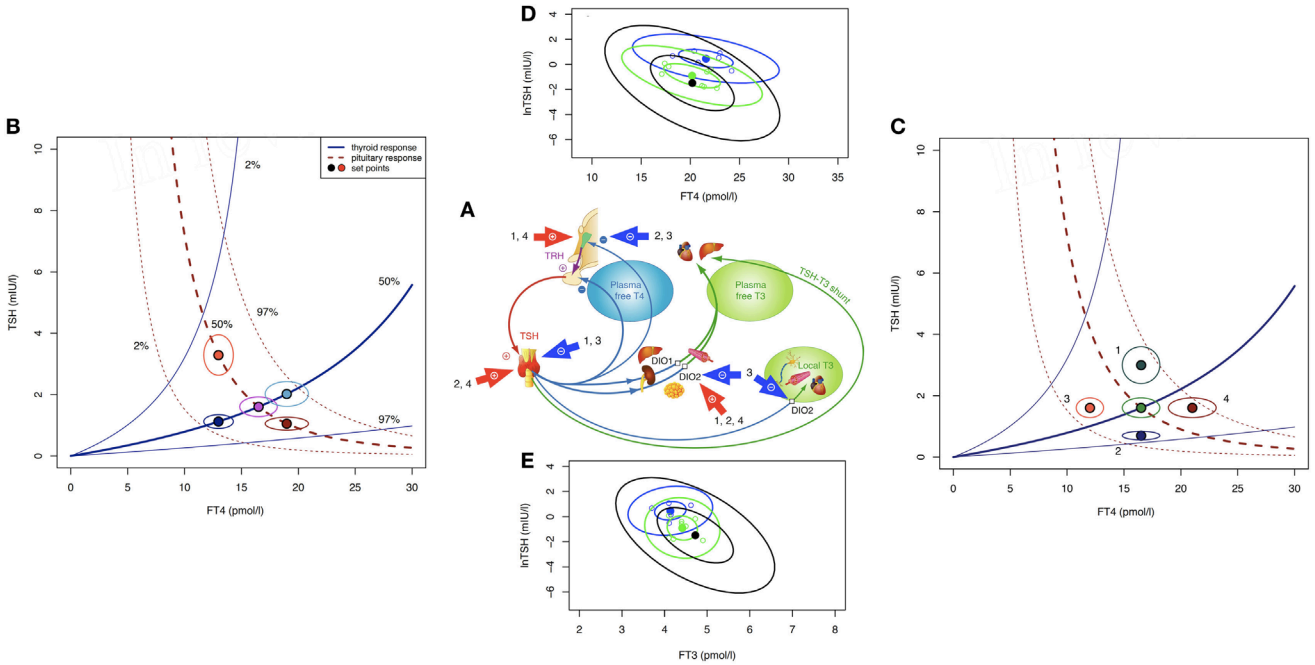

FIGURE 1 | Theoretical and observed relationships between thyroid-stimulating hormone (TSH) and thyroid hormones. The central system provides an integrated solution to the location of the setpoint (settling point) of thyroid homeostasis, as determined by the various genetic, epigenetic, and allostatic parameters (27, 36). This includes monogenic factors such as polymorphic variants of receptors and transporters, changes in deiodinase activity or variations in T4 production efficiency, and environmental impacts, e.g., nutritional factors, body weight, body composition, age, and extends to diseases of the thyroid and other organs where severe disequilibria may arise and a "euthyroid" solution is not achieved (27, 36). **(A)** Schematic overview of main regulatory pathways and control loops in the hypothalamic–pituitary–thyroid regulation. External factors of influence include (1) obesity (hyperthyrotropinemia), (2) pregnancy (hCG-mediated suppression of TSH release and stimulation of T4 secretion), (3) non-thyroidal illness (NTI) (both pituitary and thyroid function down-regulated), and (4) certain psychiatric diseases (both pituitary and thyroid function stimulated). **(B)** The response of the thyroid to TSH by hormone release and corresponding feedback on pituitary TSH secretion produces a finite equilibrium solution, thereby defining interlocking TSH–FT4 pairs (setpoints). These are characteristic for each person and show only little variation unless disturbed by internal or external strain. In the event of progressive thyroid capacity stress, irrespective and independent of other influences, setpoints for FT4 and TSH translocate along a homeostatic pituitary response curve (isocline) unique for the individual. Hence, in the case of a diminished or exaggerated pituitary response the translocation moves along the thyroid isocline. The open circles indicate the expected variation (10% for FT4 and 30% for TSH) surrounding the individual setpoint. The percentiles for the isoclines of the response curves were derived from previous data in a healthy sample and the included area between the 2.5 and 97.5 percentiles represents the population's reference range (37). **(C)** Influences additional to direct thyroid activity change (e.g., allostatic changes such as obesity, age, pregnancy, and NTI) may have dislocating effects on isoclines and setpoints. **(D)** Observed TSH–FT4 pairs (setpoints) in two individual patients (blue and green symbols) and the averaged group of 250 patients (black ellipse) followed long term on stable treatment conditions. Data ellipses indicate the 50 and 95% confidence limits for the setpoint. Levothyroxine dose was 100 µg/day, 1.5 µg/kg body weight for each of the individual patients, and 1.5 (SD 0.27) µg/kg for the whole group. Data are from a published longitudinal study (38). **(E)** Observed TSH–FT3 pairs depicted in the same patients as in **(D)**. FT3 concentrations vary with conversion efficiency and impact on the location of the TSH–FT4 setpoint (38).

(36, 39, 50, 51). Central and peripheral deiodinases are also sensitive to nutritional factors and body composition, adjusting T4 to T3 conversion to maintain T3 stability under varying conditions (39, 40). As transporters, deiodinases and thyroid hormone receptors subtypes differ in both their expressions and T3 affinities, central responses may readily diverge from peripheral equilibria (52–54). This may explain why none of these physiological changes is associated with thyroid dysfunction, although the equilibria between thyroid hormones and TSH are profoundly altered. Similar changes in control modes are observed in aging (55). In the Baltimore Longitudinal Study of Aging, some participants experienced a parallel rise/fall of TSH and FT4, whereas others showed a rising TSH with declining FT4 concentrations (55). Interestingly, the two patterns carried different mortality risks (55). Underlying mechanisms are complex and confounded by other pathologies, but pituitary responsiveness is clearly age related, as is deiodinase activity (56–58). Generally, higher TSH ranges are deemed acceptable in the elderly patient (57, 59, 60). More dramatic perturbations occur in severe non-thyroidal illness (NTI) including psychiatric disease (**Figure 1C**) where the allostatic stress response operates to alter both the setpoint and peripheral transfer parameters of the control loop, as reviewed elsewhere (36).

These lines of evidence suggest that equilibria and correlations between TSH, FT4, and FT3 are by no means invariant; rather being situationally adjusted in response to minor disturbances, and completely deranged in severe pathology (**Figure 1B**).

The pituitary–thyroid feedback loop must be revisited in the light of these findings (27). The strong log-linear correlation between TSH and FT4, observed in primary hypothyroidism, disintegrates toward the euthyroid range (61–65). The statistical correlation between TSH and FT4 is invalidated by clustering of

genetically determined TSH–FT4 pairs (**Figure 1B**), and influences other than TSH shape the interrelations and equilibria (**Figure 1C**) (37, 65). This results in a high-individuality index of TSH, which gravely limits the use of population statistics in determining the euthyroid range (66, 67). Hypothalamic–pituitary–thyroid control is more complex and frequently denies the diagnostic simplification of a fixed log-linear TSH–FT4 gradient (**Figures 1A–C**) (27, 68). Studies in levothyroxine (LT4)-treated patients with thyroid carcinoma favor cascade-type control involving FT4, FT3 and their interaction over simple proportional control (38). Setpoint simulations corroborated these clinical results (37, 38). Together these findings question, the current diagnostic use of the TSH response as a reliable mirror of thyroid status. They demand a conceptional evolution of thyroid regulation to underpin a more differentiated clinical use.

APPROPRIATENESS VERSUS NORMALITY

The appropriateness of a TSH level is a different concept from range consideration. In secondary hypothyroidism or hypopituitarism, TSH measurement may be frequently inappropriate, residing within its reference range, yet abnormally low relative to the low-FT4 level. Deranged setpoints together with normal or elevated FT4 concentrations have been well recognized in TSH or thyroid hormone resistance syndromes and TSH producing pituitary tumors. Similarly, low-FT3 and/or low-FT4 serum concentrations in the NTI syndrome are accompanied by variable TSH values within or outside the reference range (4, 5, 36, 69–72). The isolated interpretation of TSH is not diagnostically useful in these conditions. While these limitations are well known, the dominance of TSH in the diagnosis of euthyroidism and as a treatment target for primary hypothyroidism also warrants closer scrutiny.

The high-individuality index of TSH and its correlative variation with thyroid hormones cause major statistical issues, because the basic concept of statistical hypothesis testing demands that the sample is randomly drawn from a population and thus representative of the population (73). This tenet is, however, violated where a measured TSH value is not an expression of normality surrounded by some margin of error or variation within a given population, rather a heterogeneous group of individuals sharing the same TSH value with different physiological meaning. For some individuals, the same TSH value may indicate a perfectly normal situation, for others subtle thyroid failure with declining FT4 concentrations and negative correlation between the two hormones, and for others a control shift to a positive TSH–FT4 correlation induced by non-thyroidal influences. A known response heterogeneity, however, precludes the use of statistical regression models based on normality assumption, requiring multilevel, hierarchical, or latent class models instead (74). The heterogeneity bias is known as Simpson's amalgamation paradox (75, 76). Misleading and conflicting results may therefore be expected when mixing different underlying physiologies without proper stratification. Some authors reported a protective effect of TSH in the elderly, others an increased mortality risk of higher TSH within the normal range, and others no association of death with TSH, but with higher FT4 (21, 77–81). A dissociation between TSH and FT4 is also apparent in atrial fibrillation in euthyroid individuals where the risk increases with higher FT4 concentrations, but not lower TSH levels (82). We face a similar paradox in RCTs comparing T3–T4 combinations with T4 monotherapy, where patients invariably expressed their preference for the combination, but statistical analysis finds no evidence of superiority (83, 84).

We conclude that a TSH measurement representing ambiguous and overlapping categories between thyroid health and disease is by itself unreliable as a diagnostic tool. Statistical averaging or arbitrary cut-offs such as tertiles or quartiles should not be based on false assumptions of normality without further clinically meaningful stratification.

Another situation that differs from any naturally occurring condition is LT4 monotherapy for primary hypothyroidism. The homeostatic equilibria between TSH, FT4, and FT3 adequate for the previously healthy state no longer apply equally to the treated state, where dissociations between FT4 and FT3 and TSH and FT3 are apparent (**Figures 1D,E**) (31, 33, 65, 85–87). The T3/T4 conversion rate is reduced in LT4-treated athyreotic patients, compared with their rate prior to surgery (38, 86). FT3 is displaced from TSH in these patients, being relatively lower at the same TSH level (33, 85). While the consequences of the altered ratios are widely unknown and require further study (88), implications for the interpretation of TSH measurements can be readily derived.

Thyroid-stimulating hormone concentrations in normal health cannot be a therapeutic target for establishing LT4 dose adequacy (31, 89, 90). The treatment situation has to be re-evaluated on the basis of the shifted equilibria rather than pre-existing range considerations.

Can't we just rely on bringing TSH within an acceptable population range and assume peripheral autoregulation at the tissue level should take care of the adequate tissue supply with T3? There are two major issues with this popular belief in the ability of the patient's own pituitary gland—except for pituitary deficiency—to be the best judge of dose adequacy. First, this leaves patients frequently dissatisfied, because their quality of life is not generally restored with LT4 treatment to the same level seen in healthy persons despite their TSH concentrations being within the reference range (29). Second, the TSH for a patient on LT4 is not what it is for an untreated patient (65). The clinical and biochemical treatment response to LT4 turns out to be diverse and is influenced by many treatment-related or unrelated factors (**Figures 1D,E**) (38, 89). As a consequence of low-conversion efficiency, at least in some patients, the equilibrium for TSH may be shifted below the reference range of the healthy population (38, 86, 89). This poses a dilemma to clinicians.

TOWARD AN OPTIMIZED TREATMENT STRATEGY

Given the fundamental changes in thyroid control related to LT4 medication, we can no longer dismiss patient complaints when discrepancies arise between clinically hypothyroid versus

biochemically euthyroid states (23–26). A recent retrospective study in patients with thyroid carcinoma followed long term found that displaced equilibria and resulting lower FT3 concentrations were associated with a lack of symptom relief, independently of known confounders such as gender, age, body weight, and weight-adjusted LT4 dose (38). This study and others contradict the assumption that patients may invariably gain satisfaction from LT4 treatment when TSH levels are kept within or even below reference range (29, 38, 90). Unfortunately, tissue T3 concentrations or T3 receptor saturation cannot be readily determined in humans. This was, however, done in rodents where various tissues remained in the hypothyroid state on LT4 monotherapy despite normal serum TSH levels, and only T3/T4 combination could restore euthyroidism in tissues (91, 92). These experiments uncovered limiting biochemical pathways that affect local sensitivity to T4 such as ubiquitination of hypothalamic type 2 deiodinase (92).

Implications emerging from advances in the understanding of the diverse facets of pituitary control (**Figures 1A–E**), clinical studies, mathematical simulations, and experimental treatments suggest replacing the current TSH paradigm with a more inclusive interpretation. This should take into account clinical signs and symptoms, all three thyroid parameters, and their relationships. The appropriateness of the respective levels relative to each other, to the previous healthy state and to a specific condition becomes more important than a blanket categorization according to placements within or outside any set range (**Table 1**).

If required for symptom relief, considering wide variations in individual responses, clinicians may find it acceptable to suppress the TSH close to or slightly below its reference range while avoiding overt clinical or biochemical hyperthyroidism. In fact, in a recent study, patients after thyroidectomy with mildly suppressed TSH levels were closest to euthyroid, based on surrogate markers for end organ response, those with normal TSH mildly hypothyroid and those with strongly suppressed levels mildly hyperthyroid (90). T3/T4 combination therapy may be preferable to patients with persistent symptoms or a failure to sufficiently raise their FT3 concentration despite LT4 dose escalation and TSH suppression (93).

This practice should not be discouraged by incorrect statistical analyses of epidemiological studies. Many studies reported increased risks associated with suppressed TSH such as atrial fibrillation and osteoporosis but failed to properly classify the hormone status of patients into euthyroid versus hyperthyroid, and frequently did not even distinguish between treatment-induced TSH suppression and endogenous hyperthyroidism (94). Importantly, thyroid hormones, while suppressing pituitary TSH, have been reported to upregulate the locally produced osteoprotective TSHβv variant (95). Statistical associations with TSH cannot establish causality, as the opposing effects of low-TSH and low-FT3/TSHβv frequently occur together in LT4-treated patients.

However, it is equally appropriate to stress a caveat that not every patient on LT4 may require or tolerate a suppressed TSH. Unfortunately, conventional range considerations for TSH do not apply to the LT4-treated patient. There is no easy fix, but a paradigm shift could be a first step toward a solution. Until more personalized methods such as setpoint reconstruction have been evaluated (37), treatment adequacy must be judged on an individual basis by a combination of clinical and biochemical outcomes. The frequent overlapping and unspecific nature of hypothyroid symptoms presents yet another challenge (96–99). Unfortunately, reliable and readily accessible markers of the tissue effects are lacking.

However, the hypothyroid patient should not opt for under-treatment and forego symptom relief out of an exaggerated fear of over-treatment. On the other hand, long-term risks of LT4 treatment should also be carefully evaluated. Assessing scientific studies is a difficult task because statistical masking, confounding, and response heterogeneity can all be expected in a statistical

TABLE 1 | Differences between the thyroid-stimulating hormone (TSH) paradigm and the newly proposed paradigm.

TSH paradigm	New relational paradigm
Normality-based statistics	Homeostatic equilibria
Univariate normal distribution	Multivariate distributions
Population-based range	Setpoint, joined TSH–FT4 pairs
Low degree of individuality	High-individuality index
TSH is reflective of thyroid hormone status	TSH is interlocked with FT4 and FT3
The reference range is fixed across individuals and conditions	The setpoint is genetically determined and adjustable to various conditions
The parameters are treated as singularities, even when interpreted in combination	The parameters are interpreted in relation to each other
Interpreting ranges	Reconstructing setpoints
Levels are interpreted as within the reference range or outside its limits	Levels are interpreted as relatively appropriate or inappropriate
A TSH within its reference range in a healthy population indicates euthyroidism	The population-based TSH reference range is too wide to reliably define euthyroidism in a person
A high TSH indicates overt or subclinical hypothyroidism with rare exceptions	A high TSH originates from diverse physiologies
The setting of reference ranges and their interpretation is a simple process	The derivation of conjoined homeostatic equilibria is an intricate process
Subclinical thyroid disease entities are solely based on laboratory measurements and do not correspond to treatable clinical entities	The clinical change or challenge is considered primary mounting a defensive reaction that may alter the setpoint or transfer function
TSH is frequently interpreted without sufficient consideration of the clinical situation	The interpretation of TSH is tied to the clinical presentation
TSH reference range is universally suitable to judge treatment success	TSH level is inadequate as a measure of treatment success and LT4 dose adequacy
The suitable TSH range remains unchanged in LT4-treated patients	The suitable TSH range is shifted in LT4-treated patients
Exclusive role of TSH in guiding treatment targets	Supportive role of FT3 in guiding treatment targets

parameter with a high-individuality index (67, 100, 101). This includes RCTs, regarded as the highest class of empirical evidence, which are not immune from violations of the underlying statistical assumptions.

Simplified concepts such as the negative thyroid pituitary feedback control contributed a fundamental understanding of endocrine principles, but were subsequently not refined for dealing with more complex facets of the various thyroid disorders and the clinical requirements of the diagnostic process. The pituitary TSH response is too diverse to be viewed as a sensitive mirror image of thyroid function. The interlocking elements of the control system are highly individual, dynamic, and adaptive. The assumed negative TSH–FT4 association that underpins the diagnostic use of TSH becomes frequently uncorrelated or even inverted, involving various influences, and mechanisms such as statistically multivariate clustering of TSH–FT4 setpoints, non-proportional cascade-type control, control mode shifts from negative feedback to positive tracking, and prevalent extra-pituitary–thyroid modulators of the relationship. Discrepancies arise between individually appropriate TSH levels and population-based reference ranges, laboratory-based disease definitions and treatment requirements, feedback adjustments and thyroid failure, and non-shared equilibria between thyroid health and LT4 treatment (**Table 1**). These have to be conceptionally reconciled to meet the needs of patients and clinical practitioners. TSH should be viewed as a controlling element and interpreted within its physiological role as a more narrowly defined and conditionally adaptive setpoint (**Figure 1**). Novel testable concepts are emerging and await clinical study.

AUTHOR CONTRIBUTIONS

RH and JM drafted the main part of the manuscript. RL and JD edited the text and contributed additional ideas, material, and text passages. JD also contributed to figures. All authors read and approved the final version.

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REVIEW
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Torpor: The Rise and Fall of 3-Monoiodothyronamine from Brain to Gut—From Gut to Brain?

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3-Monoiodothyronamine (T1AM), first isolated from rat brain, is reported to be an endogenous, rapidly acting metabolite of thyroxine. One of its numerous effects is the induction of a "torpor-like" state in experimental animals. A critical analysis of T1AM, to serve as an endogenous cryogen, is given. The proposed biosynthetic pathway for formation of T1AM, which includes deiodinases and ornithine decarboxylase in the upper intestinum, is an unusual one. To reach the brain *via* systemic circulation, enterohepatic recycling and passage through the liver may occur. The possible role of gut microbiota is discussed. T1AM concentrations in human serum, measured by a specific monoclonal assay are up to three orders of magnitude higher compared to values obtained by MS/MS technology. The difference is explained by the presence of a high-affinity binder for T1AM (Apolipoprotein B-100) in serum, which permits the immunoassay to measure the total concentration of the analyte but limits MS/MS technology to detect only the unbound (free) analyte, a view, which is contested here.

Keywords: T1AM, thyroxine, monoiodothyronamine, apolipoprotein B-100, hibernation, immunoassay, torpor, mass spectrometry

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1. INTRODUCTION

Hibernation has fascinated scientists for centuries (1–3). Obligate hibernators, e.g., the 13-lined ground squirrel (*Ictidomys tridecemlineatus*) can survive many months under harsh conditions (no food and very low ambient temperatures) in winter. They recover in spring, apparently without any functional or organ damage. The squirrel enters this state, termed torpor, by rapidly decreasing its metabolism and lowering the core temperature down to 3 or 4°C. After a few weeks in torpor, brief periods (12–24 h) of "interbout euthermia", which are essential for survival, are observed (4). The processes of hibernation (entry and arousal) are of interest, e.g., for organ preservation in transplantation (5), ischemia–reperfusion damage and cardio protection in the context of cardiac surgery (6, 7), organ protection after hemorrhagic shock or global ischemia after cardiac arrest (8), and protection of brain from ischemic injury (9). Likewise, understanding the conservation of bone and skeletal muscle mass and performance (10) despite many months of inactivity may be of interest for NASA, planning a Mars flight, perhaps with a "torpid" crew (11). A torpor-like state, but not multiday hibernation, can be induced in mice by fasting and a cage temperature below their thermoneutral zone. The search for endogenous signals that trigger hibernation or a torpor-like state started in 1969 (12) and remains topical, with the aim of developing drugs for therapeutic hypothermia. Among the endogenous compounds mentioned in reviews (13, 14) is 3-monoiodothyronamine (T1AM). This paper shall serve as a focused review on torpor induction by T1AM in the context of its pharmacology and the mysteries of its biological origin.

2. EXCITING PROPERTIES OF THE NOVEL, THYROXINE-DERIVED, HORMONE 3-MONOIODOTHYRONAMINE

"*These unique molecules* [i.e. T1AM] *have developmental potential as cryogens for the treatment of stroke, in which rapid and prolonged cooling offers outstanding therapeutic benefit to patients*" (15). "*Such potent actions of 3-T1AM, its metabolites, and synthetic congeners are of eminent interest in emergency and critical care medicine, surgery, tissue transplantation, metabolic and eye clinics, as well as space science. Application of an endogenous biogenic cryogen derived from a hormone provides a rather safe and valuable "lead compound" to be tested and developed by the pharmaceutical industry for various medical applications…*" (16). "*From the current body of literature, potential therapeutic applications with T1AM are quite apparent, ranging from sleep/torpidity induction, conferring protection against ischemic injury, and anti-obesogenic by inducing increased metabolic reliance on lipid oxidation*" (17). "*The major endogenous thyroid hormone metabolite 3-iodothyronamine (3-T1AM) exerts marked cryogenic, metabolic, cardiac and central nervous system actions. It is bound to apolipoprotein B-100 (ApoB-100), possibly facilitating its cellular uptake via interaction with the low density lipoprotein-receptor*" (18).

3. TRACE AMINE-ASSOCIATED RECEPTORS AND 3-MONOIODOTHYRONAMINE

T1AM, after its isolation from rat brain, was tested as a putative endogenous ligand (19) for activation of trace amine-associated receptors (TAARs) (20, 21). Trace amines (e.g., octopamine, tyramine, β-phenylethylamine) received their name from their two to three orders of magnitude lower abundance in brain tissue compared to classical amine neurotransmitters such as noradrenaline, serotonin, or dopamine.

Trace amines are formed enzymatically from aromatic amino acids by decarboxylation and were, for a long time, regarded as curiosities. The situation changed when high-affinity binding sites were identified in brain membranes by classical grinding and binding experiments with radiolabeled trace amines. The sensitivity of these agonist binding sites to guanylyl nucleotide inhibition indicated their relationship to the family of Gα*s* G protein-coupled receptors, which finally led to cloning of the first prototype TAAR1 of a larger family in 2001. For a review of its discovery and properties, we recommend the review by Grandy (22). Except TAAR1, all other TAARs function as odorant receptors expressed on olfactory neurons. Mice possess 14 of such receptors in their nose epithelium (23) compared to five TAARs in humans. Among them is human TAAR5, which is activated by trimethylamine, occurring in rotten fish (24) and TAAR2, which also occurs in human white blood cells (25) and in mucosal layers of the gastrointestinal tract of mice (26). TAAR1 protein is expressed in brain but also in the periphery [e.g., heart, T-lymphocytes, stomach (27), duodenum, and pancreatic β-cells (28, 29)]. Human TAAR1 is implicated in drug addiction, eating behavior, sleep-wake balance, and neuropsychiatric disorders (30, 31). This explains the initial excitement for T1AM that was postulated to be a metabolite of T_4 and an endogenous physiological signal acting rapidly *via* cell surface receptors similar to the actions of T_4 and T_3 on $\alpha_V\beta_3$ integrins (32).

Injecting T1AM into mice induces a "torpor-like" state. This immediately fascinated scientists and even convinced a National Space Lab program in South Korea to fund research on the newly found "hibernating" drug (33).

4. PHARMACODYNAMICS OF T1AM

Rodents such as mice and rats have a large surface area compared to their volume, consequently suffering from much greater heat loss compared to larger animals (34). At cage temperatures below thermoneutrality (about 28°C for rats and 30°C for mice), the sympathetic nervous system and brown adipose tissue (BAT) are always activated (35), and a considerable fraction of the total energy expenditure is spent for cold-induced thermogenesis *via* BAT. Many small mammals have a natural defense mechanism during the colder season, upon a decline in food supply (36). The set point is lowered in the hypothalamus, and the core temperature approaches ambient temperatures. This "torpor-like" state may not be confused with hibernation (4) but is nevertheless often used as a readout for drug candidates investigated for therapeutic hypothermia. Among them are adenosine agonists (37–39) and α_2 adrenergic agonists (40).

Upon intraperitoneal injection of T1AM, the rectal temperature of mice dropped in a dose-dependent manner with an ED50 of around 25 mg/kg. Mice injected with 100 or 200 mg/kg died, suggesting a very small or even absent therapeutic window for torpor induction (19). Moreover, the heart rate dropped significantly, and a strong negative inotropic effect was observed in the isolated perfused rat heart preparation. The T1AM-treated mice are sedated, have a hunched back, closed eyes, and the tail rolled around the body. This "hibernated" state could be reproduced several times by multidose application, provided the animals were always warmed up between the applications (33). Despite their sleep-like state, T1AM-receiving rodents are under extreme metabolic stress: plasma levels of corticosterone, glucagon, and glucose increase several-fold in rats, but insulin is not responding to the raised glucose levels (41). The combination of sedation, bradycardia, and hypothermia in rodents is typical for centrally acting α_2 adrenoceptor agonists such as the approved drugs clonidine, guanabenz, the sedo-analgesic dexmedetomidine, or the Servier experimental compound S18116, which is one of the most potent and selective drugs from this class (42).

These agonists decrease sympathetic outflow from the brainstem and inhibit BAT thermogenesis in rodents (40). Guanabenz was able to maintain the torpid state in rats for up to 7 days without warming-up periods (43). Subcutaneous S18116 injection lowers the body temperature of mice by up to 10°C in the dose range from 0.1 to 40 μg/kg, with an EC50 value of 2.5 μg/kg (42). None of the aforementioned drugs killed the animals at acutely effective hypothermic doses, indicating a sufficiently high therapeutic index for this effect. Unfortunately, quantal dose–effect curves for T1AM are not available but exist for all of the drugs mentioned. T1AM is a highly potent agonist for the receptor subtype α_2A (29). This can

explain the inhibition of insulin release despite hyperglycemia (41) and provides the most likely mechanism by which the rapid drop in core temperature occurs. Blockade of the sympathetic outflow from the brain by T1AM inhibits heat generation by BAT, which is further enhanced by low cardiac output. All of the mentioned α_2 adrenoceptor agonists had much wider hypothermic windows than T1AM. The large number of metabolites (see Section 6) and already demonstrated additional targets possibly contribute to the pronounced toxicity. An excellent study with NMR-based metabolomics in obese mice (44) supported earlier findings in rats (41). Upon chronic application of T1AM (10 mg/kg/day) for 7 days, the compound initially increased lipolysis and β-hydroxybutyrate concentrations in plasma, indicating acute inhibition of insulin secretion. On days 5–7, a shift from lipid oxidation to either carbohydrate or protein metabolism as macronutrients was observed. After 7 days, the T1AM-treated mice, in contrast to the vehicle-treated animals, were still not gaining weight for additional 14 days and had increased valine and glycine concentrations in plasma. The authors commented on these toxic posttreatment effects as follows: "*The discovery that protein catabolism induction can occur after chronic application of T1AM at low concentration is important and demonstrates the power of combined analyses for anti-obesity drug evaluations to identify unexpected side effects*" (44).

Contrary to earlier expectations, TAAR1 is not responsible for the T1AM-induced torpor-like state. TAAR1 knockout mice still respond to the compound by lowering their body temperature. Classical activators of these receptors even increase the core temperature (45). Peripheral signals for the adaptive behavior of rodents upon fasting in a cold environment have been identified: lower leptin and insulin, higher ghrelin and uridine (46, 47) signal to the hypothalamus for a decrease of the temperature set point. Compared to α_2A receptor agonists (either approved or experimental), adenosine, its analogs or uridine (48), the low potency of T1AM to induce a torpor-like state in comparison to its toxic effects disqualify it as an "*endogenous biogenic cryogen*" (16).

5. *IN VITRO* PHARMACODYNAMICS OF T1AM

Synthetic T1AM is a potent activator of rat (EC50: 14 nM) and mouse (EC50: 112 nM) TAAR1, stably expressed in HEK-293 cells (19). In another cell line, the EC50 was determined as 22.4 nM for the rat and 1,510 nM for human TAAR1. These values may be compared to those of β-phenylethylamine, which activates human TAAR1 with an EC50 of 106 nM and rat TAAR1 with 206 nM (49). The low potency of T1AM for human TAAR1 was confirmed by others (EC50: 1,690 nM) (50). It is therefore unlikely that there exist any significant effects in humans *via* TAAR1. A useful comparison of these values may be drawn with data for a selective ligand, RO5166017 [(S)-4-[(ethyl-phenyl-amino)-methyl]-4,5-dihydro-oxazol-2-ylamine]. RO5166017 has EC50 values in HEK-293 cells of 3.3 nM (mouse), 2.7 nM (rat), and 31 nM (human) (51). In addition to a favorable pharmacokinetic profile, a radioligand screening of RO5166017 against 123 target proteins revealed little or no interaction with other receptors, transporters, or enzymes. To prove that the effects of such selective drugs on, e.g., animal behavior (51) or metabolism (28) are indeed mediated by TAAR1 and not *via* "off-targets", the TAAR1 knockout mouse is employed as a negative control. Alternatively, actions could be blocked by a selective TAAR1 antagonist (52).

With respect to the target profile of T1AM, α_2 adrenoceptors are activated with a similar potency as noradrenaline. Neuronal membrane as well as vesicular transporters for dopamine and noradrenaline (45, 53) and all subtypes of muscarinic acetylcholine receptors (54) are functionally blocked in the micromolar or sub-micromolar range. Other identified "targets" are cited in review articles (16, 17, 55). One very high-affinity binding site, apolipoprotein B-100 (ApoB-100), is mentioned here for two reasons. First, ApoB-100 is suggested to be relevant for delivery of the novel hormone to tissues (18) and second, its seemingly problematic nature in the context of accurate quantification of T1AM *via* MS/MS technology (56) deserves mention. ApoB-100 is a component of circulating VLDL and LDL lipoproteins and binds to T1AM in a 1:1 stoichiometry with a K_d of 17 nM (57). The concentration of serum ApoB-100 in healthy adults ranges from 500 to 1,500 nM but is considerably lowered in patients undergoing statin therapy.

In sum, T1AM may be genuinely termed as a "multi-target" compound, or in plain words: it is a "dirty" drug.

6. *IN VIVO* PHARMACOKINETICS

Unfortunately, the pharmacological profile is worsened upon a review of T1AM's pharmacokinetics and metabolism in rodents. A great deal of effort was spent with MS/MS technology to elucidate the fate of injected T1AM in rodents (58). After intraperitoneal injection, the parent drug is rapidly cleared from plasma with an apparent half-life of 7–8 min during the first hour. Thereafter, a slower elimination with a half-life of about 50 min takes place (59). Oxidative deamidation by monoamine oxidase to 3-iodothyroacetic acid (TA1) (60, 61), glucuronidation, sulfation (62), acetylation, and deiodination are observed. Within 3 h after a single intraperitoneal injection, the sum of the three main metabolites in the serum of mice is approximately 3 μM. At this time, the concentration of the parent compound (T1AM) is reduced by approximately two orders of magnitude from 16.6 to 0.19 μM. Moreover, the concentration of TA1 (17.7 μM) is equal to that of T1AM (16.6 μM) after 10 min. The authors were surprised about the extent and speed of T1AM breakdown: "[A] *rich, diverse metabolism such as this is not generally seen with synthetic drugs or xenobiotics*" (58).

In conclusion, suggestions regarding the value of such properties as a lead compound for further cryogenic drug development will probably not convince the pharmaceutical industry.

7. T1AM IS CLAIMED TO BE AN "ENDOGENOUS" DERIVATIVE OF THYROID HORMONE—BUT WHERE AND HOW IS IT MADE?

7.1. Tissue Distribution of T1AM

The claim of T1AM's endogenous nature was supported by its presence in extracts from rat brain and liver, heart as well as blood

samples from mice. Mass-spectrometric fragmentation of the isolated biological material and the synthetic compound yielded identical fragmentation patterns. No absolute concentrations were presented in 2004 (19), but the concentration of T1AM in drug naïve rat serum was later reported as 300 pM, in rat liver as 93 pmol/g, in rat brain cortex with 60 pmol/g, in rat kidney with 36 pmol/g (63), and in mouse liver with 2.4 pmol/g (64). Others reported 5.4 pmol/g in rat liver (65) or less than 0.3 pmol/g (66). In mouse brain, 48.3 pmol/g has been reported (60, 67) but in mice lacking histidine decarboxylase, T1AM could not be identified. In the corresponding wild type, 0.22 pmol/g were found. In the Djungarian hamster (*Phodopus sungorus*), the serum concentration was determined as 6 nM, increasing 3 h after intraperitoneal injection of 50 mg/kg T1AM about 10-fold (68). Presence of T1AM in brain homogenates of the hamsters was mentioned, but not reported in absolute concentrations.

Taken together, such high variability in the range of orders of magnitude is quite unusual for a T_4 derived metabolite. T_4 and T_3 levels are fairly constant in human plasma with very small circadian variation (69). Analytical errors can be excluded since, for example, the mice brain concentrations, ranging from 0 to 48.3 pmol/g, have been reported by the same laboratory for three different mouse strains. The fact that rat liver concentrations are 300-fold higher than serum concentrations, again obtained by the same laboratory, argue toward the liver as receiving input not primarily from the hepatic artery but from the portal vein. With respect to other mammals, the presence of T1AM in brains of guinea pigs was mentioned but no absolute amounts were reported (19). Human thyroid tissue does not contain a trace of T1AM (i.e., <0.30 pmol/g) (66). As other tissues, including those from ruminants, are easily available, a lack of such investigations is surprising, possibly pointing to special features of rodents that are often not considered of relevance for metabolite research.

7.2. Mass Spectrometry

Multiple reaction monitoring mass spectrometry (MRM-MS) or higher stage fragmentation techniques such as MS^3 of selected precursor ions serve as the golden standard for quantification of endogenously formed or exogenously acquired compounds occurring in trace amounts in biological matrices such as plasma, serum, or tissues. In the context of quantification, synthetic T1AM and a deuterated analog, serving as an internal standard, are available. They facilitate the calculation of recovery during extraction and aid the determination of the ionization efficiency during mass-spectrometric analysis. Obviously, they also enable the exact determination of the analyte's retention time during chromatographic separation, preceding the mass-spectrometric analysis. The lower limit of detection (LOD) for T1AM in serum or plasma was reported as 250 pM (66) or "*…lower than 300 pM*" (63). Later, an LOD of 35 pM (70) was reported, with human patients exhibiting an average T1AM concentration of 219 pM, ranging from 160 to 300 pM. Ackermans et al. could not discover any T1AM above their LOD in serum or plasma of eight human volunteers (66). The possible lack in recovery of ApoB-100-bound T1AM was properly accounted for by employing an extraction protocol including protein digestion *via* Proteinase K. Even more disturbing is the fact that Ackermans et al. could easily detect T1AM in the serum or liver of T1AM-treated animals but never in serum or liver of rats treated with vehicle (66). Their LOD in tissues was reported at 0.30 pmol/g, one to two orders of magnitude below the amounts found in rat and mouse livers by Scanlan's group. The latter laboratory commented the negative result as follows: "*Of note, a study designed similarly to ours was recently attempted and failed, because the investigators were unable to extract and detect endogenous T1AM by LC-MS/MS (32)*" (64). The above comment could be justified if the Scanlan laboratory supplied their tissue samples to the Amsterdam Laboratory of Endocrinology, which apparently never happened. As ApoB-100 is a major binder of T1AM, which is claimed to prevent sufficient extraction for the subsequent MS/MS analysis, it is noted that the rat liver, despite much lower production when compared to human liver, contains 146 mg/g of protein of ApoB-100 (71). After conversion into grams of wet weight, this amounts to about 60 nmol/g, which is three orders of magnitude higher compared to the highest levels ever reported for T1AM, being sufficient to bind an equal amount of T1AM. Moreover, Ackermans et al. ensured quantitative recovery of protein-bound T1AM in tissue samples by denaturing the proteins with acetic acetone. For the reasons given, the presence of ApoB-100 in the matrix cannot be made responsible for not discovering the analyte. For a detailed review addressing the major pitfalls in the quantification of thyroid hormone metabolites including T1AM, the reader is referred to the work of Richards et al. (72).

The published tissue and serum concentrations (see Section 7.6 for stability of T1AM in humans and (59) for rodents) are most likely correct. A few hypotheses are offered as explanations for the failure of Ackermans et al. to discover T1AM in the liver of vehicle-treated rats: firstly, Ackermans et al. kept the animals under different conditions as the Scanlan group, probably restrained and treated with antibiotics (73). Secondly, food or drinking water possibly contained traces of compounds which interfered with intestinal enzyme activities (e.g., deiodinases and ornithine decarboxylases), now shown to be involved in the biosynthesis of T1AM. Finally, levels in the liver are always a snapshot. If the input by whatever source occurred hours before the animals were sacrificed, the rapid degradation of T1AM lowered the amount below the reported detection limit.

7.3. T1AM Is Not Derived from Circulating Thyroxine

Two independent laboratories agree on the following, namely, that no peripheral or CNS conversion of injected T_4 into T1AM occurs in rodents. One group delivered T_4 as a ^{13}C-labeled compound ($^{13}C_6$-T_4) for 10 days with increasing doses by an osmotic minipump to rats, inducing different degrees of hyperthyroidism. They also employed the respective ^{13}C-labeled triiodothyronine (T_3) as a standard (66), but not a trace of newly formed ^{13}C-labeled T1AM was observed in serum or the CNS. Interestingly, this important result is almost never given credit to and was also not mentioned by Hackenmueller et al. (64). Hackenmueller et al. used $^{13}C_9$-^{15}N-T_4 ("heavy T_4") and the respective standards after induction of hypothyroidism in mice by feeding perchlorate and methimazole. The various explanations of their negative result are worth being read in the original publications but will not

be discussed here, especially in view of the interpretation given later by Hoefig et al. (74). In conclusion, there is no doubt that T1AM in rodents does not originate from circulating T_4 under conditions of drug-induced hypothyroidism or various stages of T_4-induced hyperthyroidism.

7.4. Ornithine Decarboxylase—The Missing Link

The entire enzymatic activity necessary to produce T1AM from T_4 was shown to exist in intestinal tissue of mice (74). The tissue contained the enzyme ornithine decarboxylase (ODC) that is capable of decarboxylating T_4 and its deiodinated intermediates. For analysis, the *ex vivo* everted gut sac model (jejunum) from pathogen free, but not axenic, mice was used. When the preparation (luminal side out) was incubated in solutions containing T_4, significant amounts of T1AM were produced that could be identified *via* mass spectrometry. *In vitro* human ODC was able to decarboxylate 3,5-T_2 to 3,5-T2AM and a possible sequence of enzymatic reactions leading to T1AM was presented. The authors attempted to explain the negative results by Hackenmueller et al. (64) and observed that a combined treatment with perchlorate and methimazole inhibited the intestinal expression of deiodinase 1 (DIO1) and ODC genes, which was not reversed by T_4. However, an explanation for the negative results regarding the hyperthyroid rats (66) was not offered.

7.5. Gut Microbiota, Cecotrophy, Coprophagy

In a recent review article (16), it is proposed that for T1AM formation, T_4 must enter the gut and may not be formed enzymatically elsewhere. This could indeed explain the high levels observed by some researchers in the rat liver. But this may not be the end of the story, especially considering additional important players: the gut microbiota (75). It is long known that deiodination and degradation of T_4 and T_3 by gut microbiota occur in the intestine of rodents (76). Moreover, when one partially decontaminates rats by feeding ampicillin, the metabolism of T_4 and T_3 is drastically changed (77). The intestinal wall contains ODC, but rodent gut microbes in the cecum and colon are another excellent source as *Klebsiella*, *Pseudomonas*, and *E. coli* (78) feature abundant constitutive and inducible (i.e., biodegradative) ODC. These enzymes appear to differ in some respect from the mammalian counterparts as the potent irreversible blocker difluoromethylornithine, currently in clinical cancer trials (79), inhibits ODCs of *Pseudomonas aeruginosa* but not those of *Klebsiella pneumoniae* and *E. coli* (80). Furthermore, there are enzymes in the gut microbiota of humans, and perhaps rodents, that are capable of decarboxylating aromatic amines (81). It is suggested to analyze T1AM in germ-free rats or axenic mice to refute the hypothesis about the role of gut bacteria.

If, as speculated here, the gut microbiome plays a significant role, a specific behavior of mice and rats may have contributed,

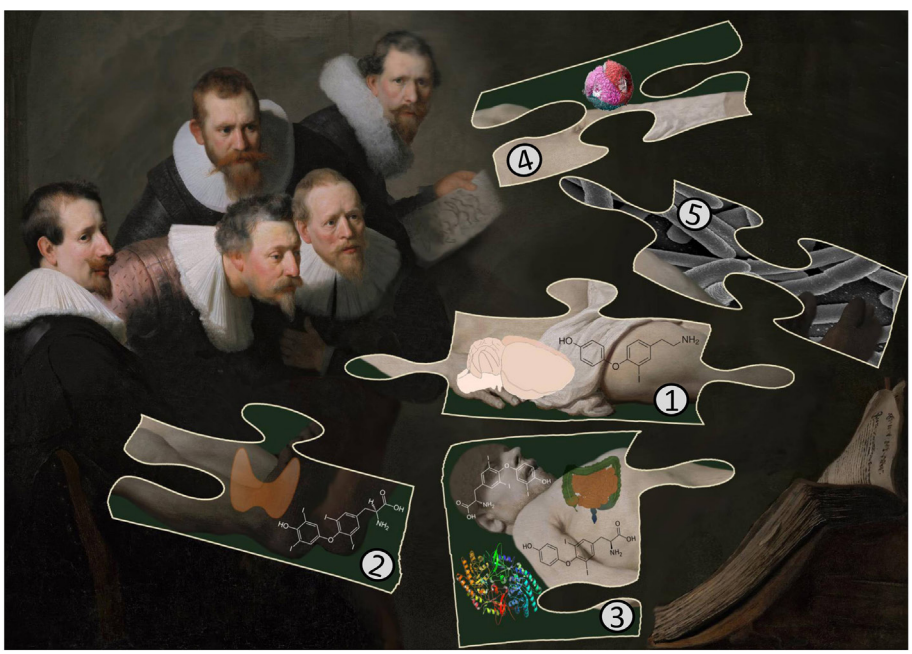

FIGURE 1 | The cartoon illustrates important findings in T1AM research as a puzzle: (1) T1AM was isolated first from rat brain and later shown to be present in liver and other rodent tissues. Surprisingly, there are no quantitative data available reporting T1AM contents in other mammalian tissues. (2) The thyroid secretes T_4 and T_3 but human thyroid tissue does not contain T1AM. (3) Rodents cannot convert externally administered T_4, labeled with ^{13}C or 13C and ^{15}N, into T1AM but T_4 is converted by mouse jejunal tissue into T1AM. The enzyme responsible for decarboxylation from deiodinated T_4 intermediates is ornithine decarboxylase (ODC). (4) Apolipoprotein B-100 in LDL is speculated to transport T1AM to target tissues and suggested to interfere with the correct determination of T1AM human serum concentrations with MS/MS but not with immunoassays [*Image adapted from Kumar et al.* (98)]. (5) The role of the gut microbiota in the biosynthesis of T1AM is unclear but speculated to be of importance.

namely the consumption of soft (or night) feces, originating from microbiota-digested cecum content. Rats ingest between 50 and 65% of their feces (82), which can enable several passages of T4 metabolites. The use of anti-coprophagy cages was not specifically mentioned in the publications about T1AM.

7.6. Serum Levels in Humans

With respect to humans, a chemiluminescent immunoassay (LIA) with a mouse monoclonal antibody for T1AM was developed. A median concentration of 66 nM in sera from healthy individuals and of 120 nM in thyroid cancer patients substituted with oral thyroxine was reported (56). In some of these patients, excessive amounts of up to 240 nM have been quantified. Most surprising was that for 10 T_4-substituted patients with pituitary insufficiency, when tested 6 days after withdrawal, the initially observed T1AM levels did not change (i.e., 97 compared to 92 nM). Somewhat lower concentrations (14.5 nM) were observed in patients undergoing heart surgery (83). For this LIA, the capture antibody for the mouse monoclonal antibody originated from goat and the reporting label was horseradish peroxidase, coupled to T1AM. Unfortunately, problems with this assay do exist, as heterophilic antibodies, especially human anti-mouse antibodies (HAMAs), may interfere (84). For reasons unknown, many cancer patients feature HAMAs (85). But a more serious problem is the human serum itself, introducing the very high-affinity binder, ApoB-100 in significant concentrations. It is suggested here that HAMAs, ApoB-100 as well as related lipoproteins observed in LDL with a K_d of 48 nM (57) may be responsible for these data, which differ from the MS/MS results by three orders of magnitude.

More recently, healthy controls were shown to have a median concentration of 8 nM whereas intensive care patients, often treated with antibiotics, had 4.8 nM (86). Here, the assay conditions have been changed as follows: the surface-bound capture is now T1AM, coupled to albumin, the mouse antibody is biotinylated, and the discovery system is Streptavidin-Europium. The authors mention ApoB-100 concentrations, apparently well aware of the aforementioned problems with the original assay. MS/MS measurements reported that T1AM concentrations in human sera or plasma are far below 1 nM (70). Roy et al. proved excellent stability of deuterated and non-deuterated T1AM in pooled human serum by incubating it for 24 hours at 37°C (57). However, in fetal bovine serum, which is often employed for cell culture experiments, pre-analytical degradation, different for internal standard and analyte (isotope effect), is suggested by preliminary experiments (87). The difference to rodent and human sera may be explained by the very high activity of soluble amine oxidases in bovine plasma. Their activity is very low in healthy humans and almost absent in rodent plasma (88).

It is hence anticipated that the ambiguities in the literature are resolved in the near future, especially in the context of proper sample preparation techniques and in adherence of analytical guidelines such as those published by the FDA or the EMEA. The publication by Rathmann et al. (89) serves as an excellent example of a thoroughly validated analytical method in this context.

8. CONCLUSION

The discovery of T1AM as an endogenous novel metabolite of T_4 exemplifies the great analytical power of MS/MS technology to identify and quantify molecules occurring in various matrices, including human plasma or animal tissues at incredibly low concentrations. The exciting finding that T1AM activates rodent TAARs with nanomolar EC50 values (19) stimulated further research. A breakthrough in the mysterious biological pathway regarding the formation of T1AM was that in addition to deiodinases such as DIO1, ODC is involved (74). One may conclude that the rise of fame of T1AM started with a rat brain extract and ended in the gut, but the question of a route from gut to brain remains (see **Figure 1**). T1AM shares an analogy with another hormone, abscisic acid (ABA). This phytohormone was isolated from pig and rat brain in 1986, guided by a highly specific antibody. The identity of ABA was proven by MS/MS and the purified compound was shown to be functionally active in a conventional ABA bioassay (90). The authors were surprised about the presence of a phytohormone in mammalian brain and kept rats on an ABA-deficient diet for a long time. To their surprise, the ABA diet-deficient rats almost doubled the content in the brain, suggesting that ABA is possibly synthesized in the absence of external supplies. Some years later, others discovered greatly reduced ABA concentrations in brain samples of ruminants but confirmed the high concentrations in rodents (91). As an explanation, the authors pointed out that ruminants had bacteria in the upper intestine whereas rats have them in the distal part. Indeed, ABA is produced by gut bacteria (92, 93). Before discarding these citations as completely irrelevant to T1AM, one should take notice that ABA is circulating in human plasma in nanomolar concentrations. ABA is not an inert contaminant from plant-derived sources. Instead, it is a powerful regulator of glucose metabolism in humans in doses of a single microgram per kilogram (94–97). One can agree with Hoefig et al. (16), that it will take much less time today compared to earlier discoveries, to unravel the mysteries of the novel T_4 metabolite.

AUTHOR CONTRIBUTIONS

HG collected the data; HG and OL wrote the paper; OL provided assistance during production of the final format of the manuscript for readers not acquainted with the subject.

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